POST GRADUATE DEGREE PROGRAMME (CBCS)
IN
BOTANY

SEMESTER-IV

Course: BOTDSET405.3

(MUSHROOM BIOLOGY)

Self-LearningMaterial

DIRECTORATEOFOPENANDDISTANCELEARNING
UNIVERSITYOFKALYANIK
ALYANI–
741235,WESTBENGAL

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Director'sMessage Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the three fold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL),University of Kalyani. Development of printed SLMs for students admitted to the DODL with in a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, NewDelhi, India under Open and Distance Mode DEB Regulations, 2020 had been our endeavour.We are happy to have achieved our goal. Utmost care and precision have been ensured in the development of the SLMs, making the useful to the learners, besides avoid in errors as far as practicable. Further suggestions from the stakeholders in this would be welcome. During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Amalendu Bhunia, Hon'ble Vice Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance. Sincere gratitude is due to the respective chairpersons as well as each and everymember of PGBOS (DODL),University of Kalyani. Heartfelt thanks are also due to the Course Writersfaculty members at the DODL, subject-experts serving at University Post Graduate departments and also to theauthorsandacademicianswhoseacademiccontributionshaveenrichedtheSLMs.We humbly acknowledge theirvaluableacademiccontributions.Iwouldespeciallyliketoconveygratitudetoallother University dignitaries and personnel involved either at the conceptual or operational level of the DODLof University of Kalyani. Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode. Self-Learning Materials (SLMs) have been published by the Directorate of Open and Distance Learning,University of Kalyani, Kalyani741235,West Bengal and all the copyright reserved for University of Kalyani. No part of this work should be reproduced in any from without permission in writing from the appropriate authority of the University of Kalyani. All the Self Learning Materials are self-writing and collected frome-book, journals and websites.

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SYLLABUS
Course: BOTDSET405.3
(MUSHROOM BIOLOGY)
(Full Marks – 50)

Course	Group	Details Contents Structure		Study hour
BOTDSET405.3	**MUSHROOM BIOLOGY**	**Unit1.** Mushroom	An introductory idea, variation in morphotypes, macroscopic and microscopic features used in morphological description.	**1**
		Unit 2. Fruiting Body Development	Stages, triggering factors, morphogens, genetic control.	**1**
		Unit3. Poisonous mushrooms	Poisonous mushrooms and their effects on human being.	**1**
		Unit4. Edible mushrooms	Edible mushrooms: Identification and nutrition value.	**1**
		Unit5. Mushroom as medicine	Mushroom as medicine and other biotechnological uses, ethno-mycological uses.	**1**
		Unit6. Mushroom cultivation technology	Infrastructure and equipments; spawn, preparation, technique of spawning; compost and composting; cultivation of button mushroom, oyster mushroom, paddy straw mushroom; mushroom processing.	**1**
		Unit7. Diseases of mushroom and management	Diseases of mushroom and management	**1**
		Unit8. Techniques for improvement of mushroom crops	Techniques for improvement of mushroom crops	**1**

CONTENTS

Mushroom Biology

Soft Core Theory Paper Credits: 2

Content Structure:

1. Introduction
2. Objectives
3. Mushroom: An introductory idea, variation in morphotypes, macroscopic and microscopic features used in morphological description.
4. Fruiting Body Development: Stages, triggering factors, morphogens, genetic control.
5. Poisonous mushrooms and their effects on human being.
6. Edible mushrooms: Identification and nutrition value.
7. Mushroom as medicine and other biotechnological uses, ethno-mycological uses.
8. Mushroom cultivation technology: Infrastructure and equipment's; spawn, preparation, technique of spawning; compost and composting; cultivation of button mushroom, oyster mushroom, paddy straw mushroom; mushroom processing.
9. Diseases of mushroom; crop management
10. Techniques for improvement of mushroom crops.
11. Suggested reading
12. Assignment

1. Introduction

The terms "mushroom" and "toadstool" go back centuries and were never precisely defined, nor was there consensus on application. During the 15th and 16th centuries, the terms *mushrom, mushrum, muscheron, mousheroms, mussheron, or musserouns* were used.

Mushrooms remained as a delicacy in human diet since time immemorial and probably predate any historical account. The therapeutic value of mushrooms has also been recognized by many of the early civilizations of Greeks, Egyptians, Romans, Chinese, and Mexican, etc., and often used them in religious ceremonies. It is now well recognized that mushrooms are not only rich in proteins but also contain vitamins and minerals, whereas lack in fats and have low carbohydrates. Furthermore, they also have high medicinal attributes like immunomodulating, antiviral, antitumor, antioxidants, and hepatoprotective properties. The importance of mushrooms is their ability to secrete an array of extracellular enzymes to convert various agro-waste materials into high-value food and valuable myco-medicinals. Therefore, mushrooms, with their abundant diversity constitute a cost-effective means, both of supplementing the nutrition of humankind and in alleviating the sufferings. The present communication deals with the biotechnology of the cultivated mushroom species with special reference to the uses of mushroom for humankind, its biology and breeding and use of molecular tools and techniques in identification, phylogenetics, and breeding improved varieties of cultivated mushrooms. Molecular markers like RAPDs, RFLPs, AFLPs, SSRs, SCAR, ESTs, microarrays, etc. permit study of any morphological, physiological or developmental process (through profiling and mapping) in which genetic variants exist with a minimum of prior information. Using genomics techniques, all the genes in an organism can be identified and genomes can be sequenced in their entirety. Moreover, using microarray and proteomic techniques activated or deactivated genes during development or in response to an environmental change can be located. Also, the molecular markers are important tools for tagging quality traits in a particular mushroom strain or variety, which can subsequently be transferred into the existing variety for the development of an improved strain with the desired quality trait. Recently, gene transfer using protoplasts fusion is a non-conventional method used to breakdown the natural barrier of gene exchange encountered in conventional breeding systems.

2. Course Objectives

To promote the possibility of self-employment after M.Sc., to enrich students' training and knowledge to practices of Mushroom Biology; to introduce the concepts of experimental designs how to develop handmade edible mushroom, and to help student's build-up a progressive and successful career in industries with a biotechnological perspective.

Learning Outcomes:

On successfully completing the course, students will be able to demonstrate a knowledge and understanding of: concept of edible and poisonous mushroom from different sources and manufacture of industrially important products as well as products with pharmaceutical importance.

3. Mushroom:

An introductory idea, variation in morphotypes, macroscopic and microscopic features used in morphological description.

INTRODUCTION TO MUSHROOM:

There is decline in income of farmers in traditional agriculture. To ensure that the farmer gets assured income year after year, there is a need for diversification in agriculture, that is, needs to cultivate different type of crops. One of the options is to grow mushrooms which can be grown on straws and other agricultural wastes. Unlike plants, mushroom cultivation is an indoor activity. It is possible to grow mushrooms in a particular season under natural conditions. It is possible to grow mushrooms throughout the year under controlled conditions. An additional advantage is that very less land is required for growing mushrooms. You must have seen that after rains many mushrooms appear from nowhere, especially in grassland, near manure heap, dung or rotting straws/ wood. We must be aware that all mushroom occurring in the nature are not edible. At present there is no simple method to differentiate an edible mushroom from non-edible type. Some mushrooms have medicinal value. A few of these are poisonous and thus it is important to cultivate edible mushrooms. In the subsequent chapters we will discuss about the cultivation of some of the common edible and medicinal mushrooms. Firstly, let us try to understand what are mushrooms and from where they come. How many types of mushrooms are there in the world, why to grow and eat mushrooms? What mushrooms are cultivated in our country and what are the basic steps in their cultivation.

When we say mushroom, many people think of only button mushroom. This is more so in West as the mushroom industry in the UK and other western countries is nearly 100% dominated by button mushroom. This could mislead you that this is the only species considered as mushroom. Actually, there are thousands of different species of mushrooms in nature. Unlike higher plants, mushrooms do not have chlorophyll (green part in leaves) which helps the plants to use water, carbon dioxide and energy from the sun to synthesize their own food. In the absence of chlorophyll, mushrooms cannot produce their own food and depend on higher plants for food. Mushrooms obtain nutrients from organic materials like straw, dead wood, manure, dung, etc.

Fig. 1.1: Button mushroom

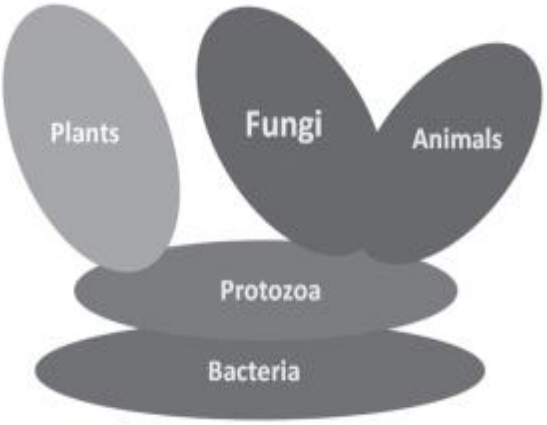


Fig. 1.2: Evolution of five kingdoms of organisms on Earth

Earlier workers considered mushrooms as plants. Now we know that these are neither plants nor animals. In evolution plants evolved from lower organisms. Soon after that the fungi and animals also got separated. Plants make their own food. Fungi and animals depend upon other organisms for food. Mushrooms have cell wall that is different from plants. Animals do not have cell wall. Because of these characters and also the method of their nutrition, growth and reproduction, etc, scientists have grouped all fungi into a separate kingdom.

Microscopic and macroscopic features used in morphological description:

Fungi are eukaryotic, heterotrophic, non-photosynthetic organisms in their own kingdom. The majority is made up of microscopic filaments known as hyphae, and the network of filaments is known as mycelium. They exist as parasites or saprophytes, absorbing organic material from their surroundings. Chitin polymer of the sugar glucosamine is found in their cell walls. Mycelium produces fruiting structures with names like sporangium, ascus, and basidium, to name a few. Sexual or asexual spores can be found in these fruiting structures. The filaments of the hyphae are haploid (1N).

Characteristics of fungi

Fungi have distinct microscopic and macroscopic characteristics that distinguish them from other organisms. Most multicellular fungal bodies, also known as molds, are composed of filaments known as **hyphae**. Hyphae can form a tangled network known as a mycelium, which serves as the thallus (body) of fleshy fungi. Septate hyphae are those that have walls between their cells; non-septate or coenocytic hyphae are those that do not have walls or cell membranes between their cells.

Macroscopic identification: mature and immature colonies of fungi

In macroscopic analysis, colony characters are observed.

- Characters of colony that are observed from the front side of the petri dish are called **obverse characters**.
- Colony characters observed from the back side of the petri dish are called **reverse characters**.

These characters are observed on the basis of:

- **Surface topography:** some fungal colonies grow freely, covering the entire surface of the agar; others grow in a restricted manner.
- **Surface texture:** fungi can be cottony or woolly (floccose), granular, chalky, velvety, powdery, silky, glabrous (smooth, creamy), or waxy.
- **Color:** Fungi can be colourless or brightly coloured. Color can be found on the fungus, its sporulating apparatus, the agar, or the colony's bottom (reverse pigmentation).
- **Colony morphology:** fungal colonies can be appeared as Rugose, Umbonate, Verrucose, Flat. Texture of fungal colonies can be Cottony, Glabrous, Granular, Velvety

Microscopic characteristics of fungi

If a possible fungus is found in a culture plate, colony characteristics are first evaluated to determine the isolate's broad group. Following the initial observations, the following microscopic criteria are used to identify the fungal isolate's genus/species.

Determine the structure of any hyphae

They are as follows:

- Whether septate or aseptate
- Whether to branch (and at what angles) or not to branch
- Pigmented or not
- The width can be even or uneven.
- Arthroconidia or pseudohyphae
- Determine the origin and structure of fruiting bodies.

Consider the following condition:

- The spores' or conidia's size and shape
- The spores' size, shape, and arrangement
- Look for the presence of any of the following special diagnostic structures: Hulle cells, pycnidia, and cleistothecia

If you only observe yeast cells:

- take note of their size, shape, and arrangement.
- Examine for the presence or absence of capsules.
- Determine whether the bastoconidiation is single or multiple daughter cells.

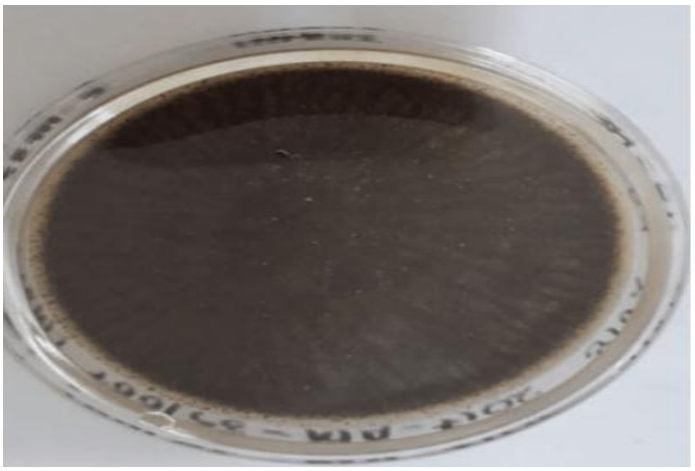
Here are microscopic characters that help to identify specific fungi:

1. Hyphae are small (3-6 micrometer) and regular in size, branching dichotomously at 45-degree angles with distinct cross-septa: Aspergillus niger
2. Hyphae are irregular in size (6-50 micrometer), ribbonlike, and septa-free: Rhizopus-Mucor-Absidia; Zygomycetes (Phycomycetes);
3. Hyphae are small (2-3 micrometers) and regular, with some branching and occasional rectangular arthrospores: only found in skin, nail scrapes, and hair: Dermatophyte class (Microsporum specie, Trichophyton specie, Epidermophyton specie)
4. Phaeohyphomyces spp., and Hyalohyphomyces spp. have regular diameter hyphae (3-6 micrometers), parallel walls, irregular branching, septate, dark yellow, brown, or hyaline hyphae.
5. Pseudo hyphae, distinct points of constriction resembling link sausages, and budding yeast forms (blastospores) are frequently observed: Candida species
6. Yeast forms, with spherical and irregular cells (5-20 micrometer), traditionally with a thick polysaccharide capsule (not all cells are encapsulated), and one or more buds that are attached by a narrow constriction: Nonencapsulated *Cryptococcus neoformans*, Cryptococcus spp.
7. Small budding yeast, 3-5 micrometers in size, with a single bud attached by a narrow base, extracellular or within macrophages: *Histoplasma capsulatum* is a type of parasite.
8. Large (8-20 micrometer) yeast forms, with cells that appear to have a thick, double-contoured wall and a single bud attached by a broad base: dermatitidis Blastomyces
9. Large, irregularly shaped (10-50 micrometer), thick-walled spherules with many small (2-4 micrometer) round endospores: Immitis Coccidioides

Examples of microscopic and macroscopic characteristics of some important fungi

Aspergillus niger

It causes a disease called black mold on certain fruits and vegetables such as grapes, apricots, onions, and peanuts etc.



Macroscopic characters:

- **Obverse character**: black in color
- **Reverse character**: white or pale in colour
- **Texture**: initially hairy that turns velvety after 10 days.
- **Margins**: circular margins
- **Top view:** color of a colony from the top appear dense

Microscopic characters:

- Its hyphae is hyaline and septate.
- Asexual conidiophores are long and globose at the tip. They are generally smooth and colorless.
- Spores are globose and have conspicuous ridges or spines not arranged in row.

Aspergillus terrus:

The filamentous fungus was isolated from a bronchial washing from one of the patients.

Macroscopic characters:

- **Obverse character**: characteristic buff or cinnamon-brown colored.
- **Reverse character**: yellow to beige-brown.
- **Texture**: powdery/flour like/crumbly due to the presence of conidia
- **Margins**: regular circular margins.
- **Top view**: the color is olive green which is white at middle.

Microscopic characters:

- Conidiophores are smooth and hyaline.
- Conidia are small, globose shaped, and smooth walled and can vary from light yellow to hyaline.
- Asexual spores are directly produced on hyphae that are larger than phial conidia.

Mucor:

Microbial genus commonly found in soil, digestive system, plant surfaces etc.

Macroscopic characters:

- **Obverse character**: new growth is white in color but turns greyish brown with aging.
- **Reverse character**: pale white.
- **Texture**: woolly growth resembling cotton-candy like.
- **Margins**: entire colony is highly dense that completely covers the plate.

Microscopic characters:

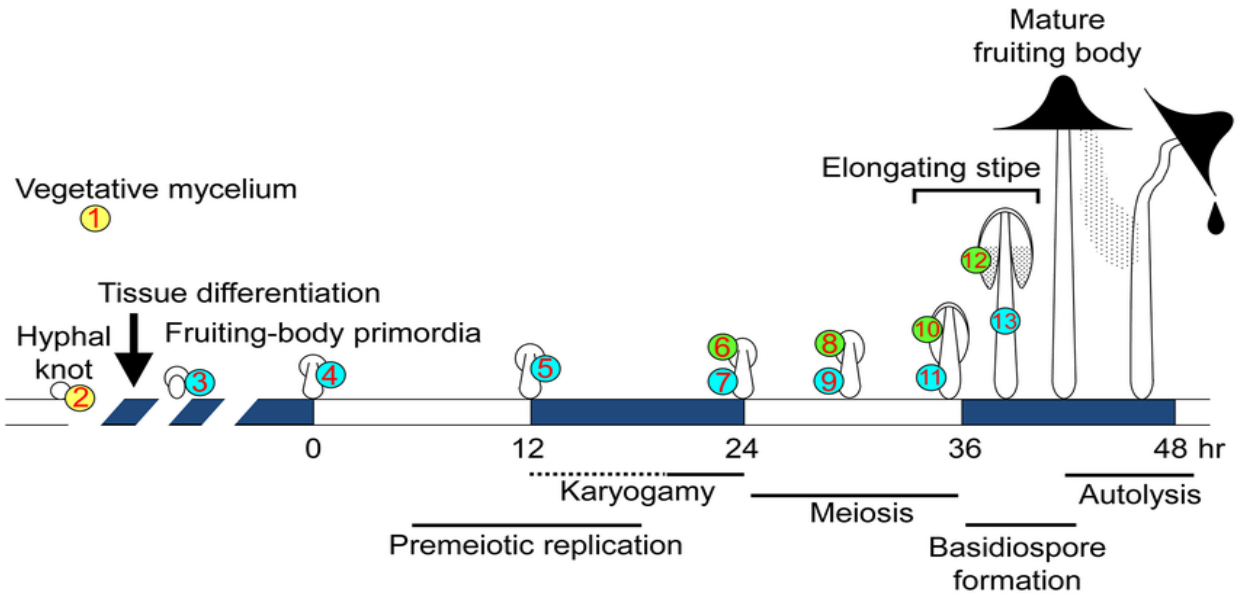
- Hyphae are non-septate or sparsely septate.
- Arthrospores are present at end of hyphae and few chlamydospores may also be present.
- Apophysis, rhizoid and stolon are absent.
- **What are the macroscopic Characteristics of fungi?**

  The macroscopic characteristics of fungi refers to the features of fungi that can be observed with the naked eye or with the aid of a hand lens or dissecting microscope, such as colony color, texture, shape, and growth pattern.

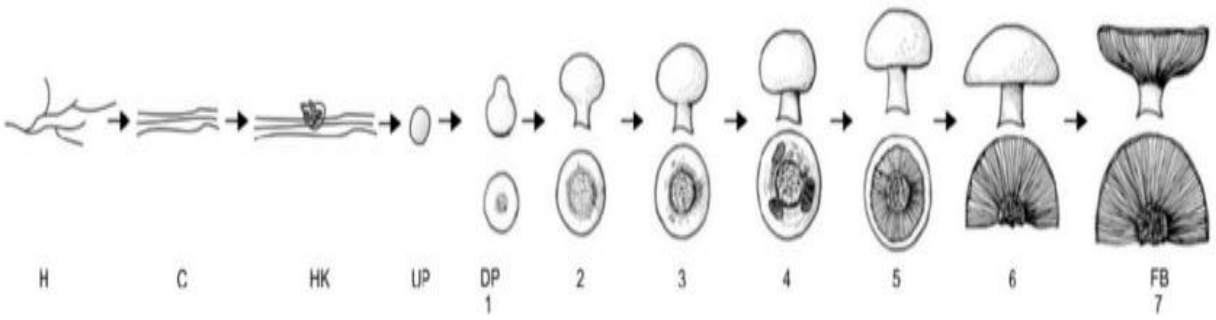
4. Fruiting Body Development: Stages, triggering factors, morphogens, genetic control.

Fruiting Body Development:

The use the term fruit bodies to encompass all the structures that develop from fungal mycelia to produce and distribute spores or other propagules, including basidiomata—the structures that release sexual spores (meiospores) in Basidiomycota, as well as a range of structures that produce asexual spores (mitospores) and some somatic (vegetative) structures, such as stromata and sclerotia, that can survive adverse conditions. Obviously, the phrase encompasses a very wide range of organs but their common feature is that they are multicellular, and their shape and form emerge as a result of a sequence of developmental adjustments. That is, they exhibit a characteristic pattern of morphogenesis.



Schematic diagram of fruiting body development in C. cinerea



Main stages in the development of a Fruiting body (FB) from hyphae (H)

Environmental factors for fruiting body induction

Environmental factors that individually or in combination influence fruiting body induction in basidiomycetes comprise physical and physiological factors. Physical factors include light, temperature, and injury. Physiological factors include nutrients, gaseous components, and hormones. The precise details of the effect of the individual environmental factors on fruiting body induction in basidiomycetes remain unclear.

Efforts to clarify these details have included the use of model organisms like C. cinerea and S. commune. These fungi can form fruiting bodies when exposed to a constant temperature with/ without light during growth on agar. In these conditions, C. cinerea forms the initial fruiting body (stage I primordia). In contrast, temperature is critical for fruiting body induction in many cultivated mushrooms (Stamets, 2000). In several species, fruiting body-inducing molecules, such as cerebrosides (Kawai and Ikeda, 1982) and saponins (Magae, 1999), have been identified. In some cultivated mushrooms, mechanical injury or scratching of the mycelia on the surface of a cultivating medium ("kinkaki" in Japanese) is effective for fruiting body induction (Yoshimura et al., 1995). In this section, environmental factors that influence the induction of fruiting bodies are discussed.

Light

Light is crucial for morphogenesis in plants and fungi for photosynthesis or morphogenesis. Its influence has been well characterized in ascomycetes (Fuller et al., 2015). It is considered that mushroom forming fungi sense light for spatial recognition for sexual reproduction, but the relationship between light and fruiting body induction in basidiomycetes has been unclear. The presence of light may not always be essential for fruiting body induction; fruiting body production can be induced under complete darkness in some basidiomycetes. However, light can induce fruiting body or promote fruiting body production; such as L. edodes (Leatham and Stahmann, 1987), Polyporus (Favolus) arcularius (Kitamoto et al., 1968), and C. cinerea (Tsusu_e, 1969). The effective wavelength for fruiting body induction includes ultraviolet wavelength (280 nm) and blue light (520 nm) (Durand and Furuya, 1985). Similar wavelengths are effective for the induction of pileus development in P. arcularius (Kitamoto et al., 1974).

More recently, it was revealed that light can induce hyphal knot formation on circle in C. cinerea cultured on limited glucose media (Muraguchi et al., 2015). These observations suggest that light can affect fruiting body induction in some basidiomycetous fungal species but is not necessarily required in some species. Light receptors that have been identified in ascomycetes include blue light receptors WC1/WC2 complex and cryA, or red light receptor phyA (Dunlap, 2006).

Temperature

Low temperature threatens life of fungal mycelia, therefore, low temperature is one of important trigger for sexual reproduction. Temperature downshift is used for fruiting body induction in many mushroom species (Stamets 2000), such as L. edodes (Nakazawa et al., 2008), A. bisporus (Morin et al., 2012), F. velutipes (Sakamoto et al., 2002), and Pleurotus eryngii subsp. tuoliensis (Bailinggu) (Fu et al., 2016), and Armillaria mella (Ford et al., 2015). Yet, fruiting body primordia can be induced without any temperature shift in model mushroom species like C. cinerea and S. commune (Muraguchi et al., 2015;). Thus, there is still a limited understanding of the molecular basis for fruiting body induction affected by temperature. Transcriptome analyses of fruiting body induction have been carried out in several cultivated mushroom species like F. velutipes (Park et al., 2014) and A. bisporus (Morin et al., 2012). However, in these studies, several environmental factors including light and aeration were active simultaneously, which made it difficult to distinguish the individual effect of temperature.

Nutrient factors and chemical compounds for fruiting body induction

Starvation is also critical signal of environmental deterioration, therefore, nutrients are also critical signal for sexual reproduction in mushroom-forming fungi. Nitrogen starvation is one of the most important factor for fruiting body induction in mushroom-forming fungi (Plunkett, 1953). Nitrogen starvation is a trigger for sporulation in S. cerevisiae and autophagy is involved in sporulation triggered by starvation. Autophagy is also involved in conidia formation in filamentous fungi (Kikuma et al., 2006a, 2006b). In several species, increased proteinase activity is correlated to fruiting body induction. and the autophagy-related gene is upregulated during fruiting body formation in Moniliophthora perniciosa (Gomes et al., 2016). However, there is no clear evidence that autophagy is involved in fruiting body induction triggered by nitrogen starvation in mushroom-forming basidiomycetes.

Carbon concentration(mainly glucose concentration) also affects fruiting body induction in several species. For example, inpresence of lowglucose (0.2%), light exposure induces fruiting body formation in C. cinerea (Muraguchi et al., 2015). Low concentration of carbon and nitrogen induces expression of galectin, cgl1 and cgl2 in C. cinerea (Boulianne et al., 2000).

In S. commune, it is reported that cAMP affects fruiting body induction (Schwalb, 1974); the amount of cAMP is increased by the addition of indole and caffeine under several conditions in S. commune (Kinoshita et al., 2002).

Gene expression during fruiting body induction Several transcriptome studies have compared vegetative mycelia and primordia in mushroom-forming fungi. Transcription factor genes have been identified in C. cinerea (Muraguchi et al., 2015) and S. commune (Ohm et al., 2011). During fruiting body initiation, genes with phospholipid biosynthesis process are also upregulated in C. cinerea (Muraguchi et al., 2015). In C. cinerea, several chromosome remodelling genes are essential for fruiting body induction (Nakazawa et al., 2016a). Upregulation of protein synthesis, energy production, and hydrophobins have been described in the fruiting body initiation stage in S. commune (Ohm et al., 2010). Stress response genes are upregulated in mycelia during fruiting body formation in several cultivated mushrooms. These observations suggest that global gene expression changes occur during fruiting body induction. However, how environmental factors, light, temperature, or nutrients stimulate these global gene expression changes for fruiting body induction remains to be investigated.

Environmental factors for fruiting body development

Aggregation of hyphae occurs in the early stage of fruiting body development, and fruiting body shape can be immediately affected by environmental factors including nutrients, humidity, temperature, carbon dioxide concentration, gravity and especially light. These environmental factors affect one another to form appropriate shape of fruiting body for effective spore dispersal. In general, light affects pileus formation and stipe elongation, and fruiting bodies that form in the dark have an abnormal shape that is characterised by a long stipe with tiny or no pileus (called dark stipe: C. cinerea (Terashima et al., 2005), etiolated stipe: C. cinerea (K€ues, 2000), or pinhead fruiting body: F. velutipes (Sakamoto et al., 2007)). Light also affects the direction of stipe elongation, which results in positive phototropism. Many mushrooms also display gravitropism, and carbon dioxide can affect fruiting body shape.

Carbon dioxidide concentration affects fruiting body shapes

The influence of the gaseous condition on fruiting body shape is relevant in commercial mushroom production. Respiration and the concentration of carbon dioxide during fruiting body formation have been investigated (Kinugawa and Tanesaka, 1990, 1994). Respiration activity increases during primordial formation in the development of fruiting bodies, and a high concentration of carbon dioxide affects fruiting body morphology. Sensitivity to carbon dioxide has been investigated in the commercially cultivated mushroom varieties of F. velutipes, P. ostreatus, Pholiota nameko (microspora), L. edodes; of these, P. ostreatus is most sensitive to carbon dioxide (Kinugawa et al., 1994). In many mushroom species, pileus is not fully developed and stipe is spindly elongated at a high carbon dioxide concentrations (Kinugawa et al., 1994). The morphology is similar to that produced in the absence of light. In the

early stage of fruiting body development, sensitivity to carbon dioxide is more pronounced (Kinugawa et al., 1994). Elevated carbon dioxide affects the synthesis of the cell wall component, R-glucan (Sietsma et al., 1977) and fruiting body cell morphology (Raudaskoski and Salonen 1984).

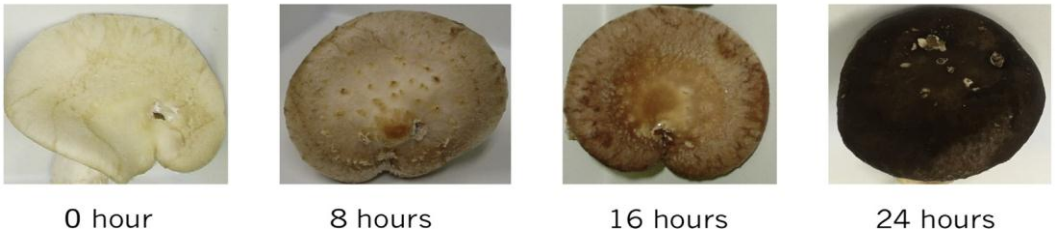
Tropism toward gravity and light

Many mushrooms possess tropism toward gravity and light, termed gravitropism and phototropism, respectively, because the angle of the gill toward the ground is critical for spore diffusion (Kern, 1999). Both have been observed in many mushroom species (Moore et al., 1996). Fruiting bodies of Agaricales that are exposed to light from one side often grow toward the light (positive phototropism), and an exception is Agaricus bisporus (Eger-Hummel, 1980). Many mushrooms possess negative gravitropism. It is suggested that the lifestyle of each mushroom (e.g. wood rotting or epigeous) affects the gravitropism or phototropism (Buller, 1909). Basically, lignicolous agaric fruiting bodies tend to develop upward under the influence of light, whereas the gills develop downward under the influence of gravity. Remarkably, when exposed to light from below, the fruiting bodies grow downward through all stages of development (Kaneko and Sagara, 2001b). Gravitropic bending involves growth inhibition at the upper side of a horizontally oriented transition zone, termed the graviperceptive region of the stipe. Pileus differentiation affects stipe elongation in mushroom-forming fungi

Induction of pileus development by light

Light is crucial for pileus differentiation in basidiomycetous mushroom-forming fungi, with a few exceptions like A. bisporus. Notably, some species can form fruiting bodies in dark.

These fruiting bodies have long and tiny pileus or no pileus on long stipes. C. cinerea fruiting bodies formed in complete darkness have tiny pileus (Tsusue, 1969). This suggests that the very early stage of pileus development can occur in complete darkness (Terashima et al., 2005), whereas further development cannot (K€ues, 2000). Light induce pileus formation, but darkness is necessary for further maturation in C. cinerea (Kamada and Tsuji, 1979).



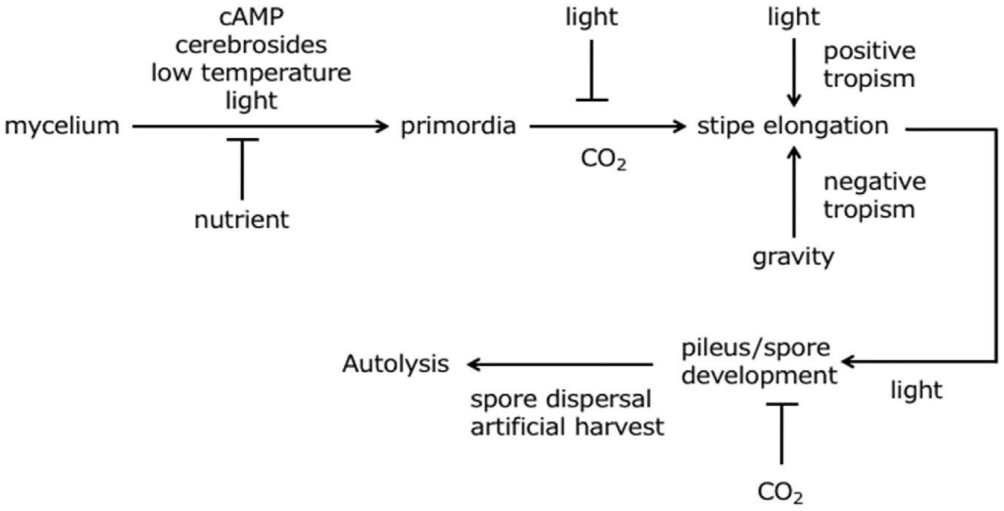
Coloration of cap in Lentinula edodes after light exposure. Light-exposed fruiting body formed in dark.

Maturation and senescence of fruiting bodies

After the pileus fully develops, the fruiting bodies will mature and senesce. In the maturation stage, fruiting body colour intensifies in many mushroom species and spores become coloured in some mushroom species. C. cinerea spores turn black, pileus autolysis occurs after spore maturation (K€ues, 2000). And some spores are diffused with droplets of the lysed pileus. Similar maturation and senescence steps occur in many cultivated mushroom species, and the quality of fruiting bodies decreases after the pileus fully expands. In cultivated mushrooms, postharvest maturation and senescence is a quality-control concern from the commercial viewpoint. Colouration (melanin synthesis) and softening (cell wall degradation) are observed in many cultivated mushroom species after harvest (Sakamoto et al., 2012).

Maturation of fruiting body and autolysis of pileus

Stipe elongation, pileus expansion, and pileus autolysis are strictly controlled by the progression of karyogamy and spore formation in C. cinerea (K€ues, 2000). For example, the light and darkness cycle is necessary for karyogamy induction in C. cinerea, and diffusible factor(s) affecting basidiocarp maturation are induced by the darkness in phase 3 (Kamada and Tsuji, 1979). Pileus autolysis will occur at the karyogamy stage, and spores are diffused with droplets of lysed pileus in C. cinerea (K€ues, 2000). It is suggested that cell wall-degrading enzymes are involved in cap expansion and autolysis. Several cell wall-degrading enzymes have been isolated from lysing pileus. 1,3-b-Glucan hydrolases (Zhou et al., 2015) and chitinase have been purified from C. cinerea (Niu et al., 2016). Lysed cell walls will be substrates for cell wall of new spores and be a nutrient for spore germination. Previously, a pileus expansionless mutant was identified in C. cinerea and shown to be defective for pileus expansion and autolysis.



Summary of developmental pathway (including exceptions) of mushroom-forming baisidiomycetes influenced by environmental factors.

Fungal Morphogenesis

Within the developing tissues of a fruit body, cells embark on a particular course of differentiation in response to the interaction of their intrinsic genetic programme with external physical signals (light, temperature, gravity, humidity), and/or chemical signals from other regions of the developing structure. These chemicals may be termed organisers, inducers or morphogens, and may inhibit or stimulate entry to particular states of determination. Chemical signals may contribute to a morphogenetic field around a structure (cell or organ), which permits continued development of that structure but inhibits formation of another structure of the same type within the field. All of these phenomena contribute to the pattern formation that characterises the 'body plan' created by the particular distribution of differentiated tissues in the multicellular structure. Pattern formation depends on positional information, which prompts or allows the cell to differentiate in a way appropriate to its position in the structure and may be conveyed by concentration gradients of one or more morphogens emitted from one or more spatially distinct organisers. Pattern formation thus involves an 80 David Moore et al. instructive process, which provides positional information, and a second interpretive process, in which the receiving cell or tissue responds. Fungi are 'modular organisms' in which growth is repetitive, and a single individual mycelium will have localised regions at very different stages of development (Andrews, 1995). Consideration of developmental regulatory systems is relevant to the current discussion because any effect of the external environment on fruit body development must operate through an influence on the control systems that determine the distribution and growth patterns of the multicellular structure. The constituent cells of a fungal fruit body are generally considered to be totipotent (able to follow any pathway of differentiation), because a mycelial culture can be produced in vitro from a fragment of a mature, fully differentiated structure, e.g. a fruit body stem. This feature results in a morphogenetic plasticity which surpasses that of other organisms and provides an intellectual challenge in terms of developmental biology, taxonomy and genetics (Watling and Moore, 1994). The only exceptions to totipotency are the meiocytes (the cells within which meiosis occurs), which are committed to sporulation once they have progressed through meiotic prophase (Chiu and Moore, 1988a, 1988b, 1990, 1993; Chiu, 1996). On the other hand, even meiocytes can be 'used' for non-sporulation functions: the hymenium of Agaricus bisporus is packed with basidia held in an arrested meiosis and serving a purely structural function (Allen et al., 1992). Differentiated fungal cells require reinforcement of their differentiation 'instructions'. This reinforcement is part of the context within which they normally develop, but when removed from their normal environment most differentiated hyphae revert to vegetative

hyphae. Hyphal differentiation is consequently an unbalanced process in comparison with vegetative hyphal growth. In most hyphal differentiation pathways the balance must be tipped in the direction of 'differentiation' by the local microenvironment, which is, presumably, mainly defined by the local population of hyphae. Another common feature is that morphogenesis is compartmentalised into a collection of distinct developmental processes (called 'subroutines' Moore, 1998a). These separate (or parallel) subroutines can be recognised at the levels of organs (e.g. cap, stem, veil), tissues (e.g. hymenophore, context, pileipellis), cells (e.g. basidium, paraphysis, cystidium) and cellular components (e.g. uniform wall growth, growth in girth, growth in length, growth in wall thickness). They are distinct genetically and physiologically and may run in parallel or in sequence. When they are played out in their correct arrangement the morphology that is normal to the organism results. If some of the subroutines are disabled (genetically or through physiological stress), the rest may still proceed. This partial execution of developmental subroutines produces an abnormal morphology. The main principles that govern fungal development as deduced from observation, experiment and computer modelling are summarised in Table 1 (from Moore, 2005). Fungal morphogenesis must be totally different from animals, because fungal cells have walls, and from plants (whose cells also have walls) because hyphae grow only at their tips and hyphal cross-walls form only at right angles to the long axis of the hypha.

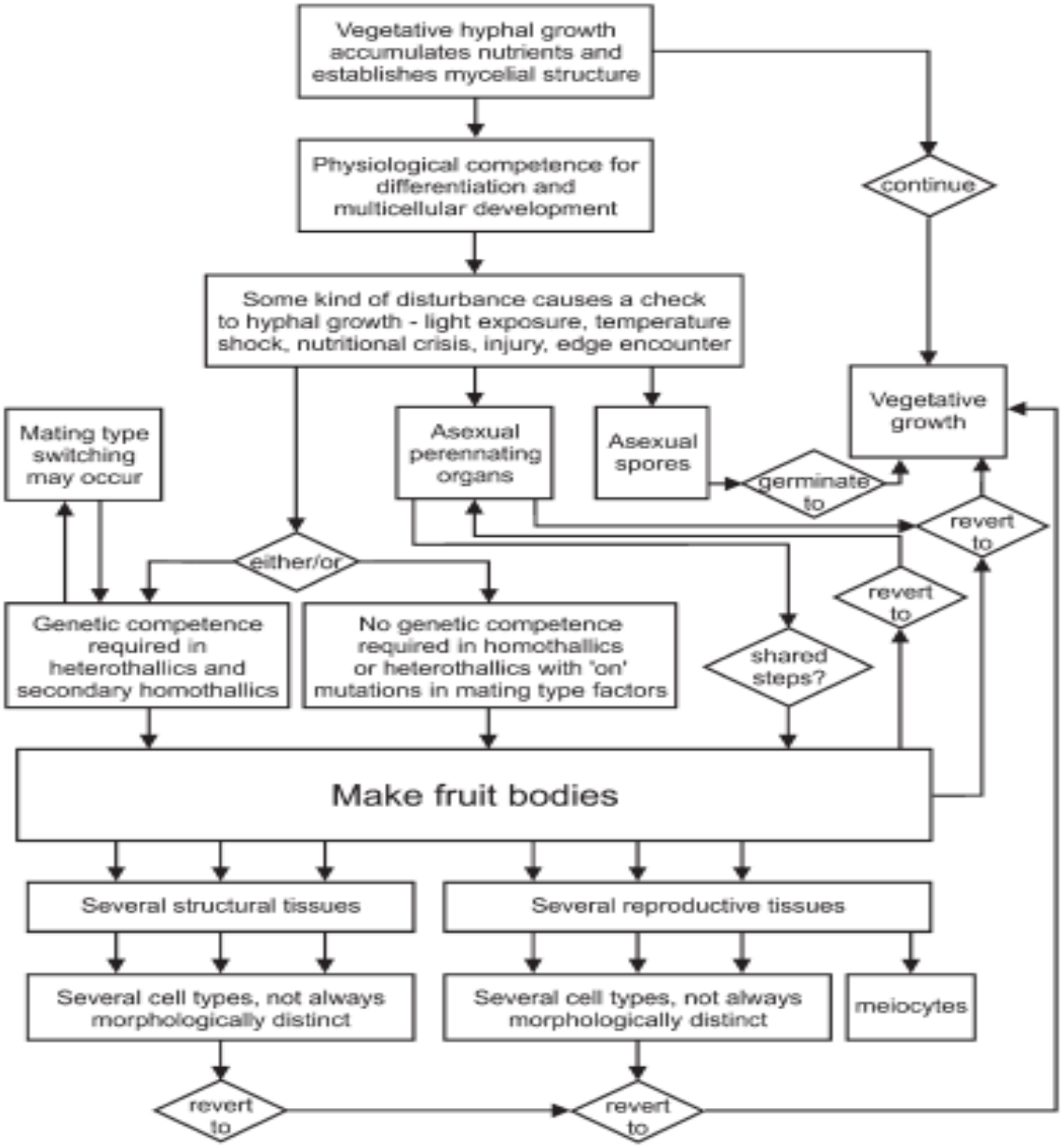


Figure 1 Flowchart showing a Simplified View of the Processes involved in Development of Fruit Bodies and other Multicellular Structures in Fungi (from Moore, 1998a).

Consequently, fungal morphogenesis depends on the placement of hyphal branches. A hypha must branch to proliferate. To form a multicellular structure, the position at which the branch emerges and its direction of growth must be controlled. A major aspect of that directional control is an autotropism—a tropism to self—in which growth direction of each hyphal branch is influenced by the position of the rest of the mycelium. Exploratory mycelia experience a negative autotropism, which causes them to grow away from the main mycelium and this maintains the outward exploration of the substratum. On the other hand, to create a multicellular structure like a fruit body, positive autotropism is essential to cause hyphae to grow together for

hyphal branches to cooperate and coordinate their activities. Tropic reactions imply a signalling system, a signal sensing system and a reaction system. Mathematical models of these systems can be created very successfully (Stockus and Moore, 1996; Meskauskas et al., 1998, 1999a, 1999b, 2004a, 2004b; Moore et al., 2006), but we know nothing yet about their biochemistry, cell biology or molecular nature. However, it is clear that what mechanisms exist must be different to animals and plants because gene sequences known to regulate development in animals and plants do not occur in fungal genomes (Moore et al., 2005; Moore and Meskauskas, 2006).

Morphogenetic Control Elements:

The only major morphogenetic control elements known in fungi are the mating type factors, which regulate pheromone production and pheromone receptors involved in mating, ranging from recognition between sexually competent cells in yeast to governing growth of clamp connections, internuclear recognition and regulation of the distance between the two nuclei in Basidiomycota (Casselton, 2002). However, not all fungi possess mating type factors, and, indeed, even in species that have a well-developed mating type system apparently normal fruit bodies can be formed by haploid cultures, and fruit body formation can usually be separated from other parts of the sexual pathway by mutation (Moore, 1998a). Generally, vegetative compatibility genes define the individuals of fungal populations, while mating type factors are usually interpreted as favouring the outbreeding of a fungal population (Chiu and Moore, 1999). Consequently, mating type genes contribute to management of the genetics of the population as 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 Table 1. to distribute viable spores, and poorly (or wrongly) differentiated cells still serving a useful function Principle 11 Mechanical interactions influence the form and shape of the whole fruit body as it inflates and matures, and often generate the shape with which we are most familiar Source: From Moore (2005). 84 David Moore et al. well as to the sexual development of the individual. Sexual reproduction generates genetic variation, offers an escape from DNA parasites and provides a means to repair DNA damage (Bernstein et al., 1985). 1.3 Importance of Sexual Reproduction The crucial step in sexual reproduction, which provides the contrast with asexual reproduction, is the fusion of nuclei derived from different individuals. If the individuals involved in a mating have different genotypes, the fusion nucleus will be heterozygous and the products of the meiotic division can be recombinant genotypes. Thus, in one sexual cycle, new combinations of characters can be created in the next generation for selection. Consequently, the most common ‘explanation’ for sex is that it promotes genetic variability throughout-crossing and that variability is needed for the species to evolve to deal with competitors and environmental

changes. There is plenty of evidence to show that asexual lineages change little in time and that out-crossing certainly does promote variability in a population, which enables the organism to survive environmental challenges (Hurst and Peck, 1996; Burnett, 2003). This, though, is a 'group selectionist' interpretation. It argues that variation generated in an individual meiosis benefits the group or population to which the individual belongs. Yet current theory prefers to emphasise that selection acts on individuals (Carlile, 1987; Dawkins, 1989). A feature that is advantageous in selection must be so because of benefit to the individual itself or its immediate progeny. As noted above, an alternative interpretation of the selective value of a sexual cycle suggests that repair of damaged DNA is the crucial advantage of meiosis (Bernstein et al., 1985). It is argued that bringing together genomes from two different individuals enables DNA damage in one parental chromosome, caused by mutation or faulty replication, to be repaired by comparison and recombination with the normal chromosome provided by the other parent. Genetic fitness would be increased but only when out-crossing ensures heterozygosis. Even an incomplete sexual cycle might be of advantage in this case. Gene mutations can be recessive and damaging, and different mutations are likely to occur in different mitotically generated cell lines. Just the formation of the diploid (or heterokaryon in most Basidiomycota) by out-crossing will benefit the mated individual if recessive adverse mutations are masked by non-mutant ('wild-type') alleles in the nuclei of the other parent. Out-crossing might also give rise to heterozygous advantage, where the heterozygous phenotype is better than either of its homozygous parents. This has been demonstrated frequently in plants and animals, and also in Saccharomyces cerevisiae (James, 1960). Clearly, the genotype of the parental mycelium makes a crucial contribution to the genetics of the progeny population, but to produce a progeny population the parental mycelium must first produce a crop of fruit bodies and to do that it must grow into and through the substratum to capture, translocate and accumulate sufficient nutrients to support the formation of what can be massive multicellular structures.

PROPERTIES OF MORPHOGENESIS IN FUNGAL CELLS

The defining morphogenetic characteristic of fungal cells is their polarity, whereby cell surface expansion and wall deposition are confined to discrete sites on the cell surface. Although hyphae and yeasts show obvious differences in their modes of growth (Fig. 1), they share three basic properties that enable polarized growth and the formation of a diverse array of cell shapes. The first is symmetry breaking, in which an initially isotropic cell generates an axis of polarized growth. The second is polarity maintenance, which refers to the stabilization of the polarity axis such that polar growth ensues. The third is depolarization, in which polarity is lost in a controlled manner. The balance between polarity maintenance and depolarization generates the

diversity of fungal cell shapes. Fungal cells, typically, are not polarized during the earliest phases of development. They usually undergo an initial period of nonpolar isotropic expansion (e.g., yeast mother cells, spores). Ultimately, however, cellular symmetry must be broken and a polarity axis generated, either for bud site selection or the development of polar structures, such as hyphae. One form of symmetry breaking relies on preexisting spatial landmarks that direct the recruitment of the morphogenetic machinery to a discrete site on the cell surface. The best-known example is the bud site selection process in Saccharomyces cerevisiae, which uses a set of landmark proteins to communicate positional information to the morphogeneticmachinery via sequentialGTPase modules (Chant 1999). In contrast, the fission yeast Schizosaccharomyces pombe uses cell end markers coupled to a microtubule-based delivery system to demarcate polarization sites (Martin 2009). Another form of symmetry breaking refers to the formation of a polar axis without any detectable, preexisting landmark (WedlichSoldner et al. 2003). Although a full consensus has yet to be reached regarding the specific details of this form of symmetry breaking (Johnson et al. 2011), it does involve the simultaneous action of multiple feedback loops that direct the accumulation of the activated form of the Cdc42 GTPase at a discrete site on the cell surface (Freisinger et al. 2013). Such feedback loops can be regulated to permit formation of multiple polarity axes based on studies in S. cerevisiae (Wu and Lew 2013). This might provide a basis for understanding how filamentous fungi can generate several hyphae from a single cell. Once an axis of polarized growth has been generated, it must be stabilized to ensure that cell surface expansion and wall deposition are confined to a discrete site. The existence of a stable polarity axis is reflected by the resulting asymmetry of cellular organization (Riquelme 2013). Vesicle exocytosis is largely confined to that discrete site, which is flanked by endocytic zones in which membrane material and proteins are retrieved for recycling. Localized actin filaments support exocytosis, whereas actin patches mediate endocytosis. Arrays of cytoplasmic microtubules are organized in parallel to the polarity axis and, generally, mediate longer-range vesicle movements to and from the growth site. Localized changes in plasma membrane composition might be one broadly applied mechanism for maintaining polarity in both yeasts and hyphae. This is consistent with the recent observation that polarity factors are spatially segregated into distinct clusters on the cell surface in both S. pombe and S. cerevisiae (Dodgson et al. 2013). Although some of these features vary across different growth forms (i.e., yeast cells vs. hyphae), they appear to be universally coordinated by Cdc42/Rho1-related GTPase modules and their numerous effectors.

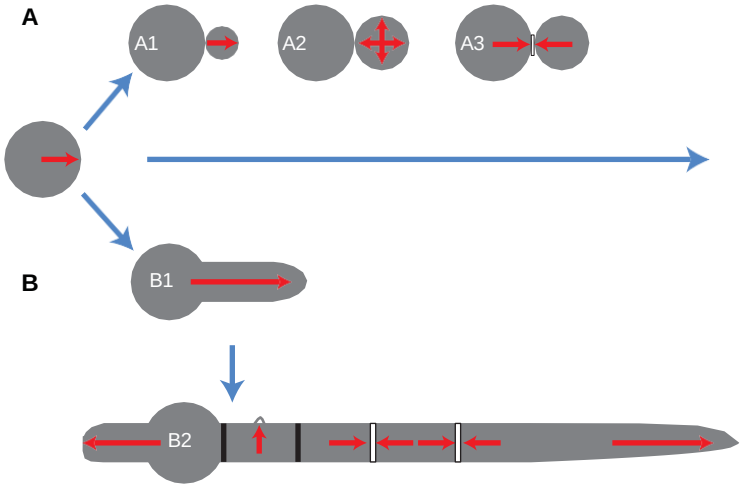


Figure1. Distinct patterns of morphogenesis in yeast and hyphal cells. Yeasts and filamentous fungi typically initiate growth as nonpolarized cells or spores. Budding yeasts such as Saccharomyces cerevisiae or Cryptococcus neoformans (A) establish an axis of polarity that directs the emergence of a new bud (A1). Following a period of polarized growth, depolarization enables the formation of an ellipsoidal bud (A2). Following nuclear division, the construction and controlled degradation of a septum (A3) results in cell separation. Filamentous fungi such Aspergillus nidulans and Candida albicans (B) establish a polarity axis that directs the emergence of a germ tube (B1). Unlike yeasts, sustained polar growth leads to the formation of a hypha that grows by apical extension. Moreover, hyphae are able to simultaneously support multiple polarity axes to allow the formation of septal cross-walls (bars) and lateral branches (B2). For many filamentous fungi, spores also generate secondary germ tubes once they are partitioned from the primary hypha by a septum.

Hyphal Morphogenesis

Hyphae are multicellular filaments in which symmetry breaking leads to the formation of a new hypha referred to as a germ tube or branch initial (Fig. 1). To date, there is no evidence supporting the existence of spatial landmarks that designate sites of germ tube or branch emergence. It seems reasonable that these sites are selected stochastically (e.g., spontaneous po- larization). On the other hand, there is evidence suggesting that secondary polarization events in germinating spores are biased toward the pole that is opposite to the original polarization site (i.e., a bipolar polarization pattern) (Fig. 1) (Harris 1999). The basis of this bias remains unknown.

The continued growth of germ tubes and branch initials results in their maturation into hyphae. This generally correlates with an in-crease in the rate of apical extension (Horio and Oakley

2005), as well as the appearance of a Spitzenkörper (apical body). The latter struc- ture is typically located proximal to the hyphal tip (Sudbery 2011), largely composed of vesicles of different sizes, micro- filaments, and ribosomes (Harris et al. 2005; Verdin et al. 2009). An extensive body of data support the view that Spitzenkörper dynamics determine the shape of the hyphal tip, dictate the orientation of hyphal extension (Bartnicki- Garcia et al. 1995; Riquelme 2013), and help maximize rates of apical extension (Kohli et al. 2008). Nevertheless, the presence of a Spitzen- körper is not an obligate requirement for the formation of polarized hyphae. It should be noted that the Spitzenkörper exists within the context of a larger assembly that has been re-ferred to as the hyphal tip complex (Taheri- Talesh et al. 2008). Components of this complex include the polarisome and exocyst, which lo- calize to the extreme hyphal apex in which they regulate exocytosis, as well as the subapical collar that consists of actin patches and the endocytic machinery (Sudbery 2011a). As char- acterized in *Aspergillus nidulans*, precise spatial organization of the hyphal tip complex does seem to be a strict requirement for polarized hyphal growth. Thus, the Spitzenkörper might be a highly structured hyphal tip complex re- quired for efficient apical growth.

A single fungal spore is capable of generat-ing a mycelium composed of an extensive net- work of branched hyphae. At the margins of the colony, individual hyphae show an avoidance response, whereas in the colony interior, hyphal fusion (anastomosis) permits the exchange of nutrients and perhaps growth signals (Rayner 1996; Simonin et al. 2012). An important con- cept that underlies mycelium formation is api- cal dominance, which refers to the suppression of secondary polarity axes in the vicinity of an actively extending hyphal tip (Rayner 1991). Apical dominance enables the precise regula- tion of lateral branching by minimizing the competition between hyphal tips for existing resources. Although not well understood, the enforcement of apical dominance in some fila- mentous fungi requires the accumulation of re- active oxygen species at the hyphal tip (Tanaka et al. 2006; Semighini and Harris 2008). It seems likely that calcium gradients would also play a role in this process (Schmid and Harold 1988). Because of apical dominance, lateral branches often emerge from subapical hyphal compartments separated from the tip by a septum. In some filamentous fungi, septa conceiv- ably could play a role as "branching landmarks" (Harris 2011a), although there appears to be no universal pattern of lateral branching. One distinct branching pattern observed in some hy- phae is apical branching, whereby an existing hyphal tip "splits" into two distinct tips. This pattern of branching is shown by rapidly grow- ing hyphae of *Ashbya gossypii* (Philippsen et al. 2005).

Yeast Morphogenesis in Ascomycetes

Yeasts generally divide either by budding or fission (Martin and Arkowitz 2014). Even in nearly spherical and symmetric yeast cells, cell polar- ity plays a major role in cell division and growth. The establishment of subcellular asymmetry is required in the context of the most polar events of yeast cell growth, for example, estab- lishing the site of new bud emergence (Fig. 1). In *S. cerevisiae*, symmetry breaking leads to local- ized cell surface expansion and wall deposition at the incipient bud site. As a new daughter cell begins to form, the mother cell must direct the trafficking of new cellular material to this growing cell. Subsequently, the morphogenetic machinery then relocalizes to the mother–bud junction to enable septum formation and cyto- kinesis. With cytokinesis, there is a transition to the more symmetric phase of isotropic growth until the cells are ready for new budding events. Therefore, yeast cell division is dependent on a dynamic and ordered transition between cytoskeletal polarization and loss of polarization to complete its budding cell cycle (Fig. 1).

Yeast Morphogenesis in a Basidiomycete

Much of the work studying the cell division events for common pathogenic yeasts uses par- adigms established in the ascomycete *S. cerevi- siae*. However, basidiomycete yeasts show both conserved and distinct features of morphogen- esis. By definition, a basidiomycete fungus (e.g., *Cryptococcus neoformans*) produces hyphal structures during sexual differentiation that dis- tinguish it from ascomycetes (Kwon-Chung 1975, 1976). Spores produced by *Cryptococcus* hyphae are quite infectious and may be the pri- mary particle inhaled during a natural infection (Giles et al. 2009; Velagapudi et al. 2009). There- fore, the transition to hyphal growth that sup- ports sporulation is required for the wide dis- semination of *Cryptococcus* in the environment, as well as for infection of its mammalian hosts. Once inhaled in the lung, cryptococcal spores germinate to produce yeast cells. Dur- ing the context of infection, this fungus grows within the human host almost exclusively in a budding, yeast-like form.

Evolution of Fungal Morphogenesis

An intriguing question in fungal morphogene- sis is the evolutionary relationship between hy- phae and yeasts. It seems likely that the cell mor- phology of ancestral fungi resembled protists (Jones et al. 2011), and hyphal growth emerged as the dominant growth form that facilitated the colonization of terrestrial habitats (Stajich et al. 2009). Yet, many filamentous fungi also show yeast-like patterns of cellular morphogen- esis, particularly during development (Cole and Samson 1979). It seems reasonable that specific ecological niches favored the loss of hyphal growth in the subphylla Saccharomycotina and Taphrinamycotina (i.e., the clades containing *S. cerevisiae* and *S. pombe*, respectively), resulting in the evolution of clades in which yeast growth forms dominate

(Harris 2011a,b). Nevertheless, many members of these clades retained the capacity to undergo hyphal (or pseudohyphal) growth in response to different environmental triggers. These so-called dimorphic fungi in- clude many prominent human and plant path- ogens whose morphotype transitions play crit- ical roles in virulence.

THE MORPHOGENETIC MACHINERY

Vesicle Trafficking

Both yeasts and filamentous fungi possess exocytic vesicles of distinct classes based on their size and presumed content (Harsay and Bretscher 1995). In filamentous fungi, each class of exocytic vesicles appears to transit through the Spitzenkörper on its way to the apex of the hyphal tip. The localization of exocyst compo- nents to the apex suggests that this is the ulti- mate site in which vesicles fuse to the target plasma membrane (Taheri-Talesh et al. 2008). However, recent studies with *Aspergillus oryzae* point to the existence of additional secretion sites, including septa and subapical sites (Ha- yakawa et al. 2011). These observations imply that the trafficking of exocytic vesicles is far more complex than previously imagined.

The specificity of vesicle interactions with their target plasma membranes is likely governed by interactions between vesicle and tar- get soluble NSF (*N*-ethylmaleimide-sensitive factor) attachment protein receptors (v- or t- SNAREs). Filamentous fungi possess a large complement of v-SNAREs and there appears to be some degree of redundancy in their func- tion (Kuratsu et al. 2007). It should be noted that alternative routes for the delivery of con- tents to the cell surface might not rely on the classical secretion pathway (Rodrigues et al. 2011).

The Golgi complex serves a pivotal role in vesicle trafficking, as it is the source of exocytic vesicles and terminus of many endosomes. Thus, in the Golgi, different classes of vesicles must be sorted and matched to their correct cargoes. The importance of the Golgi to vesicle trafficking and morphogenesis has long been established in *S. cerevisiae* (Schekman and No- vick 2004), but only recently has its importance for sustained polar growth been described for filamentous fungi (Pinar et al. 2013). Indeed, whereas the Spitzenkörper appears to be re- quired primarily for rapid rates of hyphal exten- sion, polarized growth fails completely in the absence of a functional Golgi complex.

The Cytoskeleton

The organization and function of both micro- filaments and -tubules have been well charac- terized in yeasts and filamentous fungi. Formins assist nucleating microfilament formation at polarization sites (Evangelista et al. 2003; Pear- son et al. 2004; Schmitz et al. 2006), but the formation of a stable axis of hyphal polarity can occur in their absence, as shown for *A. ni- dulans* (Sharpless and Harris 2002). On the other hand, regulators of actin patch formation and

dynamics appear to be essential for the for-mation of a stable axis (Araujo-Bazan et al. 2008; Upadhyay and Shaw 2008; Hervas-Aguilar and Penalva 2010). These observations underscore the importance of endocytosis in polarized growth, but also imply that the localized delivery of cell wall material to polarization sites can oc- cur in the absence of actin filaments. Limited evidence suggests that microtubules can poten- tially compensate for the absence of actin fila-ments (Virag et al. 2007). Normally, the key function of microtubules in hyphae appears to be positioning of the Spitzenkörper to enable proper orientation of hyphal extension (Fischer et al. 2008). However, in the absence of actin filaments at the tip, microtubules likely ensure sufficient vesicle flux to support apical exten- sion, albeit at less than optimal rates.

Septins are highly conserved throughout fungi and animals, wherein they play multiple roles in cellular morphogenesis (Hall et al. 2008). In *S. cerevisiae*, septins form scaffolds that are essential for normal polarized growth, septation, and cytokinesis (Oh and Bi 2011). In filamentous fungi, septins are needed for normal septum formation and appear to delimit growth sites (Ryder et al. 2013). For example, the ab- sence of septins in *A. nidulans* leads to the si-multaneous emergence of multiple germ tubes and hyperbranching. However, further matura-tion of branch initials into secondary hyphae is blocked. In general, septins function as scaffolds that coordinate localized cell wall biosynthe- sis with the cytoskeleton and vesicle trafficking machinery. Thus, septins likely consolidate and stabilize polarity axes, thereby preventing the formation of spurious ones.

Positional Markers

The only spatial landmarks that have been char- acterized in filamentous fungi are those that also function in *S. cerevisiae* and *S. pombe*. In budding yeast, the cell surface protein Axl2 acts in conjunction with the septin-associated pro- teins Bud3 and Bud4 to specify the axial bud- ding pattern of mating-type a or a cells, whereas a distinct set of cell surface proteins (i.e., Bud8, Bud9, Rax1, Rax2) specify the bipolar pattern observed in a⁄a cells (Chant 1999). In either case, the landmarks trigger local activation of Cdc42 via the Ras-like GTPase Bud1 (Bi and Park 2012). In fission yeast, the plasma mem- brane-anchored protein Mod5 provides a target for the delivery of Tea1 via the plus ends of cytoplasmic microtubules (Snaith and Sawin 2003). This serves to orient microtubules and allows the accumulation of a formin-containing complex that enables localized microfilament formation (Martin 2009). The relationship be- tween this pathway and Cdc42 in *S. pombe* is not clear, although some evidence suggests that they may act in parallel to promote cell polarity (Das et al. 2009).

Signal Transduction

Small GTPases, such as Ras, Rho, Cdc42, and Rac, play a fundamental role in the regulation of fungal morphogenesis. Typically, they act via multiple effectors to coordinate organization of the cytoskeleton and vesicle trafficking at po- larization sites. Cdc42 effectors include p21-associated kinases such as Cla4, the Borg-related proteins Gic1 and Gic2, formins such as Bni1, Wiscott– Aldrich syndrome protein homologs such as Las17, and the exocyst (Park and Bi 2007). Rho GTPases have well-established roles in the regulation of b-glucan synthesis and cell wall integrity (Levin 2011). Less is known about potential effectors of Ras that might mediate polarized growth, particularly in filamentous fungi. It should be noted that there are examples of effectors with important roles in polarized hyphal growth that are not well conserved in *S. cerevisiae*. A particularly prominent example is NADPH oxidase, which appears to be an effector of Rac1 in filamentous fungi (Tanaka et al. 2006; Semighini and Harris 2008), in which it regulates seemingly diverse morphoge- netic processes, such as lateral branching and infection-related morphogenesis (Tanaka et al. 2006; Egan et al. 2007). Small GTPases, such as Ras, Rho, and Cdc42, do not operate in isola- tion, but rather in a sequential manner as suggested by studies in *S. cerevisiae* and *S. pombe*. For example, considerable evidence shows that Ras GTPases function upstream of Cdc42 to regulate polarized growth (Chang et al. 1994), whereas Cdc42 and Rho1 may antagonize each other to spatially and temporally coordinate localized cell wall deposition (Gao et al. 2004). Collectively, these observations reinforce the central role of small GTPase modules as regula- tors of hyphal morphogenesis in fungi. Never- theless, the extent to which individual GTPases contribute to specific aspects of morphogenesis appears to vary across fungal species.

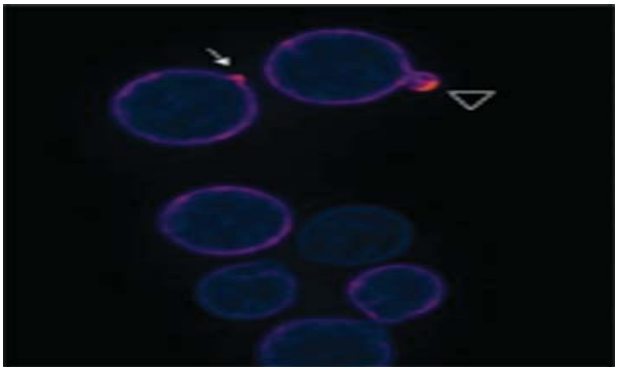


Figure 2. *C. neoformans* Rac2 localization suggests a role in cell polarity. A Gfp– Rac2 fusion protein was expressed in *C. neoformans* and visualized using an Olympus (Center Valley, PA) IX70 microscope.

The molecular events directing *C. neofor- mans* yeast cell polarity also possess conserved and unique features compared with ascomyce- tous yeasts. As in other fungi, the Ras1-Cdc24-

signaling pathway appears to control many aspects of yeast-cell polarization and budding. Ras proteins control the activity of Cdc24, a guanine– nucleotide exchange factor, which, in turn, controls the activation of downstream ef- fector proteins, such as Cdc42 (Zhao et al. 1995; Bassilana et al. 2003). In *C. neoformans*, muta- tion of either the *RAS1* or *CDC24* gene results in a mutant strain that cannot efficiently repolarize its actin cytoskeleton after exposure to stresses, such as elevated temperatures (37◦C). This defect in cell polarity manifests as tem-

perature sensitivity because of unchecked iso- tropic growth, with eventual arrest as a large, unbudded yeast (Alspaugh et al. 2000; Nichols et al. 2007). In addition to temperature ele- vations, the *ras1* and *cdc24* mutants are more susceptible to other physiologically relevant stresses, such as hypoxia (Nichols et al. 2007). Together, impaired resistance to conditions encountered in the host make these strains avir- ulent in animal models of cryptococcosis (Al- spaugh et al. 2000; Nichols et al. 2007). Studies in these mutant strains, therefore, emphasize the role of proper cell morphogenesis and bud- ding in survival within the host.

MORPHOTYPE, NICHE ADAPTATION, AND FUNGAL VIRULENCE

Unlike in multicellular eukaryotes, in which most cells are typically embedded in a rela- tively constant environment, fungal cells face the challenge of unpredictable environmental fluctuations. Appropriate responses, including morphological changes, are often needed to sur- vive, reproduce, and disperse. Cells in the hyphal form are inherently more effective in penetrat- ing physical barriers and expanding colony growth three-dimensionally. Hyphae are also more likely to show gravitropism, thigmotro- pism, phototropism, aerotropism, and galvano- tropism (Hoch et al. 1987; Crombie et al. 1990; Moore 1991; Moore et al. 1996; Aoki et al. 1998; Idnurm and Heitman 2005; Brand et al. 2007). In contrast, yeasts or yeast-like cells (including conidia, endospores, etc.) are superior in ampli- fication, stress tolerance, and dispersal in liquid or air. The ability to switch morphotypes helps fungi to survive or escape otherwise suppressive environments, contributing to the success of this kingdom in the tree of life (Institute of Med- icine (US) Forum on Microbial Threats 2011). Morphotype switching is a prominent strat- egy adopted by phytopathogenic fungi for host invasion and dissemination. In *Ophiostoma ulmi*, the causal agent of Dutch elm disease, the mycelial form is required to penetrate adja- cent xylem vessels. In contrast, the conidia/ yeast form (conidia bud in a yeast-like fashion) is used to translocate within the individual xy- lem vessels via the host transpiration stream (Kulkarni and Nickerson 1981). Production of conidia or yeast-like blastoconidia in planta by *Verticillium* species is also implicated in the rapid dissemination of the pathogen within the host vascular tissue in diverse plant species (Schnathorst 1981; Pegg and Brady 2002).

My- cosphaerella graminicola, an important wheat pathogen, switches from a yeast-like form to the infectious hyphal form to invade leaf tissue through stomata (Mehrabi and Kema 2006). In *Taphrina deformans,* the causal agent of leaf curl in peach and almond, yeast cells land on leaves and undergo a mitotic nuclear division to es- tablish a binucleate condition. The resulting dikaryotic cells subsequently switch to parasitic filamentous growth in planta (Rodrigues and Fonseca 2003). Similarly, in the biotrophic maize pathogen *Ustilago maydis,* haploid yeast cells of compatible mating types fuse to form a dikaryon, which subsequently generates the in- fectious dikaryotic hyphae to continue growth and development in vivo (Banuett 1991). Al- though the hyphal form is used by many plant pathogens to invade the host, filamentous growth itself may not be adequate, or even re- quired, for pathogenesis.

Likewise, the ability to undergo morpho- type transitions is often required for human fungal pathogens to adapt to the host environ- ment, elude host immune defense systems, and inhabit different niches in the host. The classic thermally dimorphic fungi, such as *Histoplas- ma capsulatum, Blastomyces dermatitidis*, *Para- coccidioides brasiliensis*, *Coccidioides immitis*, and *Penicillium marneffei,* grow as saprophytic molds in the environment. After host infection, conidia or hyphal fragments convert to yeasts to replicate intracellularly and disseminate through the host (Gauthier and Klein 2008).

Similarly, the yeast form of *C. albicans* is associated with the commensal state, and the appearance of filaments is associated with invasive disease. The escape of *C. albicans* from phagocytic cells requires the switch from the yeast form to the hyphal form (Lorenz et al. 2004). Failure to sustain polarized hyphal growth leads to the entrapment of *C. albicans* intracellularly (Zakikhanyet al. 2007). Although

C. albicans hyphae predominate at the infiltra- tion site, yeast cells are seen on the epithelial cell surface, as well as emerging from penetrating hyphae (Scherwitz 1982; Ray and Payne 1988).

Dermatophytes (e.g., *Trichophyton rubrum*) are known to grow as hyphae when infecting the stratum corneum or hair follicle. However, dur- ing invasive or deep dermal infections, derma- tophytes also manifest morphological diversity as broad, pleomorphic hyphae with budding yeast– like arthrospores are observed (King et al. 1975; Bibel et al. 1977; Lillis et al. 2010; Mar- coni et al. 2010; Brand 2012). Such morpholog- ical switching in the dermatophyte *Trichophy- ton mentagrophytes* can also be observed under certain in vitro conditions (Bibel et al. 1977). In contrast, *Cryptococcus* species grow as encapsu- lated yeasts in animals/humans. Its filamentous form is only occasionally seen in host tissues and considered less virulent (Lin 2009; Mag- ditch et al. 2012; Wang et al. 2012). For this intracellular pathogen, the ability to hijack host cells bypasses the requirement of the hy- phal form for host invasion. Thus, *Cryptococcus* can

translocate, invade, escape, and disseminate in the host, all in the yeast form.

In term of pathogenesis, fungal morphotype transitions can alter the host– pathogen interac- tion by the differential presentation of patho- gen-associated molecular patterns in different morphotypes. For example, in *B. dermatitidis*, *H. capsulatum*, and *P. brasiliensis*, the transition from the hyphal to the pathogenic yeast form is accompanied by increased a-1,3-glucan dep- osition in the cell wall (Kanetsuna and Carbon- ell 1971; Seider et al. 2010), which masks the immunostimulatory b-glucan (Rappleye et al. 2007). Additionally, these different fungal mor- photypes can display altered tolerance of certain host physiological conditions, as well as varying abilities to disseminate in different host tissues (Klein and Tebbets 2007).

The yeast and hyphal forms of *C. albicans* also differentially interact with the host and its immune cells. For example, the hyphal form of this fungus does not expose the immunostimu- latory b-glucan (Gantner et al. 2005). Instead, it produces a suite of hypha-specific factors such as Als3, Hyr1, and Hwp1 to assist in host inva- sion (Staab et al. 1999; Luo et al. 2010).

In *Aspergillus fumigatus*, both hydropho- bins and melanin coat the conidial surface to help the cell type evade host detection and re- main protected within host phagolysosomes (Aimanianda et al. 2009; Volling et al. 2011; Carrion et al. 2013). On germination and hy- phal growth, cell wall protein CspA is un- masked, helping to mitigate hyphal damage in- duced by neutrophils (Levdansky et al. 2010). Thus, factors produced during morphogenesis, in addition to the morphotype transition itself, help fungi adapt to host conditions.

ENVIRONMENTAL REGULATION OF HYPHAL MORPHOGENESIS

Sensing Nutritional and Environmental Signals

Pathogens must be proficient at sensing and adapting to their surroundings to survive changing host microenvironments. *C. albicans* cells undergo the yeast to hypha transition in response to many nutritional and environmen- tal signals, including an increase in temperature to 37◦C, neutral pH, serum, nutrients, *N*-ace- tylglucosamine (GlcNAc), hypoxia, and CO_2.

Sensors for many of these signals have been identified in *C. albicans* (Fig. 3) (Cottier and Muhlschlegel 2009). Many of the strong hy- pha-inducing signals are sensed and integrated by the adenylate cyclase Cyr1, which is indis-pensable for hypha growth under all conditions (Bahn and Sundstrom 2001; Rocha et al. 2001; Hogan and Sundstrom 2009; Zou et al. 2009). CO_2/$HCO_3$2 directly stimulates Cyr1p activity by binding to the catalytic domain of Cyr1 (Klengel et al. 2005). The cyclase activity is also regulated by the small GTPases (Ras1, Ras2), G-protein coupled receptor Gpr1, and Ga protein Gpa2 in response to nutrients (Maidan et al. 2005a; Zhu et al. 2009). Gpr1 in *C. albicans* is responsive to methionine, but not to glucose (Maidan et al. 2005b), suggesting that this signal is likely encountered by this fungus in vivo. The

rapid increase in temperature to 37◦C is also essential for hyphal induction, likely by reliev-ing Hsp90-mediated repression of the Ras1- Cyr1 pathway (Shapiro et al. 2009, 2012). The cAMP-dependent protein kinase A (PKA) is a major target of Cyr1, consisting of one regula- tory subunit (Bcy1) and two catalytic subunits (Tpk1 and Tpk2). Each PKA subunit has dis- tinct functions in *C. albicans* hyphal develop- ment, indicating even further adaptability of this otherwise highly conserved signaling sys- tem (Sonneborn et al. 2000; Bockmuhl et al. 2001; Cassola et al. 2004). A major function of the *C. albicans* cAMP-PKA pathway is to down-regulate the expression level of *NRG1* (Lu et al. 2011), the major repressor of hyphal morpho-genesis (Braun et al. 2001; Murad et al. 2001). This down-regulation of *NRG1* expression re-quires the transcription factors Efg1 and Flo8 (Lu et al. 2011).

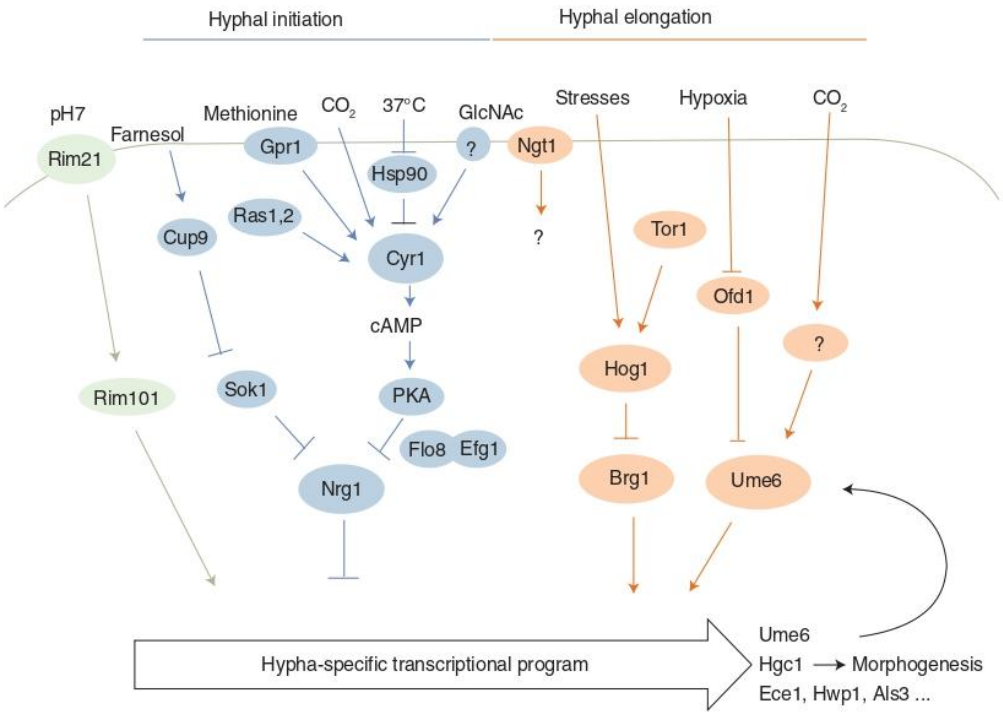


Figure 3. Signal transduction pathways integrating various signals for morphogenesis in *C. albicans*. Selective signal transduction pathways and regulators of morphogenesis are shown. Arrows indicate activation. Bars indicate inhibition. Hyphal-specific regulator Ume6 sustains the hypha-specific transcriptional program and Hgc1 promotes hyphal morphogenesis.

The yeast-to-hypha transition in C. albicans must be initiated and then maintained (Lu et al. 2011). Hyphal initiation requires temporary clearing of Nrg1, which needs a transient activation of the cAMP-PKA pathway. In contrast, hyphal maintenance requires continuous and active sensing of the surrounding environment.

During hyphal initiation, when the Nrg1 protein disappears, the expression of a GATA family transcription factor Brg1 is activated in response to serum, starvation, or treatment with rapamycin via reduced Tor1 signaling (Lu et al. 2011, 2012; Su et al. 2013). The accumulated Brg1 recruits the Hda1 histone deacetylase to promoters of hypha-specific genes, leading to nucleosome repositioning, obstruction of Nrg1 binding sites, and sustained hyphal development (Lu et al. 2011, 2012). The conserved Tor1-signaling pathway functions as a global regulator of cellular growth in response to nutrient availability, and it controls different cellular processes in fungi (Rohde and Cardenas 2004; Rohde et al. 2008). Therefore, hyphal development is controlled by two major nutrient responsive and growth-regulating pathways.

Other environmental factors also control morphological decisions in human fungal pathogens. The combination of hypoxia and high CO2, but neither condition alone, maintains C. albicans hyphal elongation, even in mutants lacking the nutrient responsive chromatin-remodeling pathway (Lu et al. 2013).

Extracellular pH is another important environmental factor that regulates fungal growth (Fig.3). A fungal-specific signaling pathway, characterized by the activation of the Rim101/ pacC transcription factor, controls the cellular response to changes in pH. First identified in S. cerevisiae and A. nidulans, this pathway is also conserved in fungal pathogens and controls important microbial interactions with the host environment. C. albicans grows in the yeast form in acidic conditions and forms hyphae in neutral/alkaline conditions.

Quorum Sensing

Quorum sensing is the regulation of gene expression and group behavior in response to changes in cell-population density. Quorum sensing in C. albicans was established based on the observation that dense cultures display a reduced propensity for the yeast-to-hypha switch (Hornby et al. 2001). The inhibitory activity is caused by the accumulation of a sesquiterpine alcohol, farnesol (Hornby et al. 2001), formed from an intermediate of the sterol biosynthesis pathway (Hornby et al. 2003). Therefore, farnesol is a quorum-sensing molecule secreted to the medium by C. albicans cells as a cell density signal (Hornby et al. 2001). At concentrations of 10–250 mM, farnesol inhibits hyphal initiation, but it does not suppress hyphal elongation (Mosel et al. 2005). Farnesol is reported to exert its inhibitory effects on germ-tube formation through Ras1-Cyr1 (Davis- Hanna et al. 2008).

Contact Sensing

In C. albicans, the growth direction of hyphae can be dictated through contact with a surface (thigmotropism) (Gow et al. 1994). Thigmotropism is seen when growing hyphae come into contact with a ridge. Instead of continuing through the ridge, hyphae modify their growth

direction (Brand et al. 2007). This response involves two plasma membrane proteins, Mid1 and Cch1, which are components of the high affinity calcium uptake system, and Fig. 1, a member of the low-affinity calcium system (Brand et al. 2007).

From Transcription to Hyphal Morphogenesis

Hyphal growth requires sustained activation of Cdc42 at the growing tips. This is achieved by hypha-specific expression of a G1-type cyclin protein Hgc1. The hypha-specific Cdk1Hgc1 phosphorylates and prevents Rgt2 GTPase-activating protein (GAP) from localizing to hyphal tips in which Cdc42 is concentrated, resulting in a local increase of Cdc42-GTP at hyphal tips (Zheng and Wang 2004; Zheng et al. 2007). Therefore, hypha-specific expression of Hgc1 is responsible for maintaining Cdc42-GTP at the hyphal tips and for sustained polarized growth in hyphae.

CONCLUSION

Morphological transitions are commonly observed among diverse fungal species. These cellular responses are accompanied by equally profound changes in cell physiology and structure. As noted above, the highly regulated process of fungal morphogenesis represents an adaptive response to specific stresses encountered in various microenvironments, including that of the infected host. Therefore, morphological and physiological plasticity allows fungi to rapidly adapt to changing extracellular conditions.

Species-specific signaling and morphological features appear to be a direct result of fungal attempts to survive as new microenvironments, and their particular cell stresses, were encountered. The concerted action of morphotype and physiological changes in the context of a particular environment are therefore critical for successful fungal adaptation (Butler et al. 2009; O'Connor et al. 2010). Defining the cellular machinery controlling fungal morphogenesis offers unique insight into our basic understanding of fungal life cycles and pathogenesis.

5. Poisonous mushrooms and their effects on human being.

Mushrooms are a type of fungus. Australia has many varieties of wild-growing fungi, many of which are edible. However, a few types are poisonous or even deadly. Contrary to popular belief, there is no home test that can distinguish between edible and poisonous varieties. The only way to tell whether a wild mushroom is safe to eat is to have it identified by a mushroom expert (mycologist).

If you are unsure if a mushroom is safe to eat, don't eat it. It is recommended that you only eat mushrooms you have bought from the supermarket, greengrocer or another reputable source.

Mushroom poisoning is poisoning resulting from the ingestion of mushrooms that contain toxic substances. Symptoms can vary from slight gastrointestinal discomfort to death in about 10 days. Mushroom toxins are secondary metabolites produced by the fungus. Mushroom poisoning is usually the result of ingestion of wild mushrooms after misidentification of a toxic mushroom as an edible species. The most common reason for this misidentification is a close resemblance in terms of color and general morphology of the toxic mushrooms species with edible species. To prevent mushroom poisoning, mushroom gatherers familiarize themselves with the mushrooms they intend to collect, as well as with any similar-looking toxic species. The safety of eating wild mushrooms may depend on methods of preparation for cooking. Some toxins, such as amatoxins, are thermostable and mushrooms containing such toxins will not be rendered safe to eat by cooking.

However, there are a number of recognized mushroom toxins with specific, and sometimes deadly, effects:

Toxin	Toxicity	Effects
Alpha-Amanitin	Deadly	Causes often fatal liver damage 1–3 days after ingestion. The principal toxin in the death cap.
Phallotoxin	Non-lethal	Causes extreme gastrointestinal upset. Found in various mushrooms.
Orellanine	Deadly	Redox cycler similar to paraquat. Causes kidney failure within three weeks after ingestion. Principal toxin in genus *Cortinarius*.
Muscarine	Potentially deadly	Causes SLUDGE syndrome. Found in various mushrooms. Antidote is atropine

Monomethylhydrazine (MMH)	Deadly	Causes brain damage, seizures, gastrointestinal upset, and hemolysis. Metabolic poison. Principal toxin in genus *Gyromitra*. Antidote is large doses of intravenous pyridoxine hydrochloride.
Coprine	Non-lethal	Causes illness when consumed with alcohol. Principal toxin in genus *Coprinus*.
Ibotenic acid	Potentially deadly	Excitotoxin. Principal toxin in *Amanita muscaria*, *A. pantherina*, and *A. gemmata*.
Muscimol	Potentially deadly	Causes CNS depression and hallucinations. Principal toxin in *Amanita muscaria*, *A. pantherina*, and *A. gemmata*.
Arabitol	Non-lethal	Causes diarrhea in some people.
Bolesatine	Non-lethal	Causes gastrointestinal irritation, vomiting, nausea.
Ergotamine	Deadly	Affects the vascular system and can lead to loss of limbs and/or cardiac arrest. Found in genus *Claviceps*.

The period between ingestion and the onset of symptoms varies dramatically between toxins, some taking days to show symptoms identifiable as mushroom poisoning.

Alpha-Amanitin: For 6–12 hours, there are no symptoms. This is followed by a period of gastrointestinal upset (vomiting and profuse, watery diarrhea). This stage is caused primarily by the phallotoxins and typically lasts 24 hours. At the end of this second stage is when severe liver damage begins. The damage may continue for another 2–3 days. Kidney damage can also occur. Some patients will require a liver transplant. Amatoxins are found in some mushrooms in the genus Amanita, but are also found in some species of Galerina and Lepiota. Overall, mortality is between 10 and 15 percent. Recently, Silybum marianum or blessed milk thistle has been shown to protect the liver from amanita toxins and promote regrowth of damaged cells.

Orellanine: This toxin generally causes no symptoms for 3–20 days after ingestion. Typically around day 11, the process of kidney failure begins, and is usually symptomatic by day 20. These symptoms can include pain in the area of the kidneys, thirst, vomiting, headache, and fatigue. A few species in the very large genus Cortinarius contain this toxin. People having eaten mushrooms containing orellanine may experience early symptoms as well, because the mushrooms often contain other toxins in addition to orellanine. A related toxin that causes

similar symptoms but within 3–6 days has been isolated from Amanita smithiana and some other related toxic Amanitas.

Muscarine: Muscarine stimulates the muscarinic receptors of the nerves and muscles. Symptoms include sweating, salivation, tears, blurred vision, palpitations, and, in high doses, respiratory failure. Muscarine is found in mushrooms of the genus Omphalotus, notably the jack o' Lantern mushrooms. It is also found in A. muscaria, although it is now known that the main effect of this mushroom is caused by ibotenic acid. Muscarine can also be found in some Inocybe species and Clitocybe species, in particular Clitocybe dealbata, and some red-pored Boletes.

Gyromitrin: Stomach acids convert gyromitrin to monomethylhydrazine (MMH). It affects multiple body systems. It blocks the important neurotransmitter GABA, leading to stupor, delirium, muscle cramps, loss of coordination, tremors, and/or seizures. It causes severe gastrointestinal irritation, leading to vomiting and diarrhea. In some cases, liver failure has been reported. It can also cause red blood cells to break down, leading to jaundice, kidney failure, and signs of anemia. It is found in mushrooms of the genus Gyromitra. A gyromitrin-like compound has also been identified in mushrooms of the genus Verpa.

Coprine: Coprine is metabolized to a chemical that resembles disulfiram. It inhibits aldehyde dehydrogenase (ALDH), which, in general, causes no harm, unless the person has alcohol in their bloodstream while ALDH is inhibited. This can happen if alcohol is ingested shortly before or up to a few days after eating the mushrooms. In that case, the alcohol cannot be completely metabolized, and the person will experience flushed skin, vomiting, headache, dizziness, weakness, apprehension, confusion, palpitations, and sometimes trouble to breathe. Coprine is found mainly in mushrooms of the genus Coprinus, although similar effects have been noted after ingestion of *Clitocybe clavipes*.

Ibotenic acid: Decarboxylates into muscimol upon ingestion. The effects of muscimol vary, but nausea and vomiting are common. Confusion, euphoria, or sleepiness are possible. Loss of muscular coordination, sweating, and chills are likely. Some people experience visual distortions, a feeling of strength, or delusions. Symptoms normally appear after 30 minutes to 2 hours and last for several hours. *A. muscaria*, the "Alice in Wonderland" mushroom, is known for the hallucinatory experiences caused by muscimol, but *A. pantherina* and A. gemmata also contain the same compound. While normally self-limiting, fatalities have been associated with A. pantherina, and consumption of a large number of any of these mushrooms is likely to be dangerous.

Arabitol: A sugar alcohol, similar to mannitol, which causes no harm in most people but causes gastrointestinal irritation in some. It is found in small amounts in oyster mushrooms, and considerable amounts in Suillus species and Hygrophoropsis aurantiaca (the "false chanterelle").

Effects of poisonous mushrooms
The 3 main effects of poisonous mushrooms are:

Hallucinations

Some mushroom species contain toxins that cause hallucinations. These psychotropic types are commonly referred to as ‘magic mushrooms’. One of the better known species is the golden top (*Psilocybe subaeruginosa*). Apart from hallucinations, other effects include:

- confusion
- muscle weakness
- agitation
- rapid heart rate
- headache.

The golden top looks very similar to some varieties of Galerina mushroom, which are potentially deadly.

Gastrointestinal illness

Many poisonous mushrooms cause gastrointestinal illness, such as:

- nausea
- vomiting
- stomach cramps
- diarrhoea.

Liver failure and death

About 9 out of 10 fungi-related deaths are attributable to the Death Cap mushroom (*Amanita phalloides*). Symptoms occur 6 to 24 hours after eating and include:

- nausea
- stomach cramps
- vomiting
- diarrhoea.

The toxin can fatally harm the liver and kidneys, and death can occur within 48 hours. Other mushrooms that have a similar effect to the Death Cap include some species of Galerina, Lepiota and Conocybe.

Poisonous mushrooms in Victoria

The Yellow-staining mushroom and the Death Cap are 2 poisonous mushrooms that grow in Victoria.

Yellow-staining Mushroom (Agaricus Xanthodermus)

The Yellow-staining mushroom (*Agaricus xanthodermus*) is the most commonly-eaten poisonous mushroom in Victoria. This species commonly grows wild in lawns and gardens, and looks very similar to edible mushrooms.

Characteristics include:

- Mushrooms grow on the ground in clusters, often clumped or in ‘fairy rings’.
- The cap is 50–200 mm in diameter.
- The cap is usually white, but can become brown with age.
- The cap of young mushrooms looks a little square.
- When damaged, the cap and stem stain yellow, fading later to a dirty brown.
- The mushroom can give-off a chemical smell, like disinfectant, iodine or kerosene. This smell can be even stronger if you cook them.

If eaten, symptoms of Yellow-staining mushroom poisoning include:

- abdominal cramps
- nausea
- vomiting
- diarrhoea (usually within 30 minutes to 2 hours of consumption).

Less common symptoms include headache, dizziness, sweating and drowsiness.

Death Cap mushroom (Amanita phalloides).

The Death Cap mushroom (*Amanita phalloides*) is potentially fatal if eaten.

Characteristics include:

- Mushrooms typically grow under oak trees.
- The cap is 40–160 mm in diameter.
- The cap ranges in colour from pale yellow to green to olive brown.
- The gills (ridges on the underside of the cap) are white.
- The base of the stem has a membranous 'cup'.
- Onset of symptoms is anywhere from 6 to 24 hours after ingestion.
- Death may occur from liver and kidney damage.
- One mushroom can contain enough poison to kill an average-sized adult.
- Cooking, peeling, drying or soaking the mushroom does not make the mushroom edible.

Prognosis and treatment:

Some mushrooms contain less toxic compounds and, therefore, are not severely poisonous. Poisonings by these mushrooms may respond well to treatment. However, certain types of mushrooms contain very potent toxins and are very poisonous; so even if symptoms are treated promptly, mortality is high. With some toxins, death can occur in a week or a few days. Although a liver or kidney transplant may save some patients with complete organ failure, in

many cases there are no organs available. Patients hospitalized and given aggressive support therapy almost immediately after ingestion of amanitin-containing mushrooms have a mortality rate of only 10%, whereas those admitted 60 or more hours after ingestion have a 50–90% mortality rate. In the United States, mushroom poisoning kills an average of about 3 people a year. According to National Poison Data System (NPDS) annual reports published by America's Poison Centers, the average number of deaths occurring over a ten-year period (2012–2020) sits right at 3 a year. In 2012, 4 out of the 7 total deaths that occurred that year, were attributed to a single event where a "housekeeper at a Board and Care Home for elderly dementia patients collected and cooked wild (Amanita) mushrooms into a sauce that she consumed with six residents of the home.". Over 1,300 emergency room visits in the United States were attributed to poisonous mushroom ingestion in 2016, with about 9% of patients experiencing a serious adverse outcome.

6. Edible mushrooms: Identification and nutrition value.

Mushrooms are epigeous macrofungi and have been highly valued in many cultures because of their nutritional value and characteristic aroma and taste. Out of _3000 edible species of mushrooms only 100 are cultivated commercially, and only ten are produced on an industrial scale. Their global economic value is, nevertheless, considerable. The prime reason for the upswing in consumption is increased health awareness. This has led to selection of foods with superior nutritional or (perceived) nutraceutical benefits.

The nutritional value of mushrooms is established; they are rich sources of complete proteins, containing all the essential amino acids and fiber and very little fat. Mushrooms also provide significant quantities of vitamins (B1, B2, B12, C, D, and E) and bioactive compounds such as unsaturated fatty acids, phenolic compounds, tocopherols, and carotenoids.

Mushroom production has increased over the last two decades. As depicted in Figure 2, China has emerged as the biggest producer of mushrooms (2.0 million tonnes). The species mostly cultivated worldwide is *Agaricus bisporus* followed by *Lentinula edodes, Pleurotus spp., and Flammulina.*

Nutritional components of mushrooms

The total carbohydrate content in the fruiting bodies of mushrooms accounts for 50 to 65% on dry weight basis. It comprises of sugars which are monosaccharides, their derivatives and oligosaccharides. The carbohydrates contain some amount of alcoholic sugar, such as mannitol and trehalose. Trehalose is known to synthesize stress-responsive factors in human cells when

exposed to environmental stresses like heat, cold, oxidation, desiccation etc by retaining cellular integrity. The possible mechanism behind this is the prevention from protein denaturation which usually degrades under stress conditions. Mushrooms rich in proteins contain all the essential amino acids and of these the rich ones are glutamic acid, aspartic acid and arginine. Besides these, the two unusual amino acids g-amino butyric acid (GABA) a non-essential amino acid and ornithine which are known for their peculiar physiological activities have also been found. Mushrooms contain unsaturated fatty acids with total lipid (crude fat) content 20–30 g kg1 DM. They are found to be rich in linoleic and oleic acids. Linoleic acid has been reported to exhibit anti-carcinogenic effects on almost all stages of tumorigenesis in animal models of breast, prostate, and colon cancers and also in reducing the tumor cell growth by altering the 5-lipoxygenase metabolite, 5-hydroxyeicosatetraenoic acid (5-HETE) and expressing the five-lipoxygenase activating protein (FLAP). Linoleic acid is also a precursor of 1-octen-3-ol (an alcoholic group of mushrooms) which is the key aromatic compound contributing to mushroom flavour. The lipid fraction of mushrooms contains tocopherol an important antioxidant component. Mushrooms are good reservoir of vitamins specially vit. B complex and vit. D. Interestingly mushrooms are the only non-animal based food that contains vit. D. Recent studies have indicated that mushrooms when exposed to UV light under certain conditions produces vit. D2 in amounts greatly higher than that that of daily requirements of vitamin D. The process of vit D2 formation takes place through a higher than that of daily requirements photochemical reaction in which fungal sterol, ergosterol, is converted to vitamin D2 through a series of photochemical and thermal reaction catalyzed by ultraviolet (UV) radiation coming from sunlight. Mushrooms are rich in potassium, calcium, phosphorus and magnesium. Sodium is relatively less in mushrooms and hence they are thought to be a good option amongst other vegetables for the hypertensive people. Fresh mushrooms with soluble and non- soluble fibers are proved to lower the total cholesterol levels and thereby good in managing cardiovascular diseases. The presence of dietary fiber (DF) with non-dietary carbohydrates (NDCs) including b - glucans, polysaccharides-protein Complexes (PSPC), chitin, hemicelluloses, mannans, xylans and galactose provides a wide range of health benefits to the humans. Mushrooms also contains large number volatile compounds which are inbuilt with some enzyme system that are capable of catabolising aromatic substrates, the major one’s are 3-octone, 3-octanol, 1- octen 3-ol, benzaldehyde, octanol, and 2-octen-1-ol.

Table 1
The comparative information about the proximate composition (g/Kg) and energy value (kcal/Kg) of some edible mushrooms.

Species	Dry matter	Crude protein	Ash	Lipid	Carbohydrates	Energy	References
Agaricus bisporus(white)	87.3	140.8	97.4	21.8	740.0	325	Kalac [20]
Agaricus bisporus (brown)	83.6	154.3	113.6	16.7	715.4	303	Kalac [20]
Pleurotus eryngii	110.0	110.0	61.8	14.5	813.7	421	Kalac [20]
P. ostreatus	106.0	284.0	86.0	47	354.0	-	Ahmadetal. [21]
Lentinus Edodes	202.2	228	60	21	644	411	Bisen et al. [22]
Volvariella volvaceae	90.3	280	10	33	503	305	Mashandete & Cuff [23]
Auricularia auricular	94	360	52	43.7	285	-	Usha & Sugune [24]
Flammulina velutipes	121.3	176.0	74.3	28.9	731	378	Reis et al. [25]
Calocybe gambosa	90.8	154.6	138.9	83	698.2	317	Vaz et al. [26]
Calocybe indica		214	131	49.5	485	320	Alam et al. [27]

Bioavailability of nutrients Certain diseased conditions require specific nutrient management. Bioavailability refers to the nutrient absorption and utilization in the body and is affected by many factors. Mushrooms are excellent functional foods and contain selenium, vitamin D2, ergothioneine, Vitamin B1 and Iron etc. It is interesting to mention that the bioavailability of any nutrient depends on the mushroom variety. Mushroom contains 0.4–2.0 mg/g (dry weight) of ergothioneine (sulfur containing amino acid). Various researchers have reported good bio availability of ergothioneine in human subjects. Calvo et al. conducted an interventional study on 37 pre-diabetics subjects and reported consumption of 100 g of fresh mushroom for 16 weeks resulted in increased ergothioneine content (317 ng/mL at baseline to 677 ng/mL) along with increased ORAC (oxygen radical absorption capacity), anti-inflammatory hormone (adiponectin) and significant decreases in serum oxidative stress inducing factors like carboxymethyllysine (CML) and methylglyoxal(MG). 16 g of button mushroom powder has also been found to significantly increase the ergothioneine content in RBCs. Other mushroom species rich in ergothioneine are king oyster, oyster, maitake, and shiitake. Mushrooms are rich in dietary selenium and are known to reduce oxidative stress. Boletus edulis has the highest selenium content (approx 20mg Se/g dry weight). Many animal model studies have been reported highlighting the bioavailability of selenium. In a study conducted by Da Silva et al. feeding selenium fortified P.ostreatus mushroom for 5 weeks resulted in increase in the total plasma Se of 64 wistar rats. Another study by Maseko et al. demonstrated significant boost in the GPx-1 and GPx-2 gene expression and colon GPx-1 enzyme activity when fed with selenium-enriched button mushrooms. The bioavailability of Se is dependent on its chemical

form of Se present in it and the same are bound to the polysaccharides in the cell wall. It is due to this reason that selenium bound to chitin might not be available to the humans. Apart from this, mushrooms contain ergosterol which gets converted to Vit. D2 under UV light exposure. Studies on 26 healthy adults having low 25(OH) D levels were conducted for 2 weeks and were fed with the UV treated mushroom soup. It was found that the levels of 25(OH) D were significantly higher than the control and it was quite comparable with the vitamin D2 supplement. Similar studies have been conducted by Stephenson et al. where higher levels of 25 (OH) D have been observed after six weeks of supplementation indicating the strong absorption in the serum. The uptake of minerals by the body indicated potential uses of mushrooms as food source, dietary supplements or as nutraceuticals. Another study reports that administration of UVB exposed mushrooms also enhances bone growth and mineralization. Some studies also emphasize on the effects of processing on the vit. D2 content in mushrooms. Report by Bogusz et al. suggests that ingestion of dried white button mushroom was equally effective in increasing the serum 25 (OH) D levels as the commercial supplements. Mehrotra et al. reported low absorption of vit. D2 in prediabetic subjects upon feeding with cooked UVB treated mushrooms as compared to the fresh ones. Other nutrients like vit. B12 in mushrooms is similar to the ones found in beef, liver, and fish, suggesting its high bioavailability and can be important for individuals consuming a vegan diet over a lifetime. Supplementation of products with dried mushroom powders like shiitake and pleurotus have confirmed to increase the blood hemoglobin concentration and liver Fe levels in vivo models.

Effects of processing on nutritional composition of mushroom.

Species	Method of storage	Incubation period	Nutrients affected	Reference
A. bisporus	Stored at 12 °C	12 days	Reduced sugar content mannitol and fructose Total free amino acids increased from 77.92 to 140.57 g kg^{-1}	Tseng and Mau [54]
A. bisporus	Blanching at 95–100 °C	15 min	Reduced mineral levels	Coskuner and ozdemir [57]
A. bisporus	Freezing at −25 °C Canning and salting	6months	Reduction in protein by 24.3% 22.2% 16.54% in freezing, canning and salting respectively. Reduction in free amino acids (tyrosin, alanine, glutamine, cysteine) and flavor 5′-nucleotides in all treatments.	Liu et al. [53]
P. ostreatus	Microwave processing Frying	– –	Reduced Fe, Zn, Mn, Ca and Cu Increased Iron content	Ziarati & Ghasemynezhad [58]
P. ostreatus	Freezer-storage	12months	Decreased levels amino acids (alanine, glycine, serine, histidine, methoinine and threo nine)	Jaworska et al. [52]
P. ostreatus	Oven dried at 60 °C Blanched at 88 °C Brining (25% brine solution)	Till the constant weight obtained For 1 min. 30–60 min	Reduction in protein content and enhanced carbohydrate content Reduction in protein, fat content and enhanced carbohydrate content Remarkable reduction in protein, fat and carbohydrate content	Mayunja et al. [59]
A. bisporus and *B. edulis*	Canning Blanching in low-methylated pectin before canning	12 months	Reduction in amino acids by 2–16% in *A. bisporus* and 3–24% in *B. edulis*. The contents were better as compared to simple canning by 9–16% *A. bisporus* and 22–27% *B. edulis*.	Jaworska et al. [60]

Bioactive nutraceutically important compounds Mushrooms nutraceuticals have been used since ancient times for treating and curing thousands of human ailments. Their nutritional and culinary characteristics attracted the researchers, pharmacists, nutritionists to haul out their usage for the common people and to be included them as one of the vegetable under food kingdom. Its excellent medicinal power such as vit. B (panthothenic acid) important to build up the nervous system, mineral like selenium and ergothioneine an natural antioxidant to protect human cells from damage and the b-glucans marked for strengthening the immune system by inducing both the adaptive and innate immune responses makes these mushrooms a wonderful key source for making nutraceuticals. Moreover the presence of wonder molecules like polysaccharides, low molecular weight proteins, terpenes, glycoprotein and bioactive compounds again reinforce the usage of such magic food for the better mankind. The current review is discussing such active compounds in detail.

Polysaccharides

Mushroom polysaccharides have already been isolated and characterized like pleuran from *Pleurotus* species, lentinan and erothionine in L. edodes, ganoderan from *Ganoderma lucidium* and agaritine from *Agaricus* and *Calocyban* from *Calocybe indica*. All the mushroom polysaccharide contains a common b-linked glucose backbone but the pattern and degree of branching vary species to species. However, some polysaccharides having galactose or mannose as their backbone have also been found in mushrooms. These polysaccharide are known to

possess various physiological activities like antitumor activity, immune modulatory action, antioxidant activity, antiviral activity, anti-inflammatory actions and anti-carcinogenic. The antitumor action of polysaccharides is mediated through a thymus-dependent immune mechanism, which involves the activation of cytotoxic macrophages, monocytes, neutrophiles, natural killer cells, dendritic cells, and chemical messengers (cytokines, such as interleukins, interferons, and colony stimulating factors) which triggers the complementary and acute phase responses. Mushroom polysaccharide extracted from *A. bisporus* exhibits excellent inhibiting action against human breast cancer. Also proteogylcans from A. blazei are known to have strong immunomodulatory properties which are therapeutically important in controlling cancers and immune deficient diseases through the upregulation of dendritic cells maturation. Recently a novel hetropolysaccharide composed of glucose units isolated from P. eryngii showed a great antitumor activity against HepG-2 cells. The poysaccahride-protein complex isolated from aqueous extract of Phellinus rimosus found to have profound effects towards radiation-induced oxidative stress. Interestingly the anti-fatigue activities has also been observed in the polysaccharides extract of *Hericium erinaceus*, which extend the utilization of these kinds of novel polysaccharides for sports nutrition. *Cordyceps sobolifera* also found to prosper in medicinally important polysaccharides and polysaccharide extract can be used to protect and treat the body from renal injuries.

Bioactive protein molecules

Proteins with biological functions show physiological activity in the gastrointestinal tract by enhancing nutrient absorption, inhibiting enzymes, and modulating the immune system to defend against pathogens. Mushroom bioactive proteins and peptides such as lectins, fungal immunomodulatory proteins, ribosome inactivating proteins, antimicrobial proteins, ribonucleases, and laccases are an important part of functional components with great value of pharmaceutical potential. Lectins are the non-immune proteins or glycoproteins binding specifically to cell surface carbohydrates and have been studied for their ant proliferative, antitumor, and immunomodulatory activities. Some other proteins which exhibit enzymatic activities are ribosomal inactivating proteins (RIPs), laccase, fungal immunomodulatory proteins (FIPs). These exhibit great potential towards inhibiting proliferation of hepatoma Hep G2 cells and breast cancer MCF-7 cells. Furthermore there is no effect of thermal, freezing, acid, alkali and dehydration treatments on the properties of lectin protein (ABL) isolated from A. bisporus, hence indicating their usage as a stable immune stimulant for nutraceutical and functional food development. Similar results have been reported in case of G. lucidum protein after giving various food processing treatments.

Table 3
Important mushroom polysaccharides and their health benefits [70].

Mushroom	Type of polysaccharides	Health benefits
Agaricus bisporus	Heteropolysaccharides	Activation of macrophages
Agaricus bitorquis	Homopolysaccharides	Activation of natural killer cells
Agaricus blazei	Glucan-protein complex	Activation of T lymphocytes
Auricularia auricula-judae	Homopolysaccharides	Anti-viral activity
Boletus erythropus	Homopolysaccharides	Antimicrobial activity
Calocybe indica	Homopolysaccharides	regulate lipogenesis
Ganoderma lucidum	Heteropolysaccharides	Induction of apoptosis
Geastrum saccatum	Glucan-protein complex	Treatment in stomach cancer
Grifola frondosa	Heteroploysaccharides Grifloan	Antitumor activity
Lentinus edodes	Heteropolysaccharides Lentinan	Antitumor activity
Phellinus linteus	Homopolysaccharides	Increase production of interleukin
Pleurotus eryngii	Homopolysaccharides	Antiproliferative effect
P. florida	Homopolysaccharides	Inhibit tumoral cell to cell adhesion
P. ostreatus	Homopolysaccharides	Increase gastrointestinal motility
Poria cocos	β-glucans type polysaccharides	Treatment of colon cancer
Polyporus rhinocerus	β-glucans type polysaccharides	Treatment of colon cancer
Schizophyllum commune	Homopolysaccharides Schizophyllan	Antitumor activity
Sparassis crispa	Homopolysaccharides	Lipid peroxidation inhibition
Termitomyces eurhizus	Homopolysaccharides	Anti-aging effects
T. microcarpus	Homopolysaccharides	Hepatoprotectiveactivity

Terpenes

Terpenes are basically a group of volatile unsaturated hydrocarbons which are responsible for the anti-inflammatory activities and have been isolated from the mushrooms widely and these terpenoids are responsible for many pharmacological activities like anticancer antimalarial, anticholinesterase, antiviral, antibacterial and anti-inflammatory activities. These are classified as monoterpenoids, sesquiterpenoids, diterpenoids and triterpenoids. Monoterpenes and sesquiterpenoids have been isolated from the Pleurotus cornicopiae and the same have been investigated for their cytotoxicity against cancer line and found positive results. Another species Flammulina velutipes has also been reported to contain sesquiterpenoids naming flammulinol, flammulinolides and were tested for their cytotoxicity against three tumor cell lines namely; HepG2, HeLa, and KB. About the triterpenoids, lanostane is the compound which has been mostly isolated from the mushrooms, and reported as an anticancer agent. Two lanostane triterpenes isolated namely methyl ganoderate A acetonide and n-butyl ganoderate H from Ganoderma lucidum with anti-acetylcholinesterase activity. Hence the usage of triterpenoids opened one more way to utilize them as a possible drug for the treatment of Alzheimer's and related neurodegenerative diseases by the pharmaceutical companies. Basically these terpenes can be effectively utilized in developing drugs for curing degenerative diseases. Antioxidants Physiological processes involve production of free radicals and other group of reactive oxygen as by-products. Presence of free radicals beyond a limit in the body alters the antioxidant balance and results in oxidative stress. This leads to the destruction of tissue and cells which

later develop into cancers. So to maintain the balance of the antioxidants in the humans' diets is an important factor to reduce theses oxidative damage. Phenolics, tocopherol, ascorbic acid and caretenoids are the responsible compounds which has been isolated from the different species of mushrooms and reported to boost the immune system, have anticancerous, anti-hypercholesterolaemic activity and anti-viral activity, and ameliorate the toxic effect of chemo- and radiotherapy. Their radical scavenging activity has been extensively studied and documented, species like *Pleurotus, Agaricus, G. lucidum and L.edodes* known for their profound antioxidant activities. New Indian emerging medicinal mushroom species *P. rimosus* has been also studied for its antioxidant power, and found that its methanol and ethyl acetate extracts are good in lipid peroxidation, hydroxyl radical, and nitric oxide scavenging inhibiting activities. The popular Chinese edible mushroom *Hericium erinaceus* is used to treat number of ailments, its hot water extract exhibit high free radical scavenging activity which strengthens its usage as nutraceuticals having natural antioxidants. The high phenolic contents and high antioxidant potential of *C. sinensis* makes it suitable for the healthy commercial preparations for the consumers.

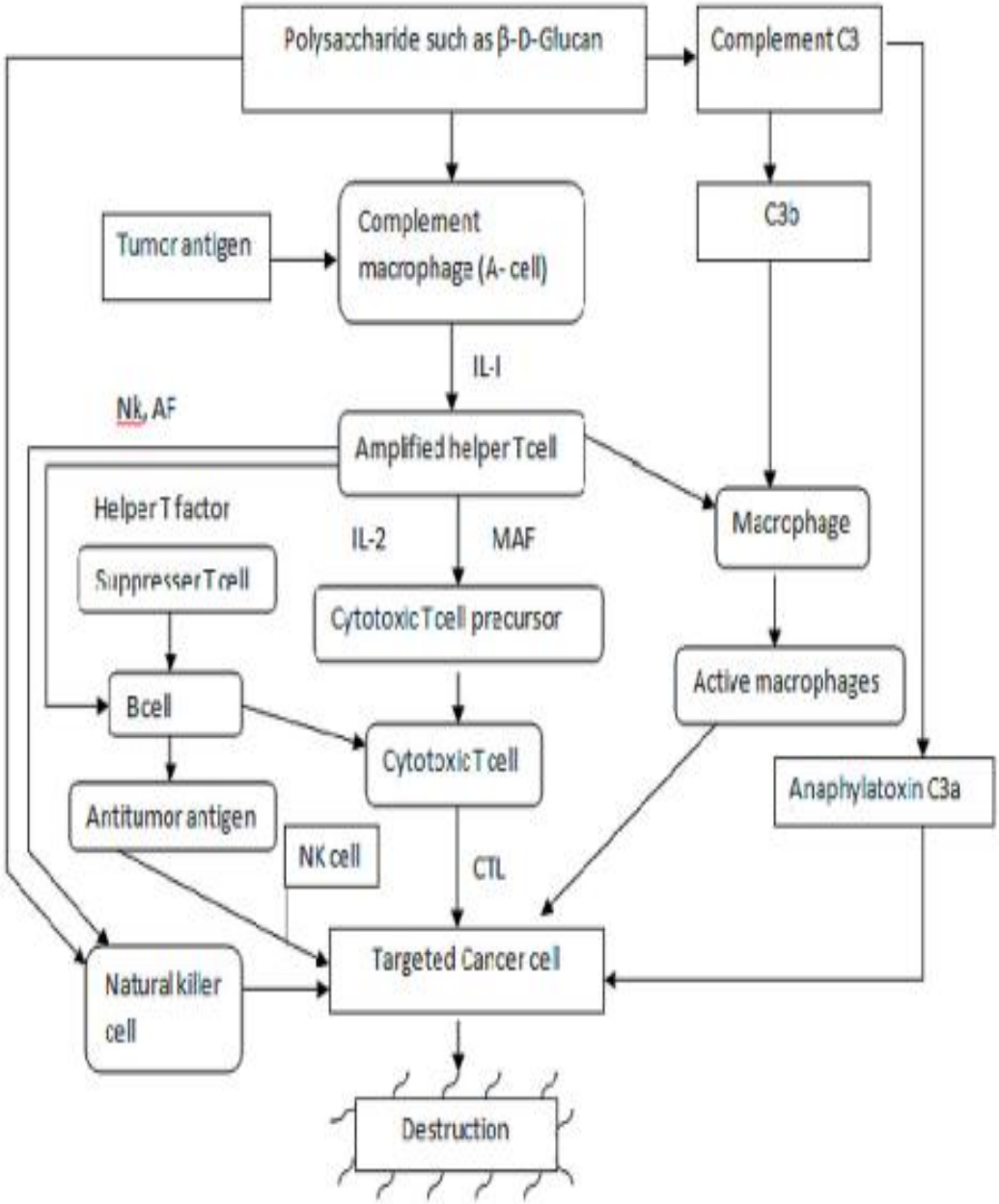


Fig. 2. Anticancer mechanism of mushroom polysaccharides [63].

7. Mushroom as medicine and other biotechnological uses, ethno-mycological uses.

For many years, mushrooms have accompanied humans both as food and medicine. Data from the literature indicate that, with the onset of hunting, mushrooms began to play an important role in the human diet. Fruiting bodies, i.n., the visible part above the substrate commonly referred to as the mushroom, are the edible elements of some filamentous fungi. Fungi form a separate kingdom alongside the kingdoms of prokaryotes, eukaryotes, plants, and animals. About 2.2–3.8 million species of fungi in the world have been identified, of which 150,000 species have been described, 2000 species are considered edible, and over 200 species of wild mushrooms are considered medicinal . Edible mushrooms, unlike medicinal mushrooms, are mainly consumed as fresh mushrooms with fruiting bodies or dried products. They can also be consumed as boiled, fried, roasted, soups, tinctures, teas, and many different dishes, while medicinal mushrooms are mostly used in biopharmaceutical applications in powdered, loose, or liquid extract forms. In culinary terms, mushrooms are wrongly classified as vegetables and are informally categorised as ‘white vegetables’. According to the USDA (United States Department of Agriculture), they can be used as a substitute for vegetables in the diet at a ratio of 1:1 (USDA, 2022). Due to their content of biologically active compounds with beneficial health effects, medicinal mushrooms have been used worldwide in folk medicine for centuries. They are particularly popular in Asian countries, e.g., China, Japan, Taiwan, and Korea. Due to the presence of numerous biologically active compounds, including polysaccharides, proteins, peptides, terpenoids, polyphenols, vitamins, and mineral elements, they are ascribed, e.g., anti-cancer, anti-inflammatory, antioxidant, hypocholesterolemic, hypoglycaemic, and immunomodulatory effects. However, it should be remembered that the consumption of medicinal mushrooms is not always advisable. The safety of their use during pregnancy, lactation, and in children is still poorly reported. The selected bioactive compounds found in mushrooms may potentially limit the absorption of nutrients, trace elements, and vitamins. As a result, it is recommended that the elderly and children avoid the excessive consumption of mushrooms. Additionally, individuals taking medications or herbs should exercise caution when using mushrooms due to the potential for interactions with their bioactive compounds. The chemical profile of medicinal mushrooms varies according to species, strain, cultivation conditions (cultured or growing wild), the degree of maturity, and the proportion of individual anatomical parts in the total mass of the mushroom. This is largely determined by environmental (access to water, light, UV radiation) and biological (type of substrate/host,

presence of competing fungi) factors. Song et al. compared the chemical composition and functional properties of wood-cultured and sackcultured Shiitake (Lentinula edodes (Berk.) Pegler) and proved that the wood-cultured fungus had a higher content of terpenoids and phenolic components and concurrently exhibited higher antioxidant and hypoglycaemic potential compared to the sack-cultured Shiitake (Lentinula edodes (Berk.) Pegler). In the case of Chaga (Inonotus obliquus (Ach. ex Pers.) Pilát), which is a parasite of various deciduous trees, only sclerotia derived from birch trunks have contained tree-specific compounds (betulin and betulinic acid) showing anticarcinogenic activity. Equally great importance for the chemical composition and healthpromoting potential of medicinal mushrooms is ascribed to the world region from which they originate. Chaga (Inonotus obliquus (Ach. ex Pers.) Pilát) sclerotia collected in France, Ukraine, and Canada were characterised by their different contents of betulin, betulinic acid, and inotodiol and showed differential biological activity in different cancer cells. The bioactive substances present in fungi are primary and secondary metabolites that can be synthesised in response to specific environmental stimuli. Their content depends on the species of fungus and their growing conditions. However, Peng and Shahidi emphasise that the cultivation of medicinal mushrooms in standard conditions offers the possibility to stimulate the synthesis of selected biologically active substances and yields raw materials with a reproducible chemical composition, comparable biological effects, and greater health safety (with a lower content of heavy metals, which are often found in excess in wild mushrooms growing in polluted environments). The existence of a huge number of medicinal mushroom species with their diverse chemical composition and content of biologically active compounds and thus multidirectional effects on the human organism could make mushrooms objects of growing consumer interest. In 2020, the size of the global mushroom market was 14.35 million tonnes; it is estimated to grow to 24.05 million tonnes in 2028. The most popular mushrooms among consumers include Reishi (Ganoderma lucidum), Lion’s Mane (Hericium erinaceus), Chaga (Inonotus obliquus (Ach. ex Pers.) Pilát), Turkey Tail (Trametes versicolor (L.) Lloyd)), Shiitake (Lentinula edodes (Berk.) Pegler), and Cordyceps (Ophiocordyceps sinensis (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones and Spataforaprior name Cordyceps sinensis). It is, therefore, expedient to compile and systematise existing knowledge on the most popular medicinal mushrooms, compare their functional potential, and discuss the possibilities of their use in the food industry.

Exploring the Uses of the Top Medicinal Mushrooms

The medicinal use of mushrooms dates back thousands of years because of their varied and uniquely adaptive benefits for health. Now, in recent times with functional medicine and holistic nutrition going mainstream, we're seeing a resurgence of interest in these marvelous superfoods. But, there are a few things you should know before running to your local supplement shop.

This article will encapsulate the top health benefits of 7 medicinal mushrooms, while also revealing why some mushroom supplements sold in today's marketplace don't contain mushrooms at all. And finally, it will explain how Real Mushrooms produces medicinal mushroom extracts for our supplements and what to shop for when you're looking to get true high-quality mushroom supplement benefits.

Overview of Health Benefits of Medicinal Mushrooms

Medicinal mushrooms are nutritional powerhouses with a myriad of health benefits including the following:

- Provide immune support*
- Full of antioxidants*
- Support a healthy inflammation response*
- Help to balance blood sugar*
- Support brain health and cognition*
- Support the nervous system*
- Increase energy and stamina*

Lion’s Mane Mushroom: The Mushroom for the Mind

Species name: *Hericium erinaceus*

Primary Lion's Mane Mushroom Medicinal Benefits: **Supports healthy brain function & neuron generation***

Lion’s Mane is full of a multitude of important compounds, such as beta-glucans, which are immuno-modulating antioxidants and neuro-protective phytonutrients*. *In vitro* research suggests that certain compounds found in Lion’s mane, namely hericenones and erinacines, may help induce Nerve Growth Factor (NGF) synthesis in nerve cells. This dovetails with findings from animal research that Lion’s mane promotes nerve tissue growth and supports motor function. And research in adults with mild memory problems associated with aging found that those taking Lion’s Mane extract had better brain function compared with control participants who did not ingest the mushroom. All of this indicates that Lion’s Mane mushroom is an amazing helper for healthy brain function and may even support neurogenesis.

Reishi Mushroom: The Mushroom of Immortality

Species name: *Ganoderma lingzhi*

Primary Reishi Mushroom Medicinal Benefits: **Sleep aid and potent immunomodulator***

The polysaccharides in reishi mushroom are associated with immune function, and if taken over time, reishi can significantly support the immune system*. It can also support restful sleep and a

calm mind, reduce occasional stress and restlessness, can support lung and respiratory health, and support balanced blood sugar levels*.

One study has demonstrated that Reishi can decrease symptom scale scores for men experiencing lower urinary tract symptoms (urination difficulty). Reishi can be used safely in adjunct with certain oncology medications and diagnoses.

Because of its well-rounded capacity to support numerous systems in the body, herbalists call reishi the King of Medicinal Mushrooms.

Cordyceps Mushroom: The Caterpillar Fungus

Species name: *Cordyceps Militaris*

Primary Cordyceps Mushroom Medicinal Benefits: **Improves lung capacity and increases energy***

The *Cordyceps* mushroom is an incredible energy-boosting fungi. Certain studies have shown that *Cordyceps* can increase the production of ATP, the compound that gives cells energy, in rodents. This is why *Cordyceps* is a good mushroom supplement to take for exercise and physical performance*. In fact, two well-controlled clinical studies have found *Cordyceps* improves exercise performance in healthy older individuals.

Preliminary research in humans, animals, and test tubes also indicates that cordyceps supports healthy levels of inflammation and immune markers.

Related C*ordyceps* species may have hormone regulatory properties shown in research studies to increase libido (in women) and improve sperm health in men. Cordyceps can support kidney health in certain populations.

Chaga Mushroom: The Mushroom that's not a Mushroom

Species name: *Inonotus Obliquus*

Primary Chaga Mushroom Medicinal Benefits: **Boosts digestion and clears/protects skin**

Chaga has been studied for its use in skin and stomach disorders. Chaga has over 200 pre-clinical animal and cell studies showing promising health benefits including such as being high in antioxidants, supporting digestion, immune support, modulating inflammation, containing key anti-microbial substances, and being adaptogenic.

Turkey Tail Mushroom: The Mushroom of Multiple Colors

Species name: *Trametes Versicolor*

Primary Turkey Tail Mushroom Medicinal Benefit: **Boosts immune system function**

Turkey tail improves immune function by stimulating cytokine production, increasing natural killer cells, and through other immune-boosting functions*.

Protein-bound polysaccharides (PBP) found in Turkey Tail have the most research-backed immune-supportive effects. These PBP compounds can enhance key types of immune cells, like T-cells, when used alone or in combination with synergistic herbs. Turkey Tail is a safe mushroom to use long-term within the context of proper medical treatment.

Shiitake Mushroom: The Fragrant Mushroom

Species name: *Lentinula edodes*

Primary Shiitake Mushroom Medicinal Benefit: **Supports cardiovascular health**

Shiitake mushroom is great for immunity and liver health, and it supports the cardiovascular system*. Lentinan, a polysaccharide in shiitake mushrooms, has shown great promise as an immune system-boosting agent. Research has also discovered a compound in shiitake, eritadenine, that can help maintain cholesterol already within a healthy range. The mushrooms are great to cook with and are also loaded with B-vitamins, helping to modulate blood sugar levels in the body and support a healthy inflammation response.

Shiitake mushrooms have been shown to increase igA, a key immune cell (immunoglobulin) associated with our first line of defense when it comes to immunity. Shiitake mushrooms can decrease C-Reactive Protein (CRP), a blood marker that detects inflammation.

Maitake Mushroom: The Dancing Mushroom

Species name: *Grifola frondosa*

Primary Maitake Mushroom Medicinal Benefits: **Helps to maintain healthy blood pressure and supports immune function**

Maitake mushrooms support immune function by stimulating the immune system's lymphocytes, natural killer cells, monocytes, and T-helper cells.

3 Bonus Medicinal Mushrooms: Oyster, *Agaricus, Tremella*

Oyster Mushroom: The Carnivorous Fungus

Mushrooms of the genus *Pleurotus*, most commonly *P. ostreatus*

Primary Oyster Mushroom Medicinal Benefits: **Supports heart and metabolic health***

As one of the most actively cultivated edible mushrooms found worldwide, oyster mushrooms boast an excellent nutritional profile, including low calories, low fat, high protein, a wide range of vitamins and minerals (including niacin), and antioxidants.

A number of research studies show that oyster mushrooms can improve immune system function against respiratory infections. Oyster mushrooms have unique compounds contributing to the lowering of cholesterol known as lovastatin, ergothioneine, ergosterol, ACE-inhibiting peptides, and chrysin. Lovastatin is a natural "statin" that inhibits cholesterol synthesis. Oysters have the capacity to help the body maintain healthy levels of cholesterol and triglycerides.

Oyster mushrooms are also renowned for their ability to help clean up environmental toxins and pests. This fascinating mushroom is one of the few species of carnivorous fungi, attacking and killing ne'er-do-wells with its extracellular toxins.

***Agaricus* Mushroom: The Button**

Species name: *Agaricus bisporus*

Primary Agaricus Mushroom Medicinal Benefits: **Supports cardiovascular health and provides excellent levels of vitamin D***

Agaricus bisporus, often called "the button mushroom" or "the white mushroom," dominates the U.S. commercial mushroom industry, representing approximately 90% of mushrooms consumed in the country. It has become so common that many refer to it simply as "the mushroom." With its pleasant taste, many people may think of this mushroom as just an ingredient on pizza or in a salad, but the agaricus mushroom provides numerous health benefits, including a substantial amount of vitamin D, the sunshine vitamin.

Agaricus mushrooms show positive modulation of PSA levels in those with prostate health concerns. These mushrooms also have the capacity to increase bacteroidetes species in the microbiome, a theorized favourable shift for gut health.

Tremella: The Snow Fungus

Species name: *Tremella fuciformis*

*Primary Tremella Mushroom Medicinal Benefits: Promotes healthy, youthful complexion through potent anti-aging properties**

It may look a bit like a loofah, but don't let its innocent appearance fool you — this mushroom may just be the beauty powerhouse you've been looking for. **The unique polysaccharides found in *Tremella fuciformis* are known for their intense hydrating properties, much like hyaluronic acid, without the cost of high-end anti-aging cosmetic products**. In fact, these polysaccharides are smaller than hyaluronic acid, allowing them to penetrate the skin more easily.

One study suggested that topical products with *T. fuciformis* polysaccharides (TFPS) had a better moisture retention rate than those with hyaluronic acid. In addition to their remarkable hydrating properties, TFPS exhibit potent antioxidant, anti-aging, immune-boosting, gut-

nourishing, and neuroprotective properties, though the mechanism behind these effects remains unclear.

8. Mushroom cultivation technology:

Introduction

Mushrooms may be edible and non-edible. Mushroom is the reproductive structure of edible fungi that belong to Basido mycotina, these may epigeal or hypogeal. Mushroom are fungi which lack chlorophyll and can't manufacture their food material, however, mushrooms can produce a wide range of enzyme that degrades the complex substrate on which they grow. Techniques to grow oyster mushrooms from culture to harvest were evaluated using locally available materials. Wheat straw, Paddy straw, and waste grass are used as substrates, Spawn for Pleurotus sajor caju was prepared from wheat and green gram. The effect of temperature and relative humidity on the growth of mushrooms was evaluated. Pinhole size, high temperature (25°C), and high relative humidity were optimal for oyster growth. 25°C is optimal for spawn running both in cultivation and spawn production. Wheat straw, Paddy straw, and waste Grasses were used as substrates; waste grass alone yielded more oysters than wheat straw, paddy straw.

Material and methods:-

Materials:

Spawn (Source: - College of agriculture Alani, Osmanabad) Paddy Straw, Wheat Straw, and Grass, Water bath, Hot Air Oven, Plastic Bags, etc.

Chemicals-

Malathion 30 EC, Carbendazim (Bavistin), Formalin, sprit etc.

Methods

Collection of Substrate: -

The paddy straw was collected from the nearest Agriculture Research Station, Tuljapur (Osmanabad). Grasses were collected from barren land near College of Agriculture Alani, Osmanabad.

Sterilization: - Sterilization is the process that is involved in the killing of micro-organisms

Heat Sterilization: Sterilization requires a minimum of 121°C steam at 15Psi (1 atm pressure) for 15-20 minutes in autoclave or dipping in boiling water of 100 °C for 30 min.

Chemical sterilization: Straws were dipped in the solution prepared by mixing 50 ml Malathion, 15 gm Bavistin, and 100 ml formalin in 100-liter water for 24 hrs.

Spawning:- 15 days old spawn with mycelium formed complete coating around the grain, the spawning is done thoroughly, before filling the substrate in polythene bags, holes of about 1 cm, and diameter is made at 10-15 cm distance all over the surface for free diffusion of gases and heat generated inside.

Incubation:-

Filled bags were incubated for the first 20 days in a dark room, after 20 days of incubation they were shifted to light (cultivation) room on a hanging bed, and watering was done daily after shifting to the light room and done arrangements to maintain 98% humidity and temperature 22-24 °C.

Providing light, proper temperature, and humidity are major challenges in light room. The humidity was maintained by arranging cooler and watering on daily basis.

Biological efficiency:-

The total weight of all the fruiting bodies harvested from all three pickings was measured as the total yield of mushrooms. The biological efficiency (yield of mushroom per kg substrate on dry wt. basis), was calculated by using the following formula (Chang et al., 1981).

Fresh weight of mushroom 699.1 gm

B.E. (%) = ---------- X 100 B. E = ---------- ×100B. E = 69 %

The dry weight of substrate 100 gm

Observations:-

Spawn running: -Spawn running took 2-3 weeks after inoculation. All substrates were inoculated on the same day. Colonization of substrate was completed in 20 days all the bags were opened for primordial formation on the same day. These results agree with the findings of Tan et al, (1981) who reported that the spawn running took three weeks and fruiting bodies appeared after 2-3 days. Kumari and Achal (2008) stated that colonization of the substrate was completed within 20 days of inoculation.

Pinheads formation: - The pinheads formation is the second stage of mycelial growth during the cultivation of mushrooms. Small pinheads like structures were observed, these pinheads were formed 6-7 days after the spawn running. These results are in agreement Ahmad (1986) who stated that Pleurotus ostreatus completed spawn running in 17-20 days on different substrates and time for pinheads formation was noted as 23-27 days. Pinhead formation starts

after 8 days of spawn run 2 kg of wheat straw and is followed by an increase in pinhead formation Shah et al., (2004) reported that primordial formation of P. ostreatus appears 27-34 days of inoculation which is consistent with the results of this study.

Fruiting bodies formation and yield of Oyster mushroom:-

This is the third and final stage during the cultivation of mushrooms. The fruiting bodies appeared 10 days after pinhead formation. These findings confirm with Quimio (1978) who reported that fruiting bodies 3-4 weeks after inoculation of spawn. The crop of Oyster mushroom was harvested in three flushes. Maximum average yield was estimated with an average size of 402.9gm of paddy straw, 324.5 gm of wheat straw, 561.4 gm of grass were obtained from a bed in the first flush.

Results and Discussion:

Harvesting	Paddy straw (gm)	Wheat straw(gm)	Grasses (gm)
First	358.4gm	217.9gm	499.1gm
Second	483.5gm	431.2gm	623.8gm

Result revealed that the weight of Grass substrate mushroom harvesting (i.e. first and second) 499.1 gm and 623.8 gm, Wheat straw substrate mushroom harvesting (i.e. first and second) 217.9gm and 431.2 gm, Paddy straw mushroom harvesting (i.e. first and second) 358.4 gm and 483.5 gm respectively. Substrate where in which Grass substrate was used yielded that is 499.1 gm and 623.8 gm mushroom and which is highest as compared with wheat and paddy Substrate. However this result was confirmed by the growth of mushrooms were the highest that is 480.2 gm per 2 kg of Grass substrate (Cohen et al., 2002).

Conclusion:-

While maintaining temperature (20-25°C), relative humidity (90-98%) and light have played an important role in the growth of mushrooms. Substrate where in which Grass substrate was used yielded that is 499.1 gm and 623.8 gm mushroom and which is highest as compared with wheat

and paddy Substrate. Therefore cultivation of oyster mushrooms on agricultural wastes grasses provides multi-disciplinary advantages for the human being, animals as well as the ecosystem. The observed differences in the substrate's yield may be due to the percentages content of cellulose materials and essential nutrients that are important for the growth of oyster mushrooms.

The cultivation procedure of some of the mushrooms is given:

Cultivation of *Agaricus brunnescens* (Syn. *A. bisporus*):

The Agaricus brunnescens (syn. A. bisporus) is commonly known as white button mushroom (Fig. 4.107, 4.108). It contributes a major share in the mushroom production of the world. It is a temperate mushroom and can grow well in temperate conditions. Optimum temperature, optimum moisture, proper ventilation and good quality of spawn are very essential prerequisites for mushroom growth.

These are:

The optimum temperature for the mycelial growth is 24°C, while it is 14-18°C for the formationand develop-ment of fruit body.

Optimum moisture requires nearly at the saturation point. However, direct application of excesswater in bed is harmful for the growing crop.

Proper ventilation is essential to remove toxic gases by the introduction of ade-quate fresh air.

Good quality of spawn i.e., the spawn should be prepared from the tissue of single fruit body and its productive capacity should be good enough.

The cultivation procedure is:

Production of spawn,

Preparation of compost,

Filling of trays with compost,

Spawning i.e., inoculation of compost,

Watering of inoculated compost filled trays,

Casing,

Harvesting of mushrooms (fruit bodies), and

Storage of mushrooms.

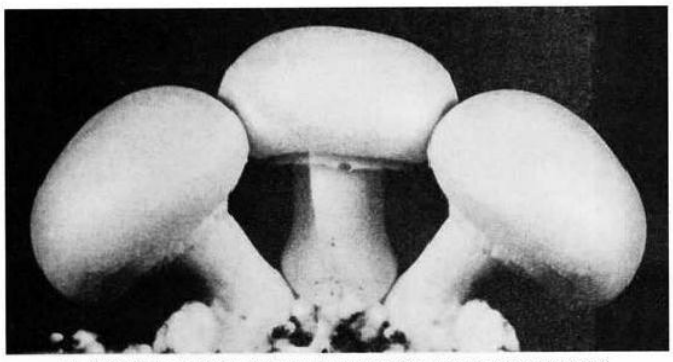


Fig. 4.108 : Mature fruit bodies of *Agaricus brunnescens* (syn. *A. bisporus*) on mushroom bed

[Courtesy : Dr. Samir Dutta]

Production of Spawn:

The spawn (seed of mushroom) is a pure cul-ture of the mycelia grown on a special medium. The medium is prepared by the grains of wheat, rye, sorghum or bajra along with some ingredients.

The preparation of spawn mainly consists of three steps:

Preparation of substrate,

Inoculation of substrate, and

Incubation of inoculated substrate for spawn production.

Preparation of Substrate Take 900 gms of grains (wheator sorghum) in 600-900 ml of water in a container and boil for 15-20 minutes, After boiling, decant the excess water and allow the grains to surface drying by spreading onpolythene sheet in shade for a few hours.

The grains are then mixed with chemicals like 2% calcium sulphate calcium (gypsum) carbonate and 0.5%(chalk) on dry weight basis and adjust the pH of the grain at 7-7.8. About 300-350 gms grains were then filled in milk bottles/ polypropylene bags.

Place a ring of tin (3.5 cm height and 3 cm diametre) towards the inner side of the open-end of polypropylene bag, tighten it with rubber band and then push the margin of the bag towards the inner side and thus a mouth is prepared.

Plug the mouth of the bottle and/or polypropylene bag with non-absorbent cotton. Then cover the mouth with brown paper and tighten it with rubber band. Sterilise the substrate by autoclave at 15lb pressure for 30 minutes for 2 consecutive days. Kept the sterilised substrate in open air to cool down near to room temperature, thus making the substrate ready for inoculation.

Inoculation of Substrate:

The substrate is then inoculated with the mycelial culture (developed earlier, either in Potato DextroseAgar i.e., PDA or Yeast Potato Dextrose Agar i.e., YPDA or Malt extract Agar and Rice bran decoction medium).

Incubation:

Incubate the inoculated container at 20-25°C in dark for 3 weeks. Shake the contai-ner after a fewdays, when the mycelial growth becomes visible on the grain.

Storage of Spawn:

Store the spawn at 0-4°C in a refrigerator for a maximum period of 6 months, if it is not nee-ded immediately.

The spawn can be purchased from any spawn-growing centre. (The spawn is also available in"National Centre for Mushroom Research and Training (NCMRT)", Chambaghat, Solan 173 213, Himachal Pradesh, India.

Preparation of Compost:

The compost used in the cultivation is of two types:

Natural and Synthetic:

Natural Compost:

The natural compost is prepared by mixing barley or wheat straw with fresh and pure horse dung (not with the dung of other animal). Mixed, rain wet or old dung is not sui-table for the preparation of compost. Commonly 100 kg of dung is mixed with 33 kg of straw. The mixture is then stacked a metre high heap.

The heap of mixture should be kept under shade in open air. After 3-4 days, the heap was turned (to release ammonia) and stacked again. The turning process is repeated 4-5 times at an interval of 5-6 days. During this process, gypsum (CaSO4.2H2O) is added @ 25 kg/tonne (1,000 kg) dung. Finally, 40 ml nemagon is sprayed and added to the mixture. The compost was then filled in the tray of 100 x 50 x 15 cm size.

Synthetic Compost:

The ingredients required for the synthetic compost are:

Chopped wheat straw (3-6 cm size) 300 kg

Wheat bran 30 kg

Calcium ammonium nitrate or Ammonium sulphate 6 kg

Urea 4 kg

Potash 1.5 kg

Calcium sulphate (gypsum) 30 kg

Sawdust 10 kg

Wet the sawdust with water by spraying and mix half of the ingredient, except wheat straw and gypsum. Next day, spread the wheat straw on the cement floor and wet it thoroughly by spraying with water. The sawdust-chemical mix-ture is then mixed thoroughly with wetted wheat straw. This mixture is then stacked under shade into a metre high heap and covered with poly-thene sheet.

After 5 days, the stack is scraped and rest half ingredient is thoroughly mixed with it and the entire mixture is then stacked again. This process is repeated six times. Calcium sulphate is added in the 3rd and 4th turning.

Normally the compost becomes ready to cultivate after 6th turning, but 2 or more turning may be given if the smell of ammonia is yet there in the compost. During last turning, insecticide like malathion (10 ml dissolve in 5 I water) is added to the pre-pared compost. The prepared compost will be brown or dark brown in colour and is sufficient enough to fill 25 trays of 100 x 50 x 15 cm size.

Filling of Trays with Compost:

Mix 3 kg of calcium carbonate with the compost prepared earlier. Fill the wooden trays with compost and compress fairly by using a wooden board (1 2 cm x 25 cm), so that a space of about 3 cm deep is left on the top of the tray.

Spawning i.e., Inoculation of Compost:

Spread the spawn on the surface of compost and then cover by a thin layer of compost. Little pressure with the fingers is given to make good contact of spawn with compost. Finally the trays are covered with old newspaper. The trays are arranged one after the other in vertical stacks in such a way that sufficient aeration between the trays is maintained.

Watering of Inoculated Compost Filled Trays:

Sprinkled water to be given on newspaper to maintain humidity. Water should be applied twice a day or less depending on the availability of moisture. The room temperature should be maintained between 24°C and 25°C for 12-15 days for the good growth of mycelium on the compost. The mycelium appears in the form of white cottony growth on the surface of bed.

Casing:

The process of covering the mycelial mat on compost, surface is made with a thin layer of soil mixed with different substances.

The casing can be done with different types of mixture like:

Soil : Sand : : 1 : 1;

Well-rotten cow dung: light soil: : 3 : 1 ;

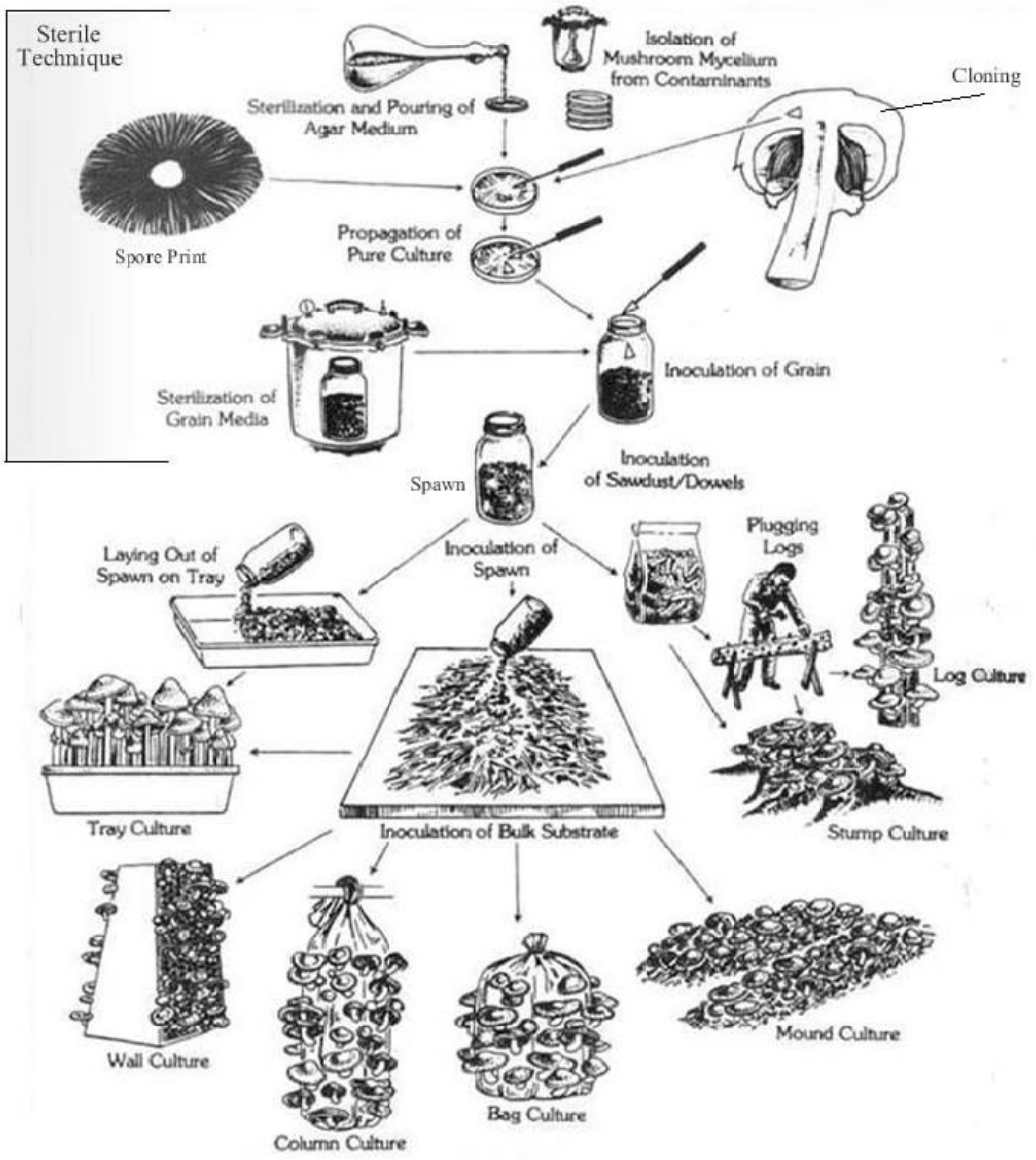
Spent compost: Sand: Slaked lime : : 4:1:1 etc.

Casing soil should be sterilised either by chemicals like methyl bromide, formalin etc. or by heating at 70-75°C temperature for 6 hours to kill the inhabiting fungi, nematodes, insects etc.

The fruit bodies of mushroom are expected to appear after 5-20 days of casing. After casing, the room temperature should be maintained between 14-18°C for the good growth of the fruit body. The fruit bodies attain the size of button stage from pinhead within 7-8 days. Next crop appears at an interval of8-10 days.

Harvesting of Mushrooms i.e., Fruit Bodies:

When the cap of the fruit body is tight with its stalk, the fruit bodies are harvested. The fruit bodiesare harvested by twisting and uprooting, after holding the basal region of stalk with fin-gers. The lower part of the stalk is cut out where the compost remains attached.



Storage of Mushrooms:

The fruit bodies may be stored at 4°C for a few days, if it is not consumed or marketed immediately.

Cultivation of Paddy Straw Mushroom (*Volvariella Volv cea*):

The paddy straw mushroom is also called tropical, straw or Chinese mushroom (Fig. 4.109). In West Bengal, it is called as 'Poal chatu'. The genus Volvariella belongs to the family Pluteaceae under the order Agaricales of Basidiomycotina.

The common edible species under this genus are *V. volvacea, V. diplasia* and *K esculenta*; those are grown commercially in different countries like Burma (Myanmar), China, Philippines, Malaya, India etc.

In addition to paddy straw, other substrates like water hyacinth, cotton waste, banana leaves, sawdust, sugarcane thrash (bagasse) etc., are used as substrate due to the presence cellulose, hemicellulose and lignin.

In India, the cultivation of this mushroom was first initiated in Coimbatore, Tamil Nadu, and now it is popular in different tropical parts due to the requirement of temperature ranges between 30-45°C.

The process of cultivation of straw mush-room is as follows:

Requirements,

Preparation of spawn,

Cultivation procedure,

Harvesting of fruit bodies, and

Preservation of fruit bodies.

Requirements:

Spawn of V. volvacea (600-800 gms grain spawn/bed),

Bricks,

Bamboo frame (1 m x 1 m),

Small water tank,

Paddy straw (preferably from aman variety), apx. 36 kg,

Loose straw 5-6 kg,

Powder of Gram or Arhar seeds 200-250 gm,

Thermometer (0-100°C scale), and ix. White polythene sheet.

Preparation of Spawn:

The spawn can be prepared following the same procedure as adopted in Agaricus brun-nescens (seepage 395). But in addition to grains of wheat or sorghum, the rice straw can also be used as substrate.

Cultivation Procedure:

Fresh paddy straw, not more than one year old and preferably from the Aman variety, should be collected from farmer or from any store. 24 straw bundles of about 1.5 kg each along with some loose straw are immersed completely in a water-filled tank by putting some weight on the bundles for about 12-15 hours. Then take out the straw bundles from the tank and keep them in stack on cement floor to drain off excess water.

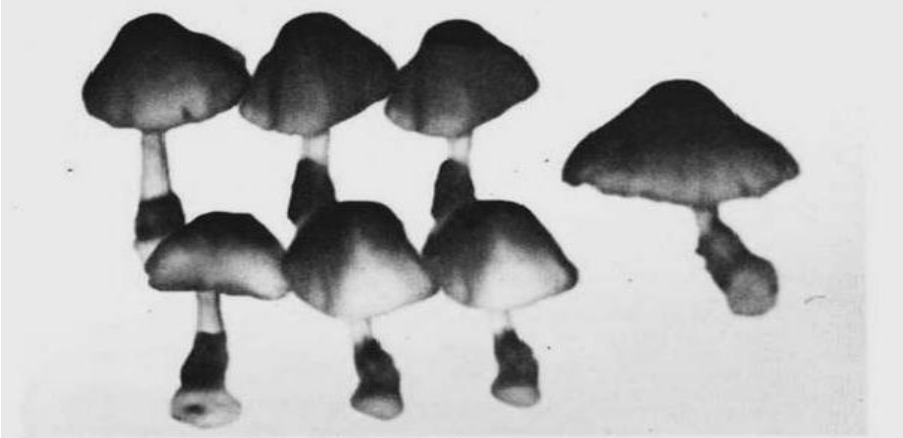


Fig. 4.109 : Mature fruit bodies of *Volvariella volvacea*

Preparation of Bed and Spawning:

One square bed of 1 m x 1 m x 1 m or 1 m x 0.75 m x 1 m is prepared with pre-soaked straw, keeping the butt ends (basal region) at one side, placed close to each other and arranged length-wise on a bamboo frame, supported on 4 pillars made of bricks. Same number of soaked straw bundles are placed on the previous one by keeping the butt ends in o posite direction. Inoculate the bed with spawn. The bids of spawn are placed about 8-10 cm inside the margin, maintaining a space of about 5 cm from each other. About 160-200 grams spawn is required for each layer. Powders of Cram or Arhar seeds of about 50 gms or more are spread along the line of spawning.

Second and third layers are arranged and inoculated in a similar process, but 2nd layer is placed at right angle to the 1st layer and the third layer is like the 1st layer. The spawn and seed powder on the 2nd layer will be given like the 1st layer, but on the 3rd layer those will be dis-tributed uniformly throughout the bed.

Finally, cover the top layer with loose straw. Loosely bind the bed with rope made of wheat straw at the three regions, one in the middle and one on each side. Press the bed with the help of woo-denboard to release the internal air and thus the spawn get compressed with the wet straw bun-dles. Coverthe bed with polythene sheet.

Watering should be done once or twice with the help of micro-sprayer. The temperature of the bed should remain 30-35°C after spawning and it should not go below 30°C during the growing season. The relative humidity should be between 80-90%.

Polythene sheet should be removed after 7-10 days of spawning for the appearance of button of the mushroom. After that the buttons quickly develop into fruit bodies.

The straw once used in the mushroom culti-vation can be used again. The bed should be pre-pared under shade away from direct sunlight and rain and also in well-aerated condition, but wind should not blow very fast.

Harvesting of Mushroom:

The fruit bodies are harvested by gentle twisting when the volva is about to rupture or is just ruptured.The production continues for 25- 30 days, but in two phases. The total production per bed is approximately 3 kg. The production of second phase is comparatively less.

Preservation:

The fruit bodies are consumed fresh or can be preserved by drying or in refrigerator for 27- 48 hours. Drying can be done either in the sun or in oven at 50-60°C temperature.

Cultivation of Oyster Mushroom (*Pleurotus*):

Species of *Pleurotus* are commonly called Oyster mushroom or Dhingri or Wood fungus (Fig. 4.110). It is the fourth important mushroom in the world ranking with an annual production of about 15,000 tones. It grows commercially in Japan, Taiwan, Italy, France, Thailand, Philippines and India, out of which the first three are the leading countries in its production.

The genus *Pleurotus* contains more than 50 species, of which *P. flabellatus, P. ostreatus, P. sajor- caju, P. sapidus, P. fossulatus, P. cornu- copieae, P. sapathulatus* and *P. florida* have been cultivated in India.

The process of cultivation of Oyster mushroom is as follows:

Requirements,

Preparation of spawn,

Cultivation procedure,

Harvesting of fruit bodies,

Preservation of fruit bodies.

Requirements:

Spawn of *Pleurotus* – 60 gms (30 gms grain spawn/kg of paddy straw),

Chopped and dry paddy straw (1-2 cm) -2 kg,

Gunny or polythene bag (40″ x 24″) – 1 piece.

Horse gram powder – 50 gms (25 gm.s/kg),

Thick polythene sheet (5 ft x 5 ft) – 1 piece,

Polythene bags (30″ x 18″) – 2 piece, and

Water sprayer. Preparation of spawn

The spawn can be prepared as per method adopted in Agaricus brunnescens (see page 395). The spawn should not be older than 1 month.

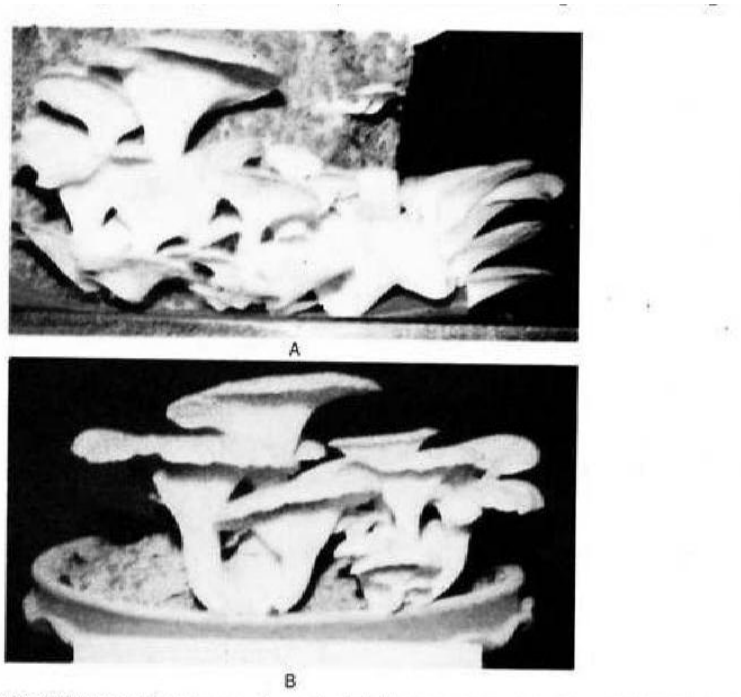


Fig. 4.110 : Fruit bodies of *Pleurotus sajor-caju* on mushroom bed : A. Mushroom bed prepared in polythene bag and B. Mushroom bed prepared on earthern pot

Cultivation Procedure:

Take two kg of chopped straw (preferably of Aman variety) in a gunny or incised polythene bag and tighten the mouth with rope. Immerse the bag completely in water (90 I water contai-ning 7 gm Bavistin, a fungicide along with 125 ml formaldehyde) filled tank by putting some weight on it for approximately 12-15 hours.

Then take out the straw bags from the tank and keep the straw pieces in a wicker basket or a scuttle (Beng. Jhuri). Put more water in the wet straw to remove dirt, rags etc. Wait for one or more hours to drain off excess water.

The wet straw pieces are then kept on polythene sheet and mixed with powder of Horse gram (20-25 gms/kg) and spawn (30 gms/kg) and if possible 10 gms ferti-liser of IFCO or P.P.L (10:26: 26) maybe added.

Take 2 polythene bags (appx. 30″ x 18″) and make 6-12 holes at the lower side of each bag. Then the entire mixture is put equally inside the two bags. Keep the filled bags on a bench or table in room at 21-30°C running. and 65-80% humidity, with sufficient light and ventilation for 15-16 days, for spawn Spray water on bed twice a day by micro-sprayer.

After 15-16 days, the straw pieces are covered with the mycelium and form a solid cylindrical mass. Remove the polythene bag and keep the mass on the same polythene bag in the same place. The compact mass should be watered 4-8 times throughout the day with the micro-sprayer.The young fruit bodies will be developed after 3-4 days (i.e., 18-20 days of spawning) from all sidesof the bed.

Within 2-3 days, the fruit bodies attain the size of harvesting. After harvesting the straw- mycelium mass again put inside the bag and tighten the-mouth with rope. Keep it for 7 days and then again remove the mass from polythene bag and keep the polythene bag as before.

Next crop of mushroom will be available within 7 days i.e., approximately 36 days after starting. Repeat the process again and the third crop will be available in 50 days. During cropping period light should be provided for 15-20 minutes/day for better yield.

Harvesting of Mushroom:

The fruit bodies are harvested by gentle twisting after holding the base of the fruit bodies with fingers. The fruit bodies can be harvested generally 3 times i.e., at 22, 36 and 50 days and the total production will be 2 kg. Afterwards the bed should be destroyed.

Preservation:

After harvesting, the fresh mushrooms can be sold in the market or they can be dried in sun (for three consecutive days) or in oven at 65°C. The cultivation of Pleurotus can also be done on earthen tray or tub.

Some Poisonous Mushrooms:

Amanita phalloides (death cap): Toxic principles are α and β-amanitin, and phalloidin.

A. virosa (destroying angles),

A. verna (fool's cap),

A. muscaria (fly-agaric) etc.

Diseases of Mushrooms:

Like higher plants, mushrooms also suffer from different diseases caused by fungi, bacteria and viruses.

8. Diseases of mushroom; crop management

Like all other crops, mushrooms are also affected adversely by a large number of biotic and abiotic agents/ factors. Among the biotic agents, fungi, bacteria, viruses, nematodes, insects and mites cause damage to mushrooms directly or indirectly. A numbe r of harmful fungi a r e encountered in compost and casing soil during the cultivation of white button mushroom. Many of these act as competitor moulds thereby adversely affecting spawn runwhereas others attack the fruit bodies a t various stages of crop growth producing distinct disease symptoms. At times there is complete crop failure depending upon the stage of infection, quality of com post and environmental conditions. General distribution of various competitor moulds an dpathogenic fungi is as follows:

I. Those occurring mainly in compost include: Olive green mould (*Chaetomium olivaceu m* and other spp.), In k caps(*Coprinus* spp.) Green moulds (*Aspergillus* spp. *Penicillium* spp. and *Trichoderma* spp.), Black moulds (*Mucor* spp., *Rhizopus* spp.) and other (*Myriococcu m praecox*, *Sporotrichum* sp., *Sepedonium* sp., *Fusarium* spp., *Cephalosporium* spp., *Gliocaldium* spp., and *Papulospora* spp.).

II. Fungi occurring in compost and in casing soil: White plaster mould (*Scopulariopsis fimicola*): Brownplaster mould (*Papulosporabyssina*), Lipstick mould (*Sporendonema purpurescens*),False truffle (*Diehliomyces microsporus*) and green moulds.

III. Fungi occurring on and in casing soil and/or on the growing mushrooms: Cinnamon mould (*Peziza ostracoderma*), wet bubble(*Mycogone perniciosa*), Dry bubble(*Verticilliu m fungicola*), Cobweb (*Cladobotryu m dendroides*), Pinkmould (*Trichothecium roseum*) and green moulds.

IV. Fungi attacking the fruit bodies only:Fusarial rot (*Fusarium* spp.).

At any phase of growth anundesirable growth or development of certain moulds can occur and can adversely affect the final mushroom yield.

FUNGAL DISEASES:

A. WHITE BUTTON MUSHROOM (Aga ricus bisporus, A.bitorquis)

a. Diseases

1. DRY BUBBLE

Pathogen : *Verticillium fungicola*

Common Name : Verticillium disease, brown spot, fungus spot, dry bubble, La mole. This is the most common and serious fungal disease of mushroom crop. If it is left uncontrolled, disease can totally destroy a crop in 2-3 weeks (Fletcher *et al.* 1986). *Verticillium fungicola* was major pathogen responsible for considerable yield losses of cultivated mushrooms in Manchuela area provinces of Cuenca and Albacete, Spain (Gela, 1993). In a disease survey of commercial mushroom houses, *V. malthousei* was isolated from 11.3% of mushroom sampled (Foree *et al.* 1974). From India the first report of the heavy incidence of dry bubble disease was from mushroom farms located at Chail and Taradevi (Seth *et al.* 1973).

Symptomatology: Whitish mycelial growth is initially noticed on the casing soil which has a tendency to turn greyish yellow. If infection takes place in an early stage, typical onion shaped mushrooms are produced. Sometimes they appear as small- undifferentiated masses of tissue upto 2cm in diameter. When affected at later stage, crooked and deformed mushrooms with distorted stipes and with tilted cap can be seen. When a part of the cap is affected harelip symptom is noticed. Affected mushrooms are greyish in colour. If the infection occurs at later stage, grey mouldy fuzz can be seen on the mushrooms. Sometimes little pustules or lumps appear on the cap. On fully developed sporophores, it produces localized light browndepressed spots. Adjacent spots coalesce and form irregular brown blotches.

Epidemiology: *Verticillium* is carried on to the farm by infected casing soil. Spread is carried out by infected equipments, hands andclothing. Phorid and sciarid flies arealso known to transmit this disease (Renker and Bloom, 1984). Under laboratory conditions sciarids and phorids were found to transmit 84- 100% and 76-100% of *V. fungicola* respectively, into two different media (Kumar & Sharma, 1998). Mites are also known to transmit the disease from infected to healthy mushroom(Fikete, 1967).

Management

a) **Physical methods:** Use of sterilized casing soil, proper disposal of spent compost andproper hygiene and sanitation are essential to avoid primary infection (Sharma, 1994). Wuest and Moore (1972) reported that treating mineral soil with aerated steam at 54.4°C for 15 minutes eliminated *V. malthousi* that had been experimently established for 17 days in axenic soil culture. Further in 1973, Moore and Wuest reported that thirty minute treatment with aerated steam at 60°C and 82°C, hindered spore germination and soil colonization by *V. malthousei* more than similar treatment at 98°C. Heat treatment of infected casing layer at 63°C for one hour completely prevented spore germination (Poppe, 1967).

b)Biological method : According to Trogoff and Ricard (1976) spraying casing soil with $100x10^6$ *Trichoderma* propagules/litre/m^2 controlled *V. malthousei* in several trials on naturally infected mushroom holdings where dry bubble disease was endemic. Under laboratory conditions, leaf extracts of *Callistemon lanceolatus, Cannabis sativus, Citrus* sp., *Euclyptus* sp., *Dhatura* sp., *Urtica dioica, Solanum khasianum* and *Thooja compacta* caused 27.77%, 13.05%, 16.66%, 22.22%, 5.55%, 6.66%, 22.77% and 27.77% inhibition, respectively of *V. fungicola* (Sharma and Kumar 1998-99) Bhat and Singh (2000) reported 5 bacterial isolates effective against *V.fungicola*.

c) Chemical methods: In laboratory trials *V. malthousei* was controlled by Zineb on a large scale, Bercema - Zineb 80 used at 0.1 - 1.2% controlled the disease when used before and between the flushes (Philipp, 1963).

V.malthousei was controlled by 3 sprays with Dithane Z-78 at 0.25 or 0.50% or Hexathane at 0.30% given at the time of casing, at pinhead formation and afterflushes of crop (Seth *et al.* 1973). Application of chlorothalonil as a drench reduced the incidence of *V.fungicola* tolerant to certain benzimidazole fungicides.

2. WET BUBBLE

Pathogen : Mycogone perniciosa

Common Name : Wet bubble, La mole, white mould, bubble, Mycogone disease

Wet bubble in white button mushroom incited by Mycogone perniciosa Magn. has been reported as one of the serious diseases from almost all the major mushroom growing countries of the world. Bubbles or mole (M. perniciosa), first described from Paris in 1888, is stated to be responsible for the heaviest losses in mushroom beds in France, England and United States (Nielson, 1932). The disease has also been reported to assume serious proportions in other major mushroom growing countries of the world such as United Kingdom, Netherlands, USA, China, Taiwan, South Africa, Brazil, Hungary, Australia and Poland from time to time. In India, this disease was reported for the first time in 1978 from some mushroom farms in Jammu and Kashmir (Kaul et al., 1978).

Symptomatology: Many workers have described Symptoms of wet bubble at different stages of mushroom development. Smith (1924) recognised two main symptom types, infected sporophores and sclerodermoid masses, which he considered to be the result of infection by *M. perniciosa* at different stages in the development of the sporophores. Thus, when infection took place before the differentiation of stipe and pileus the selerodermoid form resulted, whereas, infection after differetration resulted in the production of thickned stipe with deformation of the gills.

Symptoms of wet bubble disease

Etiology: The disease, wet bubble, is caused by Mycogone perniciosa Magn. and the perfect stage is Hypomyces perniciosa. Mycelium of the pathogen is white, compact, felt- like. Hyphae branched interwoven, septate, hyaline, 3.5 m broad. Conidiophores short , slender, branched, hyaline measuring 200 x 3-5m and having sub-verticillate to verticillate brances which bear thin walled, one-celled conidia measuring 5-10 x 4-5 m . L a r ge t wo-celled chlamydospores present; upper cell warty, thick walled, globose, bright coloured measuring 15-30 x 10-20m, lowe r cell hyalin e, s mooth a n d measure 5-10 x 4-5m.

Host Range : *Mycogone perniciosa,*though a major pathogen of *Agaricusbisporus*, is also capable of infectingother mushroom species.

Spread : Spread of *M. perniciosa*occurs primarily through casing soil but the introduction of pathogen through other agencies, like spent compost and infected trash, is not ruled out. The infection can be air- borne, water borne or may be mechanically carried by mites and flies (Garcha, 1978).

Management : As the pathogen inflicts serious damage to the crop, various attempts have been made to manage the disease through various means.

Physical : Wuest and Moore (1972) suggested that aerated steam at 54.4°C for 15 minutes can eliminate *M. perniciosa* from casing soil. Munns (1975)suggested the use of plastic pots to cover mushroom showing wet bubble symp toms during the cropping season to prevent spread of disease.

Biological : Jhune *et al*., (1990) screened 12 isolates of bacteria and 71 isolates of actinomyectes isolated from mushroom compost and casing mixture and observed AJ-117, AJ-136 and AJ-139 as promising bioagents. Though, almost negligible attempts have been made to control *M. perniciosa* through botanicals but the inhibition of fungal growth by plant extracts is not uncommon and has been reported earlier by a number of workers (Flierman, 1973; Michal and Judith,

1975).

Chemicals: Benomyl spray at 0.5-4 g/ m 2 immediately after casing has been reported very effective for protecting the crop. Fletcher (1975) advised that adequate control of wet bubble was obtained by benomyl or Thiophanate methyl at 10g a.i. at casing while TBZ wa s less affective. Kim (1975) recorded satisfactory control of wet bubble by spraying benomyle @ 0.5g a.i/ m2, 3 days after casing. Geijn (1977) suggested the control of wet bubble disease by spraying th e crop with carbe ndazim , benomyl or thiophanate methyl at100-150litre water immediately after casing. Basamid (Dazomet) and Vapam (Metham sodium) applied @ 100ppm to casing has also been reported very effective (Kim et al., 1978).

1. COBWEB

Pathogen : *Cladobotryu mden droi des*

Common Name : Milde w, Soft decay, Hypomyces mildew disease,Dactylium disease.

This disease renders extensivedamage either by causing soft rot or decay of fruiting body. Merat (1821) described this disease as *Botrytis dendroides* and transferred it in to the genus *Cladobotryum* by making a combination *C.dendroides* (Bull : Merat) W.Gams et Hoozem. Salman and Ware (1933) were the first to report *D.dendroides* being parasitic to mushrooms. According to Fletche r a n d Atkinson (1977)mushroom of any age of development would be attacked by this fungus.

Symtomatology: Cobweb appears first as small white patches on the casing soil which then spreads to the nearest mushroom by a fine grey white mycelium. A floccose white mycelium covers the stipe, pileus and gills, eventually resulting in decomposition of entire fruit body. As the infection develops, mycelium becom es pigmented eventuallyturning a delicate pink cover (Lane *et al.* 1991).

Symptoms of cobweb

Causal organism : *Cladobotryum dendroides* (*Dactylium dendroides*) imperfect state of *Hypomycesrosellus.* Sterile hyphae form a turf and are prostrate, branched, septate and hyaline with approximately opposite branches, which divide above into usually three pointed branchlets. Conidiophers are erect,similar or branched in many whorls.

Epidemiology: High relative humidity and temperature encourage the disease. Spread is mainly by conidia. The pathogen is a soil inhabiting fungus and is normally introduced into the crop by soil contamination, spores, mycelium on crop debris or by farm workers. Spores are easily spread by air movement, workers hands, tools and clothing and by water splash (Sharma, 1994). Under laboratory conditions, sciarids and phorid flies were found to transmit 4-100% of the disease in to two different media (Kumar and Sharma, 1998). A high RH and temperature range of 19-22°C and 12-15°C resulted in maximum loss in yield (Seth and Dar 1989). Optimum temperature for growth is 20°C and for spore germination is 25°C.

Management

Physical: Through disinfection of casing soil with live steam orsterilization of casing mixture at 50C for 4 hours effectively eliminates the pathogen. Regular cleaning, removal of cut mushroom stems and young half dead mushrooms after each break and controlling temperature and humidity helps in controlling the disease (Sharma, 1994).

Biological: Under laboratoryconditions, leaf extract of *Cannabis sativus*, *Ricinus cummunis*,*Callistemon lanceolatus, Citrus* sp., *Euclyptus* sp., *Dhatura* sp. and *Urtica dioica* were found to cause 5.55%, 10.55%, 18.55%, 26.11%, 34%, 19.07% and 23.33% inhibition of *C.dendroides* (Sharma andKumar, 1988).

Chemical: Terraclor (pentachloronitrobenzene) can eradicate *Dactyliu m* mildew even after the well establishment of the disease (Stoller *et al*., 1956). Bozhkor (1975) suggested annual disinfection of houses and surrounding areas with 2% bordeaux mixture or with 5% formation solution at 0.5-1.0 l/m ^{2} or fumigation with 2.0-2.5 l formation and 0.5-1.0 kg chlorinated lime/100 m^3 for controlling disease.

B. OYSTER MUSHROOM (Pleurotus spp.)

a. Diseases

There are four fungal diseases reported on oyster mushroom from I n dia. Their causal age nts, symptoms and control measures are presented in Table 1.

Table-1: Fungal diseases of oyster mushrooms in India

SN	Casual organism	Symptoms	Control	References
1.	*Cladobotry m apiculatum* *C.verticillatum* *C.variospermum*	White cottony growth on the substrate; small brown irregular sunken spots or fluffy growth on fruit bodies; soft rot and decay of sporophores emitting foul smell.	Spray bavistin 50ppm	Upadhyay *et al*; 1987;Sohi and Upadhyay 1980; Goltapeh *et al*. 1989
2.	*Gliocladiu m virens* *G.deliguescens*	Fruit bodies covered by mycelium and green spots; young pin-heads become soft, brown, pale yellow and decay. Mature fruit bodies show brown spots enclosed by yellow halo.	Spray 100ppm bavistin or benomyl	Bhardwaj *et al*. 1987; Sharma and Jandaik, 1983
3.	*Arthrobotrys pleuroti*	Fluffy growth on substrate and fruit	Spray 50ppm bavistin	Ganeshan, 1987

	bodies; infected tissues turn yellow, water logged and rot.		
4. *Sibirina fungicola*	Powdery white growth on stipe, gills and the primordia; primordia show brownish discolouration and soft rot and mature fruit bodies turn fragile.	Proper aeration and RH essential; spray benomyl twice	Sharma and Jandaik, 1983, Jandaik and Sharma, 1983.

C. PADDY STRAW (*Volvariella* spp.)

Though paddy straw mushroom (*Volvariella* spp.) was the first to be cultivated in India as early as 1943 by Thomas and his associates at Coimbatre yet very littleinformation is available on the diseases of this mushroom. This is still being cultivated outdoors in India following primitive production technology with very low biological efficiency. Paddy straw mushrooms are subject to a number of destructive diseases/competitor moulds like *Mycogone perniciosa*, *Scopulariopsis fimicola* and *Verticilliu m* spp. in other countries. In India, large number of competitor moulds and few diseases has been reported on this mushroom. *Chaetomium* spp., *Alternaria* sp. and *Sordaria* sp. have been commonly observed as contaminants on wheat, kans, maize, barely and jowar beds but not only paddy straw bundles (Gupta *et al*. 1970). A 'button-rot' disease caused by *Sclerotium* sp. has been reported by Muthukrishnan (1971) and bacterial 'button-rot' by Kannaiyan (1974). Combination of insecticide,fungicide and antibiotic (Malathion 0.025% + dithane Z-78 or benomyl 0.025% + tetracycline 0.025%) are recommended for the management of pests and diseases (Kannaiyanand Prasad, 1978).

Several othercompetitor moulds namely, *Coprinus aratus*, *C.cinereus*, *C. lacopus*, *Psathyrella* sp., *Penicilliu m* spp.,*Aspergillus* spp., *Rhizopus* sp.,*R. nigricans* and *Sclerotiu m* spp.have been reported fromthe substrate (Bahl, 1984,Rangaswmi,1978).

Partial sterilization of the straw and sprays on the beds with captan and zineb (0.2%) have been recommended for reducing the damage. Bahl and Chowdhry (1980) have reported *Podospora favrelii* as a serious competitor and inhibits the growth of mushroom mycelium completely. Bhavani Devi and Nair (1986) have also recorded *Rhizoctoria solani* on the substrate which reduces the sporophore formation and causes malformation of fruiting primordia. A serious effort is urgently needed to investigate the diseases of paddy straw mushroom and recommend the package of practices to be followed to the growers to achieve good yields.

D. OTHER MUSHROOMS

Sporadic attempts have been made to cultivate few other mushrooms like giant mushroom (*Stropharia rugoso-annulata*), black ear mushroom (*Auricularia polytricha*), shiitake (*Lentinula edodes*) and milky mushroom (*Calocybe indica*) in different parts of the country and the competitor moulds/diseases recorded on them are briefly mentioned below:

Sohi and Upadhyay (1989) have reported *Mycogone rosea* parasitizing *S. rugoso-annulata* under natural conditions.

The main symptoms are white cottony growth on gills, light brown spots on stipe and deformity of the sporophores. *Cladobotryum verticillatum* has been reported on *Auricularia polytricha* (Goltapeh *et al.* 1989) producing white fluffy growth on substrate and fruit bodies resulting in 9-96% yield loss. Spraying carbendazim (50 ppm) has been reported effective for controlling the disease. *Trichoderma viride*, *Trichoderma* sp., *Aspergillus* spp. and *Fusarium* sp. have been commonly recorded as competitors (Sharma and Thakur, unpublished) during the cultivation of winter ear mushroom. During the cultivation of *C. indica*, several competitor moulds namely, *Aspergillus niger, A. flavus, A. fumigatus, Rhizopus stolonifer, Mucor* sp., *S. rolfsii, T. viride, T. haematum, Fusarium* spp. and *Coprinus* spp. have been isolated from the substrate (Doshi *et al.* 1991). In addition Sharma and Thakur (unpublished) have also recorded very high incidence of *Cladobotryum* and *Oedocephalum* spp. from the casing mixture.

Incidence of *T. viride* has been recorded from 15-25% in the supplemented bags as compared to 5-10% in unsupplemented ones in *L. edodes* cultivation (Thakur and Sharma, 1992).

VIRAL DISEASES:

In recent years, viruses haveincreasingly been found inassociation with fungi, an association that has taken one of the two forms. In the first, the fungus is the vector of the virus and in the secon d,fungus is the host of the virus. Here only the second form of association i.e. fungi, especially the mushrooms, as hosts of viruses will be considered in detail which has been reviewed earlier by Raychaudhury (1978), Sh a rm a (1991) a nd Sh a rm a a ndK um a r (2000). Al thoug h th e presence of viruses in fungi has long been suspected (Sinden and Hauser, 1950) experimental evidence was not forthcoming until 1962 when virus particles were demonstrated in diseased mushroom (Ga ndy a nd Hollings, 1962; Hollings, 1962). To date viruses or virus-like particles (VLPs) have been reported to occur in over 100 species from 73 genera of fungi, but only a small number of th e m h ave been isolated and characterised. Several terms have been used for the viruses of fungi inclu di n g mycoviruses, fungal viruses, mycophages,double stranded RNA (dsRNA) plasmidsand virus-like particles (VLPs).

The term mycophage is clearlyunsuitable since virus infection hasvery rarely been associated with lysis in fungi. Although mycoviruses may share some of the characteristics of plasmids, their morphology, nucleoprotein composition and the possession of virion-associated RNA polymerase activity are consistent with a viral nature.

The termplasmid has already been abused incurrent literature as pointed out by Reanney (1976) and to denote theviruses of fungi as plasmids would not find ready acceptance. The termVLPs and mycoviruses have beenused by some authors (Boza rth, 1972; Saksena and Lemke, 1978),with the understanding that thefirst term applies to those particles occurring in fungi and having a virus-like appearance in electron-micrographs but which have notbeen isolated and cha racterised,whereas the second term denotesthose which have been isolated and shown to have the morphology andnucleoprotein composition generally. attributed to viruses.

This distinction offers an operation alconvenience and has been widelyadopted. Since mycoviruses have not conclusively p rove n to be infectious as purified particles, some workers prefer to apply theterm VLPs in all cases.

Virus and VLPS reported in different mushrooms

S. No.	Host/Disease	Shape	Size	Country
I	*Agaricus bisporus* La France, Watery stipe,	Spherical	25nm 29nm	Australia, England,
	X-Disease, Die-back, mushroom disease		35nm 40- 50nm	Holland, America, France GDR, India
		Bacilliform	18x50nm	U.K.
		Club shaped	60-70nm dia or 120-170 long with a spherical body of 40-50 nm & a cylindried tail 20-30nm in dia	France, W. Germany S. Africa
		Rods of varing longth	19x9-90nm 19x35nm 20x130nm	Poland GDR China
II	*Pleurotus* spp.			
	P. colombinus *P. ostreatus*	Spherical	26 + 2nm	France India
	P. pulmonarius	Spherical	24nm	China
	P. sapidus			China
	P. florida	Flexuousrods 40-600nmlong		
III	*Volvariella volvacea*	Spherical	35nm	China
IV	*L.edodes*	Spherical	20nm, 23nm 36nm, 45nm 30nm	China, Japan
		Stiff		or Japan

		17x200x1200n m	
		15x700-900nm	China
		18x1500nm	
		15x16x200-300nm	

MANAGEMENT OF MUSHROOM VIRUSES

For adopting suitable management strategies for mushroom viruses, one has to keep in mind that the disease is spread by viable mycelium and spores of diseased mushrooms; early infection is dangerous, especially an infection simultaneous with or shortly after spawning. Upto the time of casing, the compost and mycelium must be protected. Owing to the lack of useful resistance with the species, control of the disease is based largely on the use of hygienic practices directed at the elimination of dead mycelium and basidiospores from the production (Schisler *et al*., 1967, Van Zaayen, 1976). Dieleman/van Zaayen (1986) has suggested various approaches to reduce the spread of mushroom virus diseases which have been summarized below:

When the disease is not present

1. Steam the compost for 12 hours at a temperature of 70°C. At emptying, remove the compost quickly.
2. Spray the wood with 2 per cent sodium pentachlorophenate to which 0.5-1.0 percent soda (sodium carbonate) has been added, after drying spray with water.
3. Disinfect doors, little holes in the floor, shutters, racks, floors and walls with formaldehyde (not with sodium pentachlorophenate). Also cleanthe manure yard and adjacent patches of ground withformaldehyde.

   Before filling, fit spore filters, during growing time these spore filters should be replaced once or twice according to the amount of dust in the air. Use a fan forextracting air.

4. Immediately after spawning, use a pesticide against flies and cover the compost with paper. Keep the paper moist. Wet the paper twice a week with a 2 per cent solution of the 40 per cent commercial form aldehyde. Repeat till a few days before casing. Never use sodium pentachlorophenate here. Moisten the paper before removing it carefully.

5. Quickly remove cuttings and litter and destroy.

6. The entire farm and it's surroundings should bemaintained very clean and stay so. In the working corridor formaldehyde should be sprayed. Machines, refrigerator and other utilities should be disinfected with a formaldehyde solution.

7. At the first sight of contamination, the disease can be controlled best by immediately steaming out the concernedroom.

When the disease is alreadypresent

1. Adopt practices 1,3 and 4menmioned under when the edisease is not present.
2. Immerse the wood in a 4 per cent sodium pentachlorophenate solution to which 0.5-1 per cent sodium carbonae has beenadded.
3. Pick the mushrooms when still closed.
4. Keep each room as a separate entity with separate clothes, shoes, steps, buckets, picking knives, picking racks, fans etc. Kill off diseased patches with salt and cover with plastic, make the limits of the patches rather big. First pick from the healthy parts then from the diseased patches. Wash hands often.
5. Admit as few visitor s in the diseased rooms as possible and keep the door towards the working corridors closed. Kill off pests in particula r. Have a short picking period only (not more than 4 weeks).

Heat Therapy

When infected cultures weregrown at 33C for 2 weeks, and hyphaltips then sub cultured and returned to 25C, many of the latter showed normal growth and did not contain virus (Gandy and Hollings, 1962). However, these findings were not conclusively proved by Dieleman van Zaayen (1970). Rasmussen and co-workers(1972) also obtained increased sporophore yields when tissue and spore cultures derived from symptomatic sporophores of white and two cream strains were incubated at 32C for 2 weeks. Wuest and Mataka (1989) have observed more extensive spawn run on horse manure compost with the symptomatic spawn incubated a t 30C than the spawn incubated at 23 or 27C.

BACTERIAL DISEASES:

Broadly, the mushroom is defined as macro-fungus with distinctive fruiting body which can be either epigeous or hypogeous. In this article the term mushroom has been used to denote edible cultivatedmushroom s. Mor e than 2,000species of fungi are reported to be edible throughout the world (Chang and Miles, 1982).

Out of these about 16 genera representing more than 25 species have been successfully domesticated.

In India, three mushrooms namely white button mushroom(*Agaricus bisporus*), dhingri oroyster mushroom (*Pleurotus* species) and paddy straw mushroom (*Volvariella volvacea*) are being exploited for commercial cultivation. In addition to this, recently *Calocybe indica* which is commonly known asmilky mushroom is also gainingpopularity in some parts of the country and is suited for cultivation in warmer areas where *A. bisporus* cannot be cultivated. These mushrooms like any other living organism are attacked by several pahogens.

The expression of disease symptoms in mushroom depends upon the stage of development of the fruit body at the time of infection and cause of the disease/inoculum potential present.

The bacterial diseases have been reported from all over the world on fruit bodies of *A. bisporus*, *A. bitorquis*, *Pleurotus* species, *Volvarella* species, *Lentinus edodes*, *Flammulina velutipes* and *Auricularia* species and are given along with their causal organism(s) and distribution in Table 1.

The bacterial pathogens induced varieties of symptoms like blotch, mummy, pit, drippy gill, soft rot, yellowing and immature browning but in India, bacterial diseases has been reported only on fruit bodies of *A. bisporus* and species of *Pleurotus* and *Auricularia*. The various bacterial diseases reported from India are discussed as under:

Bacterial diseases of edible cultivated mushrooms

Mushroom	Disease	Causal organism	Distribution	Reference
Agaricus bisporus	Bacterial blotch	*Pseudomonas tolaasii* *P. fluorescens*	Worldwide	Fletcher *et al.* (1986)
	Ginger blotch	*P. gingeri***	UK, Netherlands	Fletcher *et al.* (1986)
	Drippy gill**	*P. agarici*	UK, Netherlands	Fletcher *et al.* (1986)
	Mummy	*P. aeruginosa*	UK	Wuest and Zarkower (1991)
A. bitorquis	Bacterial blotch	*P. tolaasii*	Worldwide	Fletcher *et al.*

				(1986)
	Soft rot	*Bukholdria gladioli pv.* agaricicola	Worldwide	Guleria *et al.* (1987)
Oyster mushroom (*Pleurotus* spp.)	Bacterial rot	*P. alcaligens* **	India	Biswas *et al.* (1983)
	Brown blotch	*P. tolaasii*	Japan, Australia Netherlands	Fermor (1986) Ferri (1985)
	Yellow blotch	*P. agarici*	India, USA	Jandaik *et al.* (1993b) Bessette *et al.* (1985)
	Fist-shaped Fruit bodies*	*P. fluorescens*	Belgium, Italy and Europe	Poppe *et al.* (1985)
Other mushrooms				
Volvariella spp.	Bacterial rot	*Pseudomonas* sp.	India	Kannaiyan
			Indonesia	(1974) Fermor (1986)
Lentinus edodes	Browning*	*P. fluorescens*	Japan	Komatsu and Goto (1974)
Flammulina velutipes	Brown soft rot*	*Erwinia* sp.	Japan	Phawicit (1985)

Bacterial disease(s) of *Agaricus* species

Bacterial blotch

Bacterial blotch of mushrooms is also known as brown blotch and bacterial spot.

Occurrence and losses

Blotch is one of the most common and serious diseases of *A. bisporus* and is responsible for considerable losses. The disease also affects. *A. bitorquis*. The disease was first described by Tolaas (1915) from America and later Paine (1919) identified the organism as *P. tolaasii*. From India, it was first reported in 1976 (Guleria, 1976). Bacterial blotch disease reduces crop yield because lesions develop on the surface of mushroom caps making the mushrooms unmarketable. The disease has been reported from almost all mushroom growing countries of the world. The disease causes 5 to 10 per cent losses in yield (Fermor, 1986; Vantomme *et al*., 1989). In Australia, bacterial blotch is second in economic importance only to the virus disease complex (Nair, 1969) and substantial losses occurred particularly after harvest and overnight storage of mushrooms at low temperature.

Symptoms

Bacterial blotch of white button mushroom is characterized by brown spots or blotches on the pilei and in more severe cases, on the stipes. Circular or irregular yellowish spots develop on or near the margins of the cap which enlarges rapidly under favourable conditions and coalesce to form rich chocolate brown blotches that are slightly depressed. The most characteristic symptom of bacterial blotch is the occurrence of dark brown areas of blotches on the surface of the cap. These may be initially light in colour but may eventually become dark brown. Severely affected mushrooms may be distorted and the caps may split where the blotch symptoms occur. Brown and slightly concaved spots appear ed on the surface of the diseased fruit bodies. Light infection of mushroom caps produced a yellow light brown spotting on the surface, but the common symptom associated with infection was appearance of brown, slightly sunken lesions of variable size and mushroom tissues were usually affected to a depth of 1 to 3 mm. Mushrooms often become infected at a very early stage in their development. The enlargement of the spots on the cap surface is dependent upon environmental conditions and is favoured by temperatures of atleast 20^{o}C together with the presence of water film.

Casual organism

Tolaas (1915) described a causal organism as a pathogenic strain of *Pseudomonas fluorescens* , but Paine (1919) while working with other isolates found differences in their action on nitrates and starch and as such proposed the name *P. tolaasii* Paine.

Lelliot *et al*. (1966) showed that *P. tolaasii* was indistinguishable from some isolates of *P. fluorescens* and suggested that *P. tolaasii* could be considered as one of the natural constituents of microflora of mushroom beds. Fahy (1981) observed that members of *P. tolaasii* contained both pathogenic and non-pathogenic strains which werecommon on mushroom.

Olivier *et al.* (1978) reported the appearance of both smooth and rough forms of *P. tolaasii* and claimed that the smooth form was non-pathogenic. Wong and Preece (1979) proposed the white line in agar and mushroom tissue rapid pitting tests for the identification of *P. tolaasii* . They observed that a sharply defined white line of precipitate was formed in *Pseudomonas* agar F between the opaque white colonies of *P. tolaasii* and translucent colonies of certain unidentified pseudomonads. The visible interaction has been utilized as specific and reliable method for the identification of *P. tolaasii* .

Epidemiology

Casing and airborne dust are the primary means of introducing the blotch pathogen into a mushroom house. Even after pasteurization the bacterial pathogen is present in most casing materials. Occurrence of the disease is associated with the rise in the bacterial population on themushroom cap rather than in the casing. Blotch can develop on cap,stipe or both at any stage ofmushroom development. Bacteria present on mushroom surfacereproduce in moist conditionsespecially when moisture or freewater film persists for more than 3hours. Once the pathogen has been introduced at the farm, it maysurvive between crops on the surfaces, in debris, on tools andvarious other structures. It is also a natural inhabitant of both peat and chalk. When the disease is presenton the farm, its secondary spreadmay take place through workers, implements, ingredients, mushroom spores, debris etc. Sciarids and mites are also important carriers of thepathogen beside water splashes.

Management

Ecological management: Manipulation of relative humidity,temperature, air velocity and air movements are of great significance in managing the disease. Temperature above 20^{o}C and relative humidity of more than 85percent should be avoided.

Additional ventilation and aircirculation after watering canensure the quick drying of mushrooms. Temperature fluctuations at higher relative humidity leading of water condensation must be avoided.

Biological management: Isolates of *P. fluorescnes* and other antagonistic bacteria have resultedIn 30 to 60 percnt control of bacterial blotch. Many selective bacteriophages have also been found effective against *P. tolaasii* without any significant effect of *P. fluorescens*. Spraying the casing soil with a mixture of *P. fluorescens* and bacteriophage has resulted in more than 80-per cent control of blotch symptoms.

Chemical managemet: Application of terramycin 9 mg per square foot, streptomycin (200 ppm),oxytetracycline (300ppm),kasugamycin and kanamycin has been found effective in managing the disease.

Physical management : Pasteurization of casing soils by steam/air mixture and short wave length irradiation have been reported effective in eliminating the bacterial pathogen but over-heating should be avoided otherwisebiological vacuum will be createdand successive invasion of moulds would be very high. The introduction of water retentive acrylic polymers as a component of casing soilmixture is also claimed to reduce the disease.

Other bacterial diseases of *Agaricus* species

Bacterial pathogen other than *P. tolaasii* recorded on *Agaricus* species are *P. agarici*, *P.aeruginosa* and *Bukholder gladioli* pv. *agaricicola*. However, *P. gingeri* is considered to be a part of the *P.tolassii* (Miller and Spear, 1995).

Bacterial disease(s) of oystermushroom

Till date, four bacterialpathogens namely, *Pseudomonas alcaligens, P. tolaasii, P. agarici* and *P. fluorescens* have been reported parasitising *Pleurotus* fruit bodies and causing considerable economic losses to the growers. Among these, *P.agarici* and *P. alcaligens* (not valid name) have been reported from India and are described as under:

Yellow blotch

Occurrence and losses

In India, heavy incidence ofyellow blotch was reported (Jandaik *et al*., 1993) which esulted in complete failure of crop in some of the mushroom units.

Symptoms

The disease appears as blotches of varying sizes on pilei sometimes depressed, yellow, hazel-brown, fawn or orange in colour. When the disease appears at primordialformation or pinhead stage, it affects the total group of early fruit bodies or only a part of them.

Infected fruit bodies turn yellow and remainstunted. The slimy appearance of the infected fruit bodies under high relative humidity (more than 90%) is a common symptom. If the relative humidity is less than 75 per cent, the blotched fruit bodies give appearance of burnt ulcers.

Causal organism

Pseudomonas agarici is a gram negative rod shaped and motile. The colony is buff, circular, pulvionate,semiopaque and 2 to 6 mm in diameter. Oxzidase and catalase tests were positive and starch hydolysis and nitrate reduction were negative. The bacteria can utilize benzoate, citrate and glucon ate efficiently.

In carbohydrate media, acid was produced from glucose, maltose and fructose. There was no acid production in sucrose, sorbitol, inositol and cellobiose.

Epidemiology

The disease incidence is more under warm and humid conditions. The pathogen is easily spread inside the mushroom farm through water splashes, workers, tools and mushroom flies. When the humidity is more than 90 per cent the fruit bodies gave slimy appearance and finally fruit bodies start rotting and smelling foul within next twenty four hours. Presence of water film on the surface of fruit bodies is quite favourable for earlier appearance of symptoms.

Management

Environmental manipulation : High rrelative humidity and continuous persistence of water film on the surface of pilei enhance bacterial multiplication. Hence, proper ventilation and careful watering coupled with monitoring f temperature in the mushroom unit help in limiting the diseaseincidence.

Use of chemicals: The regular application of chlorinated water containing 100-150 ppm of freely available chlorine (FCA) at 3 to 5days interval help in minimizing losses due to bacterial pathogen, Use of oxytetracycline and streptocycline have also been reported.

Biological management: Biocontrol of yellow blotch of oyster mushroom appears to offer a viable proposition, especially with theincreasing awareness among consumers about the use of chemicals in mushroom units. The possibility of using bacteriophages ascontrol agent for plant diseases caused by various bacterialpathogens including *Pseudomonads* has been reported and it may haveapplication in mushroom industry aswell.

10. Techniques for improvement of mushroom crops.

It is known that many Substances may be utilized for growth by fungi. For commercial mushroom production com post rich in biomass protein and humicacid nitrogen complexes derived from bacterial and fungal composting of Straws, manures etc is used as Substrate for the mushroom mycelium grains and they are allowed to colonise the growing bed. After some 14 days at 25°C. The bed is covered (cased) with a layer consisting of 6 parts by Volume of peat and 1 part by volume of sugarbeet fine chalk to a depth of 5-6 centimeter.

This nutritionally sterile layer is often mixed with particles of mycelium on a low nitrogen carrier in order to speed the link up and penetration into the casing layer of the compost mycelium.

When the casing layer has been penetrated by the compost mycelium the mushroom bed is cooled until primordia are formed and one week or so later the mushrooms are ready to harvest.

In order to get maximum yields of mushrooms per ton of compost, growers add extra nutrients to the compost. This may be in the form of formaldehyde denatured Soya products, feather meal or protein treated with antibacterial agents or carbohydrate, lipid and protein mixtures with calcium/amine salts of carboxylic acids.

These Supplementary nutrients are normally added at a rate of from one to two percent on the weight of compost. It is essential to mix the Supplementary nutrient very evenly throughout the compost bed. Any area with excess supplement will cause very dangerous heating effects and result in reduced yield and mycelium kill.

Areas with little or no supplement will of course not produce any yield increase. The overall effect of uneven mixing of Supplements is actually a reduced yield and high risk of disease within the growing bed. Even very well mixed supplemented growing beds show heat Surges which have a high cost in terms of cooling technology in order to maintain optimum growing tempera tures.

However in recent times the improved preparation of com posts has raised the levels of naturally occurring protein nitro gen from an average of 2% in the 1980s to close to 3% at the present time.

Amounts of humic acids and partly soluble lignin humic acid nitrogen residues have also improved and the combined effect is for an improvement in natural nutrient levels in the compost which has meant that adding Supplementary protein nutrients even at higher usage rates to the compost has had progressively less effect.

Up to 40% of the mass of a mushroom growing bed can be the casing layer. The function of this layer is to persuade the compost mycelium mass to form fruit bodies by virtue of the absence of nitrogenous nutrients in this top layer. As the mushrooms form in or on the casing layer the compost mycelium starts to transfer tissue nutrients from throughout the compost mass up to the casing layer to form the fruit bodies therein.

Early experiments to introduce particles of fully colonised compost into the casing in order to speed up the colonisation of the casing layer (cacing) frequently failed if nitrogen nutrient was introduced along with the mycelium particles on compost.

This resulted in cacing materials being developed using minimum nitrogen on Vermiculite carrier for the mycelium. It was clearly established that even very small amounts of nitrogen containing nutrient in the casing layer prevent the development of primordia—certainly below a threshold where any contribution to overall nutrition could be expected.

The mushroom industry subsequently regarded the casing layer as an area of nutritional sterility, which must be totally devoid of all nutrient value in order to function. Salts of the carboxylic acids are also utilized by mushroom mycelium as an important nutrient source. Many of the car boxylic acid salts also have very active anti-mould properties.

There is a need for a novel method to substantially improve mushroom yields even at ultra-low dose rates utilizing modern high nutrient composts that will not cause heating problems or disease risks for the mushroom grower.

A method and composition for improving the performance of a mushroom bed comprises adding a much reduced but effective amount of carboxylic acid salts or mixtures thereof to the casing layer or on the Surface of the compost layer at the interface with the casing layer.

A method of improving the yield of mushroom growing beds comprising Supplementing the casing layer or compost/ casing interface with between 20 grams and 350 grams per square meter of mushroom bed surface area with an effective amount of a salt of aliphatic, aromatic, alicyclic, or heterocyclic carboxylic acid or mixtures thereof, the said salt being of potassium, magnesium or calcium or mixtures thereof wherein the nutrient salt or mixtures thereof must not be admixed with the compost layer more usually between 60 grams and 300grams of carboxylic acid salts per square meter of mushroom bed Surface area are used.

The effectiveness of this invention is a function of the Surface of the growing bed and its micro Supplementation and not in its admixture with the compost layer.

The carboxylic salt nutrient is not mixed into the compost and so does not result in any significant heating effects. Surprisingly it was found that a dose of one tenth of the normal Supplement rate if mixed in with the casing layer or on the Surface of the compost at the casing interface produces the same increase in yield as the normal amount of commercial Supplement mixed throughout the compost. It is suggested that the much enhanced yields obtained from a comparatively very small amount of nutrient is due to the placing of the supplement at or very near to the site of fruit body formation. That is, the mycelium in the compost does not have to transport the nutrients from deep within the com post layer to form the fruit body in the casing layer or compost interface.

The carboxylic salts may be derived from mono di or tri basic, aliphatic, hetrocyclic, aromatic or alicyclic, Saturated or unsaturated, hydroxy or keto, straight or branch chained carboxylic acids or mixtures thereof. Examples of such acids would be Propionic, Acetic, Lactic, Butyric, N-Valeric, Pelargonic, Oleic, Linolenic, Linoleic, Salicylic, Gluconic, Hippuric, Malic, Maleic, Fumaric, Aze laic, Adipic, D & L Tartaric, Succinic. Furoic, Pyruvic, Glutaric, Citric or mixtures thereof. In one embodiment the nutrient product may be prepared from readymade salts utilising cations such as potassium magnesium and preferably calcium and or mixtures thereof.

The product may also be formed in situ in the casing by adding acid salts or acids as granules, crystals, powders or Solutions to the casing material with or without extra calcium cation depending on casing formulation. The interface between compost and casing may also be utilised as a site for micro Supplementation with salts of carboxylic acids as Solutions, solutions/Suspensions, Suspen sions, powders or granules.

In a further embodiment the carboxylic salts utilised as Supplements on the compost/casing interface may include ammonia and organic amines as the cation. The ammonium and organic amine salts must not be used for incorporation into the casing layer as Supplement. The carboxylic salt containing nutrient may be incorpo rated into the casing layer in the form of powders or granules or in solutions or suspensions in water modifying the solubility of the carboxylate salt powder or granules provides some control of availability of the nutrient over or up to four flushes. Liquid carboxylic nutrient salts are best prepared by utilising the generally good solubility of the potassium salts of carboxylic acids but are best used as blends or double salts with calcium as the major cation. An effective method of utilizing the carboxylic nutrient salts is to water on a solution or Suspension or combination of both on top of a casing layer two to three days after application of the casing when bridging has occurred between com post and casing. The liquid nutrient may be applied as one application at a concentration of, for instance, ten percent or four or five daily applications at two percent to maintain casing moisture after day two after casing.

It is important to restrict the volume of nutrient solution in order to limit the penetration of the supplement into the sur face of the compost. Excessive penetration much reduces the yield increase and allows heat Surge to develop.

The carboxylic salt nutrient may be applied on the surface of the compost at the time the bed is spawned best results at this stage are by using the carboxylic salt nutrient as a granule or pril1. The carboxylic salt nutrient may be applied to the surface of fully-grown phase two compost prior to casing, which may be as granules, powder, Solutions or Suspensions. Phase three compost may be surface supplemented with carboxylic Salt nutrient prior to casing in any form prior to casing. The carboxylic salt nutrient may be mixed with other nutrient substances in order to improve the performance of the nutrient mixture or to obtain specific nutrient effects.

In a further embodiment the carboxylic salt nutrient or mixtures thereof may be admixed with emulsifiers, clays, extenders, binders or absorbents in order to stabilize or modify the availability of the nutrient to the mushroom mycelium. In a further aspect of our invention due to the well-documented anti-mould properties of the lower molecular weight carboxylate salts significant amounts of carbohydrates Such as dextrin's may be incorporated into the micro Supplements. Another aspect of our invention would include incorporation of lignin chemicals such as lignosulphonates derived from the paper industries in order to improve the nutritional balance of the Supplement.

A further group of beneficial nutrients may be utilized in the Supplement as described to be used on the compost Sur face but not as a casing Supplement these are the soluble and semi-soluble amino acids and polypeptides. A further embodiment of the invention is that significant amounts of unsaturated long chain carboxylic acid oils or salts thereof may be incorporated in the nutrient salts in order to improve mushroom quality and flavour.

The presence of lower molecular weight monocarboxylic acid salts in the critical areas of the casing and compost interface increases the selectivity of the growing bed against many fungal infections preferably no animal or vegetable products are added to the mushroom growing beds.

Mushroom breeding strategies

Mushrooms have gained the reputation of being difficult organisms to work with and it was widely acknowledged that the mushroom, particularly *Agaricus bisporus* is not easy to manipulate through breeding. During early attempts at genetic improvement in the cultivated mushroom *A. bisporus*, there was not much understanding of the natural breeding system. The mushroom is now known to be a "secondarily homothallic" species with a single multiallelic mating type factor (Elliott and Langton, 1981). This understanding can evaluate the breeding methods previously used and to suggest alternatives.

Strain selection based on single spores, multispores or tissue culture may give improvement in the short term but is not as effective as methods with controlled crossing. Mixing fertile strains may produce hybrids but they are sometimes difficult to identify. It is better to use non-fertile isolates because only the hybrids show fruiting. Early recognition of hybrids can be done using markers that can be expressed only in hybrid cultures and the incorporation of genetic resistant trait is especially useful for this.

Karyotype Analysis

Karyotypic analysis of various mushroom species using microscopy and pulse fieldgel electrophoresis (PFGE) will be helpful to determine the number of chromosome of the species and for the identification of species. Sometimes the importance of karyotypic analysis is not justified as chromosomal polymorphism among the strains. This may be due to chromosomal aberration during growth and development of any fungi. Initially electrophoretic karyotype analysis was used as a potent tool to reveal such type of chromosomal aberration in many fungi like *Coprinus cinereus* (Pukkila andLu, 1985).

However,the results are sometimes ambiguous,for example,thenumber of chromosomes in *Pleurotus ostreatus* varying from 6 to10 (Sagawa and Nagata, 1992).This problem can be solved with the optimization of PFGE separation forfungal chromosomes (Sagawa and Nagata, 1992)that allowed the analysis of molecular karyotype of mushrooms and assigning genes to chromosomes and applied in *Agaricus bisporus* consisting 13 chromosomes in genome with 31 Mbp size (Sonnenberg et al., 1996) and also in *Pleurotus ostreatus* containing 11 pairs of chromosomes in genome (Larraya et al., 1999).While, light microscopic observations and electrophoretic karyotype analysis have suggested that this fungus contains at least eight chromosomes in the haploid genome (Tanaka and Koga, 1972; Nakai, 1986; Arima and Morinaga, 1993).There is no report for other mushrooms in this respect and the genomic organization of different species remains poorly understood.

Goals of mushroom breeding

Increasing the yield and quality of crops as well as resistance to diseases are the primary goals for mushroom breeders and mushroom research. Other goals include reducing production costs and the efficient use of compost for growth. Methods of mass selection based on natural chance mutation and programmed mutation by ionizing radiations such as γ-rays, X-rays and chemicals as well as cross breeding and transgenic breeding are some of the methods carried out for this purpose. However, cross and transgenic breeding are more effective and have shown greater promise and progress in the last few decades (Fan et al., 2006). Areas of research for mushroom breeding relate directly to commercial benefits such as problems associated with cultivation, distribution and storage, senescence-induced browning and disease resistance. Another aim of breeding in mushrooms is to incorporate various improved crop growing qualities such as shorter growth cycle and prevention of spore formation.

Traditionally mushrooms produce billions of spores floating in the air which cause health problems such as lung allergy and fever attacks. Spores also lead to the blocking of climate installations and result in higher energy costs. A new sporeless oyster mushroom has been developed by Plant Research International, Wageningen, Netherlands by using molecular marker technology. After crossing various oyster mushroom cultivars, this analytical technique can be used to rapidly identify progeny with the highest chance of sporelessness (Okuda et al., 2009). Other research areas for crop improvement in mushrooms are utilization of grain-based substrates substituted for traditional manure-based compost which will address issues related to outdoor composting and compost disposal and potentially offer sterilized substrate for biopharmaceutical manufacturing (Bechara et al., 2006). To increase yield, various strategies have been carried out. Many older strains which grow profusely and produce a large number of pins but never mature are hybridized with strains with less growth.

Production of hybrids with increased thickness and density of mushroom cap as well as prolific nature of the hybrid strains has increased yield potential. Two methods successfully employed for breeding in the lab are protoplast culture and spore germination (Horgen et al., 1991, Kerrigan et al., 1992). Improvement of strains for commercially grown fungi was boosted 30 years ago with the development of methods for isolation of protoplasts (Chang et al., 1993). This was performed using microbial enzymes that could digest the fungal cell walls and also provide an osmotic support for the naked protoplast. Methods to fuse protoplast from different sources followed later. Djajanegara and Masduki, (2010) carried out protoplast fusion between white and brown oyster mushrooms to obtain high productivity and long storage life. The regeneration of protoplasts is a time consuming and difficult process but has been performed successfully in species of Pleurotus and Volvariella (Reyes et al., 1998).

Breeding for disease resistance

Disease outbreaks can severely reduce the yield and productivity of commercial mushroom production. Many mushroom pathogens are resistant to benzimidazole fungicides or tolerant to prochloraz. New pathogens such as Trichoderma aggressivum, Cladobotryium mycophilum and the mushroom virus X have emerged at regular intervals over the years. Approved pesticides have been significantly reduced in Europe due to consumer and environmental concerns. Moreover, it is difficult to control many fungal diseases since mushroom itself is a fungus. This combination of circumstances makes controlling disease outbreaks in mushrooms more challenging. Disease control primarily depends on effective hygiene measures and sanitation. Knowledge of disease cycle and epedemiology of specific pathogens can provide growers with insight into how they are transmitted and spread. The control of growth environment by regulating the temperature and relative humidity has resulted in containing the spread of some air-borne fungal pathogens without the application of genetic resistance or fungicides. But the development of fungicide resistant strains and restriction on the use of pesticides has increased requirement for resistant cultivars (Fletcher, 1992).

Companies trying to develop disease resistant cultivars or pesticides cannot afford to undertake expensive research programs for these crops. There are thus, few cultivars that are bred specifically for disease resistance and available to the growers. *Agaricus bisporus* and *A. bitorquis* are valuable horticultural crops cultivated throughout the world with numerous strains and hybrids. There is not much work reported on comparing spawn strains and disease incidence and strains marketed that are disease resistant. Van Zaayen and Van der Pol-linton, (1977) studied different strains of *A. bitorqui* and found five strains that were less susceptible to false truffle disease (Diehliomyces microsporum). Peng, (1986) also noticed differences in disease susceptibility and found strains of A.bisporus that were naturally more resistant to Verticillium fungicola. He also found strain variation in response to bacterial blotch (Pseudomonas tolasii).

The occurrence of viral diseases in A. bisporus varies with strains but it may also be due to incompatibility between strains that prevents anastomosis which is a mode of transmission for the disease inoculum. Selecting and breeding of the naturally resistant strains was carried out. Challen and Elliott, (1987) bred novel strains of A.bisporus which were resistant to four fungicides. Research on disease control and diagnosis by PCR tests of major disease-causing organisms of mushrooms such as Verticillium, Trichoderma and La France disease virus has been performed in many labs (Chen et al., 1999). The existence of a viral complex associated with La France disease and characterization of three RNA viruses in A.bisporus have been identified by molecular methods. The genetic engineering of mushrooms with Bacillus subtilis bacteria that can control the growth of disease-causing fungi such as Trichoderma is an area of promise for future mushroom research.

Hybrid breeding

The hybrid mushroom strains introduced in the 1980s were well received and popular and have limited the choice of production characteristics and range of tolerance to environmental and cultural stresses. Cross breeding has been carried out since 1983 in mushrooms with the production of hybrids in Lentinula (Zhang and Molina, 1995), Pleurotus and Agaricus (Fritsche, 1983). Hybrid strains have not only given mushrooms that show resistance to diseases and pests but also reduced the dependence and risks of environmental and cultural stresses. Hybrids obtained by pairing monosporic cultures are cultivated to evaluate the production characteristics accompanied by RAPD and RFLP analysis. Mushroom breeding requires a large investment of capital and patience from both the breeder and grower. A number of specific industry standards have been adopted to grow strains available to the public for the last 30 years. For a new strain to be successful, some modifications in growing parameters are required for optimal growth. Traditionally growers had to adapt growing systems to accommodate cultural needs such as modifying flushing regimes, watering patterns and harvesting practices to optimize strain performance. Modifying cultural practices such as frequency and timing of irrigations are required for successful future strain development.

Marker Assisted Selection breeding (MAS) in mushrooms

Breeders now use DNA molecular markers to identify and select specific genes to locate superior traits. Repetitive DNA sequences are used to generate markers by PCR based techniques (Khush et al., 1991) as well as RAPD methods (Milad et al., 2011) Computer softwares are used to take the experimental data and generate genetic maps (Foulongne-Oriol et al., 2011). The use of genetic markers is easier for monogenic traits that segregate in distinct phenotypes in mushrooms. Previously white and off-white mushrooms strains dominated the industry and each strain had certain favourable and unfavourable traits associated with them.

The off white strains were better for mechanical harvesting but not conducive to canning due to discoloration whereas the white strains were less prolific but did not discolour on slicing and canning. Fritsche (1986) combined both strains in a breeding program to produce a hybrid with best qualities of each strain. By the introduction of DNA molecular markers, mushroom spawn producers can identify and fingerprint their strains providing greater patent protection and provide resources to expand breeding programs (Zhang et al., 2010a). Today MAS allows for a fast, easy and cheap method for the screening of cultures and selection in mushroom breeding (Kerrigan, 2000). The markers are associated with agronomic traits such as cap shape, color and quality (Miyazaki et al., 2010). MAS can identify the homokaryons with desirable traits without waiting for the fruiting stage, making it easier and faster than conventional breeding. The use of genetic markers for monogenic traits that segregate in distinct phenotypic characters such as cap colour have been used to introduce brown colour of a wild variety into commercial hybrids by introgression breeding. Also, the introduction of sporeless trait to commercial strains of Pleurotus was done by this method (Okuda et al., 2009). Complex traits such as yield, disease resistance and quality characteristics are usually inherited quantitatively. These traits are found to be associated with quantitative trait loci (QTL). Many QTL loci that are responsible for causing diseases such as bacterial blotch and dry bubble have been located. Intercrossing and backcrossing has given rise to hybrids that are less sensitive to these diseases. A large number of natural genetic variability already exists in wild populations of A. bisporus and it is not always necessary to modify or genetically engineer strains (Loftus et al., 2000). Thus, utilizing traditional breeding methods to explore traits from wild isolates can expand the genetic base of the cultivated mushrooms. But it is not clear which traits exist in the wild germplasm collection and if they can be successfully bred into commercial strains.

Genetic Linkage Map

A genetic linkage map is a partial representation of the genome that shows the relative position and distances between markers and genes along a chromosome in terms of genetic distance (in cM). Genetic linkage map can be developed by using a large number of genetic markers distributed within genome and has been analyzed for various organisms like plants (Maheswaran et al., 1997; Harushima et al., 1998; Hayashiet al., 2001), animal and fungi (Tzeng et al., 1992; Forche et al., 2000; Larraya et al., 2000) etc.

These linkage maps will be a suitable support for whole genome sequencing and for localization of genes of interest or quantitative trait loci (QTL) breeding and map-based cloning of genes of interest (Farman and Leong, 1998). By studying the segregation of genetic markers and the relevant trait in the population in which genetic markers are tightly linked to gene loci associated with that particular trait, we can easily select the population bearing desire trait without any phenotypic testing or phenotypic expression.

The generation of a genetic linkage map consists of three basic steps: 1) production of mapping population 2) identification of polymorphic molecular markers and their genotyping on mapping population and 3) statistical analysis of segregating data and correlation of genotypic data with phenotypic one for the construction of linkage map. The primary requirement for construction of linkage map is to have a segregating mapping population. Unlike the use of backcrossed and F2 basedmapping population in plant breeding, in mushroom breeding one can obtain individual post meiotic products directly from each generation to study the marker segregation. Since the principle of genetic linkage map is based on the analyses of the allelic reshuffling caused by meiosis, population derived from sexual reproduction are more appropriate for mapping (Foulongne-Oriol, 2012c). Therefore, F1homokaryotic progenies derived from meiotic spore are mainly used in fungal mapping studies (Foulongne-Oriol, 2012c). In mushroom breeding we can easily get these meiotic spores from each generation and single spore isolates are vegetatively propagated for the segregation analysis of genetic markers. Unfortunately, obtaining such progenies (i.e. homo- or heterothallism) is associated with specific constraint. For heterothallic species most of the SSIs are haploid and can directly be used for linkage analysis. Relevant genetic information present in one of the constituent parental lines of the wild isolate is introduced into both parental lines of the commercial strain via two backcrosses (BC1/BC2) and suitable genetic markers are used to select the traits and for commercial genome analysis. For secondarily homothallic species like *A. bisporus* var biosporus, this is quite problematic. Almost all the basidiospores of *A. bisporus* are binucleate and selffertile, containing two nuclei of opposite mating types. Only a low percentage of basidiospores are uninucleate that produce homothallic species which can be used as mapping progenies. Although there is no clear cut morphological distinction between homo- and heterokaryons, homokaryotic single spore isolates can be selected based on their slow growth in comparison to heterokaryotic single spore isolates. By apre-selection for lower growth, usually sufficient homokaryons are obtained (Fritsche, 1986; Kerrigan et al., 1992) and can be screened successful based on multi-locus test (Kerrigan et al., 1992). Othertypes of meiotic progenies have been occasionally used for linkage map construction in other fungi. For example, a genetic linkage map based on tetrad analysis has been reported for Lentinula edodes(Miyazakiet al., 2008). The second important step for genetic linkage map construction is to identify polymorphic markers that will be used for genotyping the entire mapping population.

Previously, isoenzymes based markers are commonly used for genotyping (Bowden and Royse, 1991). The use of these types of markers is, however, not very efficient. With the advancement of molecular technologies, various DNA based molecular markers are now commonly use for genotyping.The construction of the first generation of linkage maps was based on DNA markers like restriction fragment length polymorphism (RFLP) orrandom amplified polymorphic DNA (RAPD) (Kwan and Xu, 2002).

Second-generation linkage maps are now developed through advanced high-throughput genotyping techniques using AFLP, CAPS, STS, etc based markers. Among these, amplified fragment length polymorphism (AFLP) markers were advantageous since no sequence information was required fortheir development and a large number of markers could be rapidly generated to provide high resolution mapping (Vos et al., 1995). These markers are also more reproducible than RAPD markers. Thus, this marker has been used increasingly to develop genetic linkage map of mushroom fungi like Lentinula edodes (Terashima et al., 2002a,b), Pleurotus eryngii (Okuda et al., 2012) etc. However, their conversion into sequence specific PCR markers likeAFLP-converted markers (ACM), cleaved amplified polymorphic sequences (CAPS) and sequence characterized amplified regions (SCAR) etc. enhanced their usefulness in linkage mapping (Terashima et al., 2006;Okuda et al., 2009; Foulongne-Oriolet al., 2011a).Microsatellites, also knownas simple sequence repeats (SSR), are presently the most popular markers for genetic linkage mapping in mushroom fungi (Okuda et al., 2009). The final step ofthe constructionof a linkage mapis the analysis ofthe genotyping data to the finalized map. Two approaches to linkage analysis were followed. One is statistical analysis of the pair wise segregation of all genetic markers using chisquare (c 2) test to analyse for deviation from the expected Mendelian segregation ratio in the mapping population. That can be performed manually for a few markers, but it is notfeasible to determine linkages between large numbers of markers that are used to construct maps.The computer programs or software packages like Mapmaker/ EXP (Lander et al., 1987; Lincoln et al., 1993), MapManager QTX (Manly et al., 2001) and Join Map (Stam, 1993) are required for this purpose. Linkage between markers is usually calculated using odds ratios (i.e. the ratio of linkage versus no linkage). This ratio is more conveniently expressed as the logarithm of the ratio and is called a logarithm of odds (LOD) value or LOD score (Risch, 1992). LOD values of >3 are typically used to construct linkage maps. A LOD value of 3 between two markers indicates that linkage is 1000 times more likely (i.e. 1000: 1) than no linkage (null hypothesis). LOD values may be lower edinorderto detect a greater level of linkage or to place additional markers within maps constructed at higher LOD values. Linked markers should be grouped together into ‘linkage groups’ present within a chromosome (Collard et al., 2005). The ordering of the markers in the linkage groups can be carried out using MAPL. Referring to the road map analogy in linkage mapping, linkage groups represent roads and markers represent signs or landmarks.

Transgenic breeding

At present there are no transgenic mushroom strains available commercially but several research groups are working towards that direction with good progress. The use of recombinant DNA technique for creating transgenic mushrooms has created numerous possibilities and opportunities. Importing genes from unrelated sources is now possible and it is not restricted to searching for desirable genes only within the species.

Transformation techniques used with other filamentous fungi are being adapted for the mushroom (Van de Rhee et al., 1996a). Various techniques such as polyethylene glycol (Li et al., 2006), electroporation and particle bombardment have been used to incorporate DNA into protoplasts, mycelium or basidiospores. An efficient homologous site-directed integration of the transformation plasmid was done by isolating the tyrosinase genes responsible for mushroom browning from *Agaricus bispous* and introducing it in antisense orientation (Van de Rhee et al., 1996b). However, the multinuclear nature of fertile Agaricus mycelia presented a problem for stable transgenic mushrooms. Another gene isolated and identified in mushrooms was the mannitol-dehydrogenase (MtDH) gene and its 3- dimensional structure has now become available (Sassoon et al., 2001). Isolation of this gene can allow the production of mushrooms with altered mannitol profiles and ultimately yield strains with higher dry matter content or better pathogen resistance (Stoop and Mooibroek, 1998). The use of direct gene delivery techniques such as particle bombardment has also been carried out as an alternative method for genetic transformation in mushrooms (Li and Horgan, 1993). This process involves the bombardment of intact tissues with tungsten or gold particles coated with donor DNA and penetrating the recipient tissue. It has the advantage of being less laborious and often the problematic production and regeneration of protoplasts can be avoided. In many laboratories, attempts have been undertaken to introduce hygromycin-B resistance and other selectable markers by particle bombardment. However, this technique has not yet resulted in the selection of stable transformants or an applicable system.

Agrobacterium-based transformation

While many other transformation techniques are not very reliable or stable, the use of the soil bacterium, Agrobacterium tumefasciens for transformation reportedly yields stable transformants. The Agrobacterium system allows transformation of both homokaryons and heterokaryons and both karyotypes of a heteroharyon can be transferred simultaneously (Mikosch et al., 2001). The use of A.tumefasciens for efficient transformation and induction of its virulence gene with the plant hormone acetosyringone was first carried out in Agaricus bisporus by De Groot et al. (1998). But the limitations of this method were that it was not reproducible, showed false positives, low level of integration and DNA modification after integration. Also important is the necessity to include redundant DNA in the transformation vector that have no function in Agaricus but are needed for gene transfer only.

A successful Agrobacterium-mediated transformation was done by infecting the fruiting gill tissue with Agrobacterium strains carrying the gene construct of interest and use of a vector with homologous promoter (Chen et al., 2000). In most cases, the multinuclear nature of mushroom mycelia has restricted the advances of genetic breeding to yield strikingly improved features (Stoop and Mooibroek, 1999). Many transgenic manipulations with mushrooms will require the transfer of the gene to both parental lines so that the offsprings mimic the natural inheritance process by carrying duplicate copies of the gene.

In others, introduction of a single copy of the gene is sufficient and the resulting transgenic line may require further selection before introduction as commercial strains. Areas for transgenic breeding possibilities include importing cry genes from Bacillus thuringiensis for insect resistance and synthetase resistance from Agrobacterium for glyphosphate herbicide resistance.

Sequencing the mushroom genome

With the onset of the Genomics era, the nucleotide sequences of entire genomes have been determined (Yu et al., 2002; Buell et al. 2003). With regard to mushrooms, a proposal honoured by the US Department of Energy and carried out by "Joint Genome Institute" of America is underway for sequencing the genomes of the button mushroom, Agaricus bisporus (Kerrigan, 2009) and also the oyster mushroom, Pleurotus ostreatus (Pisabarro et al., 2006). The proposal draws up a document that substantiates the importance of sequencing the mushroom genome since mushrooms are now regarded as being very important for the environment. This is in view of the fact that mushrooms help in degradation of plant material into less harmful substances, are also used to remove heavy metals from waste flows and also play a role in production of biofuels (Thwaites et al., 2007). Sequencing work in Schizophyllum commune, a wood-degrading fungus was already completed this year (Ohm et al., 2010) by an international consortium of mushroom researchers and will now be available in public domain. After sequencing, investigations will continue into how sets of functionally related genes are organized and clustered into chromosomes. Also how these genes are distributed in related and non-related organisms will be observed. It will open up possibilities as to when and where a particular gene will become active and why. Applying functional genomics to look at gene expression, promoter, transcriptional and other regulator elements by using tools of gene silencing are other areas of research. For breeders using DNA markers, a more detailed and directed breeding of new mushroom strains can now be done. It opens the possibility of identifying regions of DNA associated to more complicated traits such as disease resistance and cellulose metabolism. Breeding advances made in other crops after mapping their genomes can also be realized in case of mushrooms.

Future scope

The use of genetic engineering in mushroom industry will be determined by economic factors related to necessity and resources. Due to funding constraints, mushrooms are very much lagging behind other crops in terms of advancement in molecular biotechnology. Public acceptance of genetically modified foods and greater consumption of mushrooms can increase research efforts (Snow and Palma, 1997). Traits controlled by single genes such as viral and insect resistance and resistance to fungal and bacterial pathogens and pesticides can be targeted first since they are simpler to tackle. With mapping of the mushroom genome and understanding of the functional genomics in mushrooms, complex traits such as yield, size, colour, shelf-life, and physical stress which are controlled by more than one gene can be undertaken in the future. Mushrooms can also be utilized as bioreactors in industry for the synthesis of

proteins and pharmaceutical compounds (Tang et al., 2007). A higher biomass of mushrooms can be produced on low-cost waste materials in a secure containment facility with the option of automation and mechanical harvesting (Lee et al., 2002). The proteins manufactured in mushrooms will also have higher specific biological activities in humans than those produced from plants (Lum and Min, 2011). The production of new mushroom cultivars with novel and improved traits will provide the industry with options for solving food problems (Zhang et al., 2010b) and increase the production efficiency. Improvement of tools available to the breeder, decoding mushroom genome and commercial pressure facing the industry can propel efforts for new strain development in the future.

11. Suggested Readings

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Kaul, T.N.2001. Biology and conservation of Mushrooms. Oxford and IBH Publishing Company.

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R.Singh and U.C.Singh 2205. Modern Mushroom Cultivation. Agrobios.

12. Assignment

1. What is Mushroom Biology?
2. Discuss in detail about the role of fungi as food procedures.
3. What do you mean by spawn? Differentiate b/w inoculation and incubation. Mention the required ingredients for the synthetic compost.
4. Write an essay on cultivation procedure of oyster mushroom.
5. Give the nutritional elements of mushrooms.
6. Name 2 poisonous mushrooms.
7. Give the classification of Agaricus bisporus.
8. What are the basic requirements for growing mushrooms?
9. What is spawn?
10. Give 2 methods of storage of mushrooms.
11. Name some foods prepared from mushrooms.
12. What do you mean by cost benefit ratio?
13. Where in India is the mushroom research centre located?

14. Define compost.
15. Describe the composting technology in mushroom production.
16. Write a note on marketing of mushrooms.
17. Describe the paddy straw mushroom cultivation in brief.
18. Give the medicinal benefits of mushrooms.
19. Give symptoms, etiology, epidemiology of blast disease of Rice, Citrus canker and Brown rot of Potatoes.
20. Write an essay on viral plant diseases, pathogens involved, their diagnosis and control.
21. Discuss the defense mechanism against plant pathogens in plants.
22. Describe the medicinal value of mushrooms.
23. Discuss the process of preparation of spawn.
24. Give a comparative account on 'short-term' and 'long-term' storage of mushroom.
25. What are the requirements used in mushroom bed preparation?
26. Make a chart about the nutritional value of mushroom in brief.
27. What do you mean by the Fruit body of mushroom? Describe the morphology of it.
28. Describe the cultivation technique of Agaricus bisporus. What are the precautions to be adopted in this cultivation process? (8+2)
29. Give the scientific names of paddy straw mushroom, milky mushroom and oyster mushroom. Give a general account on poisonous mushrooms. (3+7)
30. Describe the cost and benefit ratio of small scale cultivation of oyster mushroom in polythene bags. (10)
31. Discuss the nutritional value of mushrooms. Name two mushrooms producing antibiotics. (8+2)
32. What do you mean by long term storage? Explain how you can preserve the mushroom by drying process. (2+8)
33. Name the foods prepared from mushrooms. Discuss the importance of Regional and National Research Institutes of mushroom cultivation.
34. Mushroom Morphology: Different parts of a typical Mushroom and variations in mushroom morphology, key to morphometric identifications of Mushrooms. Identification of Edible and Poisonous mushrooms.
35. Mushroom growth and reproduction with process of perennation.
36. Studies of Nutraceutical properties of Mushrooms.
37. Spawn preparation and Cultivation techniques of Oyster mushroom / Paddy straw mushroom.
