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CONSENSUS DOCUMENT ON THE BIOLOGY OF PLEUROTUS SPP. (OYSTER MUSHROOM)

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Series on Harmonisation of Regulatory Oversight in Biotechnology

No. 34

Consensus Document on the Biology of *Pleurotus* spp. (Oyster Mushroom)

Environment Directorate

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FOREWORD

The OECD's Working[1] Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of ***consensus documents*** which are mutually acceptable among Member countries. These consensus documents contain information for use during the regulatory assessment of a particular product. In the area of crop biosafety, consensus documents are being published on the biology of certain crop species, on selected traits that may be introduced into crop species, and on biosafety issues arising from certain general types of modifications made to crops.

This consensus document addresses the biology of *Preaurotus* spp. (L.) (Oyster Mushroom). Included are descriptions of general information; taxonomy and natural distribution; agronomic practices; lifecycle and growth; sexual reproduction and grosses; genetics of *P. ostreatus*; and pests and diseases.

Korea served as the lead country in the preparation of this document. The document has been revised on a number of occasions based on the input from other member countries. It is intended for use by regulatory authorities and others who have responsibility for assessments of transgenic organisms proposed for commercialisation, and by those who are actively involved with genetic improvement and intensive management of the genus.

At the 15th meeting of the Working Group (held 16-18 June 2004) it was agreed that the document be forwarded to the Joint Meeting of OECD's Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology, which agreed that this document be declassified.

[1] In August 1998, following a decision by OECD Council to rationalise the names of Committees and Working Groups across the OECD, the name of the "Expert Group on Harmonization of Regulatory Oversight in Biotechnology" became the "Working Group on Harmonization of Regulatory Oversight in Biotechnology."

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PREAMBLE

OECD member countries are now approving the commercialisation and marketing of agricultural and industrial products of modern biotechnology. They had previously therefore identified the need for harmonisation of regulatory approaches to the biosafety assessment of these products, in order to avoid unnecessary trade barriers.

In 1993, **Commercialisation of Agricultural Products Derived through Modern Biotechnology** was instituted as a joint project of the OECD's Environmental Policy Committee and Committee on Agriculture. The objective of this project is to assist countries in their regulatory oversight of agricultural products derived through modern biotechnology - specifically in their efforts to ensure safety, to make oversight policies more transparent and efficient, and to facilitate trade. The project is focused on the review of national policies, with respect to regulatory oversight that will affect the movement of these products into the marketplace.

The first step in this project was to carry out a survey concentrating on national policies with regard to regulatory oversight of these products. Data requirements for products produced through modern biotechnology, and mechanisms for data assessment, were also surveyed. The results were published in *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results* (OECD l995a).

Subsequently, an OECD Workshop was held in June 1994 in Washington, D.C, with the aims of improving awareness and understanding of the various systems of regulatory oversight developed for agricultural products of biotechnology, identifying similarities and differences in various approaches, and identifying the most appropriate role for the OECD in further work towards harmonisation of these approaches. Approximately 80 experts in the areas of environmental biosafety, food safety and varietal seed certification, representing 16 OECD countries, eight non-member countries, the European Commission and several international organisations, participated in the Workshop. *The Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology* was also published by the OECD in 1995 (OECD, 1995b).

As a next step towards harmonisation, the Working Group on Harmonisation of Regulatory Oversight in Biotechnology instituted the development of **consensus documents**, which are **mutually acceptable** among member countries. The goal is to identify common elements in the safety assessment of a new plant variety developed through modern biotechnology, to encourage information sharing and prevent duplication of effort among countries. These common elements fall into two general categories: the first being the biology of the host species, and the second, the gene product.

Safety issues that could give rise to a safety concern are identified in the consensus documents on the biology of a specific crop and include the potential for gene transfer, weediness, trait effects, genetic and phenotypic variability, biological vector effects and genetic material from pathogens (OECD, 1993a). They make no attempt to be definitive in this respect, however, as the many different environments in which the crop species may be grown are not considered individually.

This document is a "snap-shot" of current information that may be relevant in a regulatory risk assessment. It is meant to be useful not only to regulatory officials, as a general guide and reference source, but also to industry, scientists and others carrying out research.

To ensure that scientific and technical developments are taken into account, OECD countries have agreed that consensus documents will be updated regularly. Additional areas relevant to the subject of each consensus document will be considered at the time of updating.

Users of this document are therefore invited to provide the OECD with relevant new scientific and technical information, and to make proposals concerning additional areas that might be considered in the future. ***A short, pre-addressed questionnaire is included at the end of this document. The information requested should be sent to the OECD at one of the addresses shown.***

SECTION I GENERAL INFORMATION

1. Oyster mushroom is regarded as one of the commercially important edible mushrooms throughout the world. It consists of a number of different species including *Pleurotus ostreatus*, *Pleurotus sajor-caju, Pleurotus cystidiosus, Pleurotus cornucopiae, Pleurotus pulmonarius, Pleurotus tuber-regium, Pleurotus citrinopileatus* and *Pleurotus flabellatus*. They thrive on most of all hardwoods, wood by-products such as sawdust, paper, pulp sludge, all the cereal straws, corn and corn cobs, coffee residues such as coffee grounds, hulls, stalks, and leaves, banana fronds, and waste cotton often enclosed by plastic bags and bottles. The oyster mushroom is the second most important mushroom in production in the world, accounting for 25% of total world production of cultivated mushrooms. Oyster mushroom is grown world-wide, and China is the major producer. *P. ostreatus* was first cultivated in the USA in 1900 and several other species of the oyster mushroom such as *Pleurotus sajor-caju* were initially cultivated in India after the late of 1940s. The oyster mushroom has been regarded as one of the most profitable cash crops in Korea, accounting for 65% of total domestic mushroom production.

2. This consensus document which describes the main aspects of the biology of Oyster Mushroom was prepared by the lead country, Korea, to provide background information for science-based decision making in consideration of future release of transgenic mushrooms into the environment. Included are description of the taxonomy and natural habitat of the genus *Pleurotus* and morphological description of *Pleurotus ostreatus*, the agronomic practices, the life cycle and sexual reproduction, and genetics. *Pleurotus ostreatus* is the main focus of this document, but other species of the oyster mushroom are also covered in this consensus document.

SECTION II TAXONOMY AND NATURAL DISTRIBUTION

2.1 Taxonomy and nomenclature

3. Oyster mushroom, *Pleurotus* spp., belonging to the genus *Pleurotus* (Quel.) Fr., tribe Lentineae Fayod, family *Polyporaceae* (Fr.) Fr., is widely distributed throughout the Northern Hemisphere, such as Europe, North Africa, Asia and North America (Singer, 1986). To date, approximately as many as 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently although some of these are considered identical to previously recognised species. The genus *Pleurotus,* which was first recommended as a tribe within genus *Agaricus* by Fries (1821), was proposed as a genus by Quelet (1886). Three genera of this group, *Pleurotus*, *Lentinus*, and *Panus,* were possible to be separated according to their anatomic characters of the sterile tissues of the hymenophores as being homogeneous taxonomic groups. Hilber (1982) recommended that crossing of monospore cultures is a valuable basis for *Pleurotus* studies. *Pleurotus ostreatus* (Jacq: Fr.) Kummer is the most cultivated species among the oyster mushroom and the type species of the genus *Pleurotus*.

4. Recently, the majority of mycologists have followed the proposition made by Singer (1986) which divides the genus *Pleurotus* into six sections: Sect. *Lepiotarii* (Fr.) Pilat, Sect. *Calyptrati* Sing., Sect. *Pleurotus* Sing., Sect. *Coremiopleurotus* (Hilber), Sect. *Lentodiellum* (Murr.) Sing. and Sect. *Tuberegium* Sing.. *Pleurotus ostreatus* was placed in the Sect. *Pleurotus* based on the absence of veil and with the monomitic hyphal system.

2.2 Morphological description

5. Species identification within the genus *Pleurotus* is difficult because of the morphological similarities and possible environmental effects. Mating compatibility studies have demonstrated the existence of eleven discrete intersterility groups in *Pleurotus* to distinguish one species from the others. *P. columbinus, P. florida, P. salignus,* and *P. spodoleucus* are the synonyms or subspecies taxa for the species of *P. ostreatus*.

2.2.1 Macroscopic features of Pleurotus ostreatus *(Jacq.: Fr.) Kummer*

- Pileus: 40-250mm broad, oyster-shape, spatulate to lingulate when young, convex then later becoming conchate to flabellate, surface smooth, grey lilac, violet-brown to lilac blackish when young later becoming cream-beige, but usually very variable in colour, margin smooth when young, later somewhat undulating and striate. For descriptions of macroscopic features of fruiting bodies, descriptions and illustrations of microscopic characters, and distribution of this taxa, references of Breitenbach and Kranzlin (1991), Donk (1962), Imazeki and Hongo (1987), and Moser (1983) were referred to respectively. Colour names were taken from Kornerup & Wanscher (1983).

- Context: white to grey-white, thin to thick, fleshy, radially fibrous, odour fungoid, taste mild.

- Lamellae: long-decurrent, crowded, whitish to cream or pale greyish, edge smooth, later somewhat undulating, lamellulae 1- or 3-tiers.

- Stipe: 10-20×10-25mm, rudimentary, usually lateral, severa concrescent, surface longitudinally striate, whitish villose-pilose, context solid.

Figure 1. Macroscopic feature of *P. ostreatus*

2.2.2 Microscopic features of Pleurotus ostreatus *(Jacq.: Fr.) Kummer*

- Spores: 6.5-9×2.8-3.5μm, cylindric to cylindric-ellipsoidal, smooth, hyaline, with vacuoles.
- Spore print dingy grey or pale lilac grey.
- Basidia: 23.6-27×5-7.5μm, slenderly clavate with 4-spored and a basal clamp connection.
- Hymenophoral trama: regular to irregular, trama monomitic.
- Cystidia: absent or cystioid, rarely seen.
- Pileipellis: composed of irregular, densely interwoven, flexuous and branched hyphae, usually 2-4μm across, with brown pigment, somewhat gelatinized, septa with clamp connections.
- Habit & Habitat :Usually gregarious, clustered on the dead hardwood in park and both side of road, rarely on conifers, Suwon, Pochon, Cholwon, Whasong in Kyunggi Province , Gyeryong-san, Chilgap-san in Chungnam Province, Chiak-san in Kangwon Province, Kangjin in Chonnam Province and Hanla-san in Jeju Province in Korea. Spring to autumn.
- Distribution: Europe, America, North Africa, and Asia

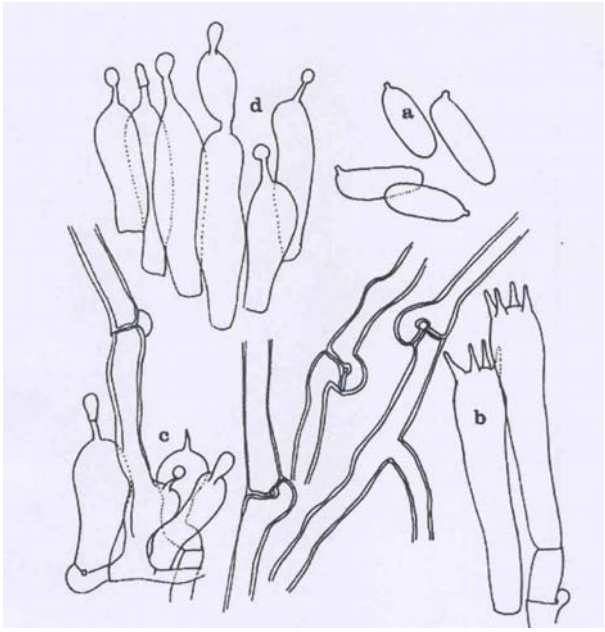

Figure 2. Microscopic feature of *P. ostreatus* (a: spores, b: basidia, c. cheilocystidia, d: pleurocystidia)

2.3 Natural habitat

6. The geographic distribution of the oyster mushroom varies according to its species. For example, *P. pulmonarius* and *P. cystidiosus* are known to be distributed in the tropical and subtropical region, while *P. eryngii* are found in southern Europe, North Africa and central Asia. It has many subspecies and similar taxa such as *P. fuscus* var. *ferulae* from China. *P. ostreatus* is widespread in the temperate zones such as Korea and Japan because it forms fruit-bodies at relatively low temperature compared to other *Pleurotus* species. The geographic distribution of *P. tuber-regium* includes most of equatorial Africa, India, Sri Lanka, Southeast Asia, North Australia, and the southern Pacific countries as well (Table 1).

7. Commonly grown on broad-leaf hardwoods in the spring and fall, especially cottonwoods, oaks, alders, maples, aspens, ash, beech, birch, elm, willows and poplars are favoured natural habitat for oyster mushroom. Although seen on dying trees, *P. ostreatus* is thought to be primarily a saprophyte, but behaves as a facultative parasite at the earliest opportunity. Occasionally, it grows on composting bales of straw and in Mexico, on the pulp residues from coffee production. The most abundant fruiting of this species is in low valley riparian habitats (Stamets, 1993).

Table 1. Classification of the genus *Pleurotus* and its geographical distribution (Singer, 1986)

Sect.	Species	Geographical Distribution
Lepiotarii	*P.dryinus* (Pers: Fr.) Kummer	Japan, USA, Swiss, Germany, Sri Lanka, Portugal
	P.dryinus (Pers: Fr.) Kummer var. *tephrotrichus* (Fr: Secr.) Gill.	
	P.rickii Bres.	
	P.lindquistii Sing.	
Calyptrati	*P.calyptratus* (Lindb.) Sacc.	China
Pleurotus	*P.ostreatus* (Jacqu: Fr.) Kummer	Korea, China, Japan, USA, UK, Switzerland, Netherlands, Germany, Sri-Lanka, Portugal, Slovakia
Pleurotus columbinus Quel.	*P.ostreatus* (Jacqu: Fr.) Kummer var. *columbinus* (Quel. Apud Bres.) Quel.	Japan, USA, Germany, Slovakia
	P.pulmonarius (Fr.) Quel. : Fr.	Korea, China, Japan, Germany, Portugal, New Zealand
	P.citrinopileatus Sing.	Korea, China, Japan
	P.ostreatoroseus Sing.	
	P.opuntiae (Dur. & Lev.) Sacc.	
	P.macropus Bagl.	
	P.laciniatocrenatus (Speg.) Speg.	
	P.euosmus (Berk.) Sacc.	
	P.phellodendri (Sing.) Sing.	
	P.araucariicola Sing.	
	P.pantoleucus (Fr.) Sacc.	
	P.prometheus (Berk. & Curt.) Sacc.	
	P.yuccae Maire	
	P.convivarum Dunal & Delile	
	P.parthenopejus (Comes) Sacc.	
	P.salignus (Schrad.) Quel.	
	P.importatus Henn.	
	P.gemmellari (Inz.) Sacc.	
Coremiopleurotus	*Pleurotus cystidiosus* O.K. Miller	
	P.abalonus Han, Chen & Cheng	
Lentodiellum	*Panus concavus* Berk. *Pleurotus concanvus*(Berk.)Sing.	China, Japan
	P.levis (Berk. & Curt.) Sing.	
	P.strigosus (Berk. & Curt.) Sing.	
	P.fockei (Miquel) Sing.	
	P.calyx (Speg.) Sing.	UK
	P.sajor-caju (Fr.) Sing.	China, USA, Sri-Lanka, Australia
	P.squarrulosus (Mont.) Sing. Ex Pegler	USA, Sri-Lanka
	P.floridanus Sing.	UK
	P.subtilis (Berk.) Sing.	
Tuberregium	*P.tuber-regium* (Rumph.Fr.) Sing.	China, Sri-Lanka, Australia

SECTION III AGRONOMIC PRACTICES

8. *Pleurotus* spp. is generally referred as the oyster mushroom because the pileus or cap is shell-like, spatulate and the stipe is eccentric or lateral. *Pleurotus ostreatus* (Jacq.: Fr.) Kummer is one of the best known species among the oyster mushrooms. Other commonly cultivated species include *P. sajor-caju, P. cystidiosus, P. eryngii* and *P.tuberregium* (Chang and Miles, 1989). Various species of these wood-rotting fungi are found all over the world and this mushroom is especially appreciated in Asia for its edibility.

9. The oyster mushroom has many advantages as a cultivated mushroom: rapid mycelial growth, high ability for saprophytic colonisation, simple and inexpensive cultivation techniques and several kinds of species available for cultivation under different climatic conditions. In addition, oyster mushroom is low in calories, sodium, fat and cholesterol, while rich in protein, carbohydrate, fibre, vitamins and minerals. These nutritional properties make this mushroom as a very good dietary food. In addition, consumption of oyster mushroom has positive effects on the general human health because of a number of special substances (Kues and Liu, 2000). Owing to these attributes during recent years, the production and consumption of this mushroom has increased tremendously and is ranked second to the button mushroom. The high ability to degrade the lignin-cellulose of *Pleurotus* spp. was also used in eliminating of the xenobiotic pollutants such as pentachlorophenol (PCP), dioxin, polycyclic aromatic hydrocarbons (PAHs). This suggests the possibility of new usage of this mushroom for environmental bioremediation (Kubatova *et al*., 2001; Hirano *et al*., 2000).

10. Despite its usefulness as food and bioconversion materials, three notable disadvantages persist in the cultivation of oyster mushroom. First, the oyster mushroom is quick to spoil and so is presentable to the market for only a few days. Secondly, the spore load generated within the growing room can become a potential health hazard to workers thus pickers can become allergic to the spore. Sporeless strains, which tend to have short gills and are thicker fleshed, prolonging storage, are highly sought after by oyster mushroom growers. Thirdly, the growers must wage a constant battle against the intrusion of mushroom flies. Oyster mushroom attracts Sciarid and Phorid flies to a far greater degree than any other group of mushrooms.

Table 2. Production of oyster mushrooms under commercial cultivation in some countries

(Chang, 1993; Kues and Liu, 2000)

(Unit: Mt)

Oyster mushroom production			
Countries	**1991**	**1994**	**1998**
China	800,000	654,000	
Japan	33,475	20,441	
USA	695	900	
Indonesia	15,000	1,000	
Thailand	7,000	15,000	
Spain		100	
Netherlands		150	
Italy		1,500	
UK		150	
Germany		1,000	
France		2,000	
South Korea	51,782	72,810	75,684
Taiwan	3,500		
India	600		
Hungary	2,500		
Total	914,552	696,241	

SECTION IV LIFE CYCLE AND GROWTH

4.1 Life cycle of Pleurotus ostreatus

11. The major events in the life cycle of *P. ostreatus* could be described as follows (Fig. 3). A single basidiospore germinates to be a mass of homokaryotic mycelium, each cell of which contains a single haploid nucleus. The homokaryotic mycelia continue to grow until the hypha fuse with the other hyphae which have compatible mating type. After fusion between compatible homokaryotic hyphae, reciprocal nuclear migration occurs and a heterokaryotic mycelium is formed. The subsequent growth involves the synchronous division of the two nuclei in each compartment and their regular distribution as nuclear pair throughout the mycelium via clamp connections. Heterokaryotized mycelia with enough mycelia mass and appropriate environmental stimuli (cooling 10 - 21℃, relative humidity 85-90%, and light requirement 1000-2000lux, CO_2 < 1000 ppm) can form the fruit bodies. During fruit body formation, nuclear fusion and meiosis occur only in the specialised basidia. Haploid nuclei migrate into a tetrad of basidiospores, external to the basidium. Each basidium has commonly four monokaryotic basidiospores. Occasionally five or more have been observed. These spores germinate into homokaryotic hyphae

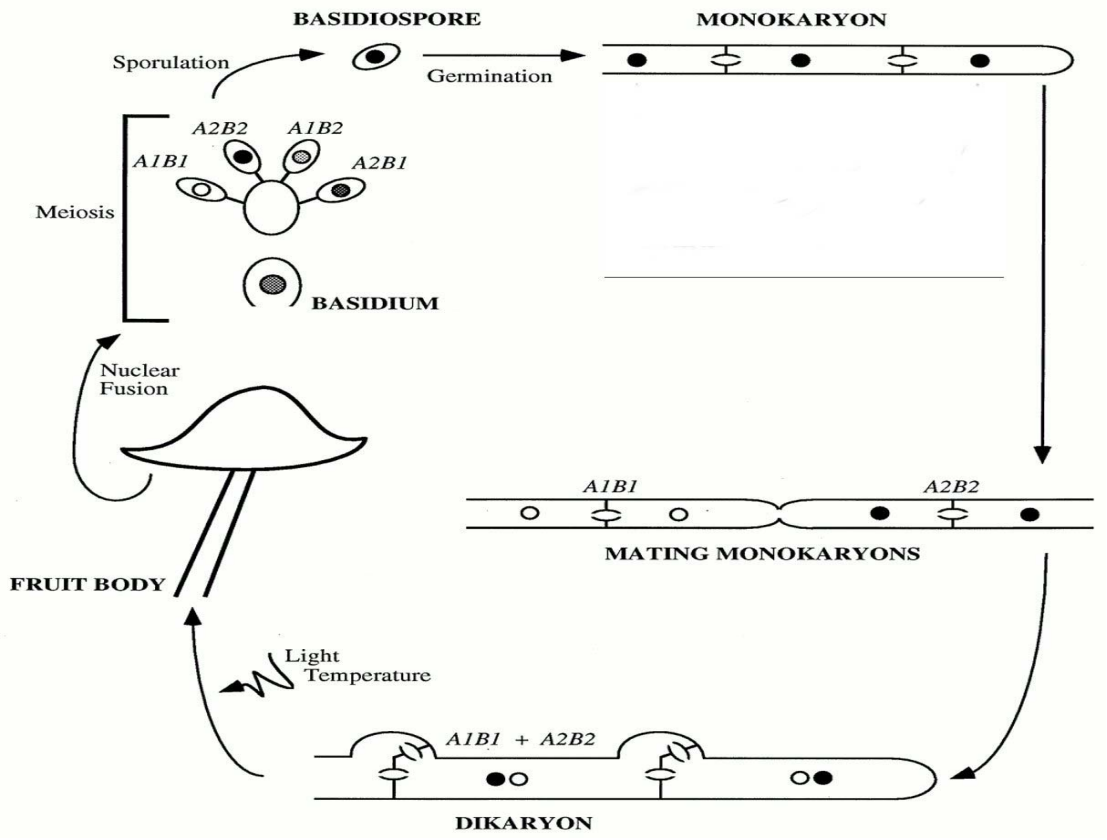

Fig. 3. Life cycle of the *Pleurotus ostreatus* (Casselton, 1995)

4.2 Requirement for mycelial growth

12. The carbon sources suitable for mycelial growth are starch, glucose, fructose, maltose, mannose, sucrose, pectin, cellulose and lignin. Ethanol is also a source of carbon for mycelial growth; however, citrate, oxalate and other organic acids are not beneficial to the growth of the mycelium. The nitrogenous sources utilised by *Pleurotus* spp. are peptone, corn steep liquor, soybean cake powder, yeast powder, ammonium sulfate, asparagine, serine, alanine and glycine. The utilisation of urea is rather poor.

13. The optimal temperatures for growth of the mycelium are around 25-28 °C and the range of pH is about 5.5 to 6.5. The tolerance of mycelia for CO_2 is rather strong. The mycelia of *Pleurotus* spp. can still grow flourishingly at the carbon dioxide concentration of 15 to 20%. Only when the concentration of CO_2 is raised to 30% does the growth of mycelia rapidly decrease (Chang and Miles, 1989).

4.3 Requirement for fruit body formation

14. For fruiting body formation, CO_2, light and temperature is key environmental factors. When the CO_2 concentration in the mushroom house or growing bags is higher than 600 ppm (0.06%), the stipe elongates and the growth of the caps will be prevented. The requirements for light are different for the various stages of growth. The growth of mycelium does not need any light and cultivation of the oyster mushroom in a dark place is better than in a bright place. The formation of primordia and the growth of fruiting bodies require light. The former requires light of 200 lux intensity for over 12 hrs. The growth of the fruiting body requires light of 50 to 500 lux intensity. The colour of the caps is closely related to the intensity of light, and if it is low, then the colour will be pale. The optimal temperatures for the development of fruiting bodies can range from 10 to 18 °C (Chang and Miles, 1989). Growers can choose a suitable strain for their own natural environment. Each *Pleurotus* species needs different environmental conditions for fruitbody development as illustrated in Table 3 (Stamets, 1993; Kang, 2004).

Table 3. Environmental parameters for fruiting of oyster mushroom

Species	Temp. (°C)	Relative humidity (%)	CO_2 (ppm)	Light (lux)
P. pulmonarius	21-29	90-95	<1,000	500-1,000
P. cystidiosus	21-27	85-90	<2,000	500-1,000
P. djamor	20-30	85-90	500-1,500	750-1,500
P. eryngii	15-21	85-90	<2,000	500-1,000
P. euosmus	21-27	90-95	<1,000	750-1,500
P. ostreatus	10-21	85-90	<1,000	1,000-1,500
P. pulunonarius	18-24	85-90	400-800	1,000-1,500
P.tuberregium	30-35	85-90	<2,000	

SECTION V SEXUAL REPRODUCTION AND CROSSES

5.1 Mating system and gene flow potential

15. *P. ostreatus* is heterothallic (self-sterile) and sexual reproduction is governed by the mating type genes. Mating type genes prevent mating between genetically identical cells. *P. ostreatus* has a bifactorial tetrapolar incompatibility mating systems which has two unlinked mating type factors designated A and B (Eugenio and Anderson, 1968). Factor A controls nuclear pairing, clamp cell formation, coordinate cell division and clamp cell septation whereas factor B is responsible for the control of nuclear migration, septa dissolution and clamp cell fusion. Two monokaryotic mycelia are compatible if they have different alleles at both loci. Multiple allelism for mating type genes was first noted by Terakawa (1957) and amply demonstrated in a sample of over 20 dikaryons collected from nature by Eugenio and Anderson (1968). The latter investigators estimated that there are a total number of 63 A types and 190 B types in the natural world-wide population of this species. Because of this multiple allelism of mating type, the out breeding potential is estimated close to 100% in nature and the inbreeding potential can be as low as 25%.

16. The spore of *P. ostreatus* usually gets off the gill and away from the mushroom cap. Once the spores have cleared the bottom of the cap, air currents carry them away. When the spores are a few millimetres away from the cap they can be picked up by the faster winds and carried considerable distances thus enabling them to cross with the same species. However, no data are available regarding how far they can travel into the open air. Due to its nature of heterothallism, the spores of *P. ostreatus* behave like open pollinated crops. Therefore, appropriate measures should be taken to avoid unwanted gene flow when *P. ostreatus* is cultivated.

5.2 Interspecific cross

17. Interspecific cross was reported among *P. ostreatus, P. florida, P. columbinus* and *P. sapidus* (Peberdy *et al*, 1993). These species are ambiguous in specification of *Pleurotus*. Some scientists said that the species are the same species. There are several reports concerning interspecific crosses involving *Pleurotus* species based on protoplast fusion (Yoo and Cha, 1993).

5.3 Monokaryotic fruiting

18. Monokaryotic fruiting has been reported on more than 34 species in basidiomycetes (Stahl and Esser, 1976). *P. ostreatus* has also been found the monokaryotic fruiting (Kim, 2000). Esser *et al.* (1979) proposed that two genes, fi1+ and fi2+, are responsible for initiation of fruiting, and Kim (2000) demonstrated the mating type switching in the homokaryotic fruiting stains.

SECTION VI GENETICS OF *P. OSTREATUS*

6.1 Genome size

19. The study of genome organisation in *P. ostreatus* has been hampered by the small size of fungal chromosomes. Different authors reported different chromosome numbers and genome sizes for this species (Sagawa and Nagata, 1992, Peberdy *et al*., 1993, Chiu, 1996). Recently, by using Pulse Field Gel Electrophoresis and linkage mapping, eleven chromosomes were resolved per haploid genome which added up to a total genomic size of 35Mb in average as shown in Table 4. Each chromosome has size from 1.4Mb to 4.7Mb. The use of chromosome-specific single-copy probes resolved the ambiguities caused by chromosome co-migration (Larraya *et al*., 2000).

Table 4. Estimated chromosome size of *Pleurotus* spp. (Perberdy *et al*., 1993)

Chromosome	***P.ostreatus***	***P.florida***	***P.sajor-caju***	***P.pulmonarius***	***P.columbinus***	***P.sapidus***
I	4.70	5.1	5.70	5.70	5.70	5.50
II	4.35	4.7	5.10	5.30	4.70	4.60
III	4.55	4.1	3.10	5.10	4.30	4.30
IV	3.55	3.8	2.50	4.50	3.60	3.80
V	3.45	2.7	2.00	3.10	3.10	3.30
VI	3.10	2.2	1.60	2.70	2.50	2.30
VII	3.15	1.6	-	2.00	1.80	1.40
VIII	2.95	1.1	-	1.60	1.40	0.90
IX	2.10	0.7	-	-	-	-
X	1.75	-	-	-	-	-
XI	1.45	-	-	-	-	-
Total genome size (Mb)	**35.1**	**26.00**	**20.00**	**30.00**	**27.10**	**26.10**

6.2 Linkage map

20. Using 80 monokaryons derived from one commercial strain, segregation of 196 markers was studied. The linkage analysis allowed to associate the markers into 11 linkage groups which span a total of 1000.7 cM. Also this linkage map was used for QTL mapping associated with growth rate of monokaryon and dikaryon (Larraya *et al*., 2000).

6.3 Transformation

21. Although commercial transgenic mushroom strains are not available, molecular breeding studies of the mushrooms have been carried out world-wide. The Netherlands, the United Kingdom, Japan, Spain and the United States are among the leading countries in mushroom biotechnology including the development of transformation systems. Possible target genes for transformation include: senescence genes to improve mushroom quality; substrate utilisation genes to enhance yields; and developmental genes to control mushroom fruiting. There are numerous potential pest and disease resistance targets, including

genes involved in response to fungal pathogens, toxicity to insects and natural pest resistance. In addition, transformations with mating type genes that regulate inter-strain compatibility can alter breeding behaviour.

22. Transformation of *P. ostreatus* was firstly reported by Peng *et al*. (1992). Peng *et al*. transformed the homokaryotic strain using the protoplast and electroporation. They used the pAN7-1 vector which is a common vector used in ascomycetes and has a hygromycin selection marker. Yanai *et al*. (1996) reported the transformation using bialaphos selection marker. Kim *et al.* (1999) developed the transformation system using uracil auxotrophic mutant and the corresponding gene. Honda *et al.* (2000) developed the carboxin resistance gene using *in vitro* mutagenesis of iron-sulfur protein subunit of succinate dehydrogenase gene. Currently, Irie *et al.* (2001) reported the genetically modified *P. ostreatus* strain with an expression system for recombinant genes.

6.4 Conservation of genetic resources

23. Storage at ultra low temperatures has proved to be the most successful method for the prevention of degenerative changes in filamentous fungi. Therefore, for long term storage, liquid nitrogen storage is generally used for *P. ostreatus*. International Mycological Institute (IMI) reported the successful storage of *P. ostreatus* mycelia in liquid nitrogen for 23 years (Smith, 1993).

SECTION VII PESTS AND DISEASES

24. Although the mushroom itself is a fungus, it can in turn be affected by a range of fungal pathogens, bacterial diseases, viral diseases and insect pests listed as follows:

7.1 Fungal pathogens

Pleurotus ostreatus

Bolbitius coprophilous (Peck) Hongo

Chrysonilia sitophila (Mont) Arx: Red Bread Mould

Cladobotryum apiculatum (Tubaki) W. Gams & Hooz.: Brown Spot, White Soft Rot

Cladobotryum dendroides (Bulliard: Merat) W. Gams & Hoozemans: Cobweb, Cobweb Disease, Cobweb Mould, Mildew, Soft Decay, Soft Mildew

Cladobotryum variospermum (Link) Hughes: Cobweb

Cladosporium spp.

Fusarium equiseti (Corda) Saccardo(1886)

Fusarium pallidoroseum (Cooke) Saccardo (1886): Pleurotus Wilt

Fusarium spp.

Gibberella fujikuroi (Sawada) Ito (1931): Pleurotus Wilt

Gibberella zeae (Schweinitz) Petch (1936): Pleurotus Wilt

Gilmaniella humicola G.L. Barron

Mucor spp.

Penicillium spp.: Blue-Green Mould, Green Mould

Rhizomucor spp.

Trichoderma hamatum (Bonord) Bain: Green Mould, Grune Schimmel

Trichoderma spp.: Green Mould, Grune Schimmel

Verticillium fungicola (Preuss) Hassebrauk: Dry Bubble, Fungus Spot, Lamole, Verticillium Brown Spot, Verticillium Disease

Verticillium spp.

Pleurotus

Aphanocladium album (Preuss) W.Gams

Arthrobotrys pleuroti

Calcarisporium spp.: Cobweb Disease

Cephalotrichum sp.: Black Mould

Chaetomium spp.

Cladobotryum spp.

Coprinus spp.: Ink Cap, Inky Cap

Dactylium spp.

Doratomyces sp.: Black Mould

Mucoraceae spp.

Nigrospora spp.

Peziza spp.

Trichurus spp.: Black Mould

7.2 Bacterial disease

Pleurotus ostreatus

Pseudomonas aeruginosa (Schroeter 1872) Migula 1900: Brown Blotch, Mummy Disease

Pseudomonas agarici Young (1970): Brown Blotch, Drippy Gill, Yellow Blotch

Pseudomonas fluorescens Migula 1895 Biovar: Brown Blotch

Pseudomonas fluorescens Migula 1895 Biotype G (=Biovar V): Bacterial Mummy Disease

Pseudomonas gingeri Preece & Wong 1982 (not validly published): Bacterial Blotch, Ginger Blotch

Pseudomonas tolaasii Paine 1919: Bacterial Blotch, Bacterial Brown Blotch, Brown Blotch, Mushroom Blotch

Pleurotus

Pseudomonas spp.: Pseudomonad

7.3 Insect pests

Pleurotus ostreatus

Cyllodes biplagiatus Le Conte: Beetle

Hexarthrius davisoni Waterhouse: Beetle

Hypogastrura (Ceratophysella) *armata* (Nicolet, 1842): Mushroom Springtail, 'Gunpowder Mite'

Leiomyza laevigata Meigen: Fly

Leucophenga maculata (Dufour): Vinegar Fly

Lycoriella auripila (Winnertz): Mushroom Sciarid, Black Fungus Gnat

Lycoriella bispinalis Yang and Zhang: Mushroom Sciarid

Lycoriella epleuroti Yang and Zhang: Mushroom Sciarid

Lycoriella jipleuroti Yang and Zhang: Mushroom Sciarid

Lycoriella jingpleuroti Yang and Zhang: Mushroom Sciarid
Lycoriella pleuroti Yang and Zhang: Mushroom Sciarid
Lycoriella yunpleuroti Yang and Zhang: Mushroom Sciarid
Lycoriella spp.: Black Fungus Gnat
Megaselia flavinervis (Malloch): Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Megaselia rubescens (Wood): Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Megaselia spp.: Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Monoclona sp.: Fungus Gnats
Mycetophila oculus Walker: Fungus Gnat
Mycophila spp.: Mushroom Yellow Cecid Fly, Gall Midge
Mycophila speyeri (Barnes): Mushroom Yellow Cecid Fly, Gall Midge
Oxyporus (Pseudoxyporus) lateralis Gravenhorst 1802: Rove Beetle
Oxyporus (Oxyporus) rufipennis Leconte 1863: Rove Beetle
Oxyporus stygicus Say 1834: Rove Beetle
Oxyporus (Oxyporus) vittatus vittatus Gravenhorst 1802: Rove Beetle
Pheidole nodus Smith: Ant
Phorodonta flavipes Meigen: Black Fungus Gnat
Rhymosia domestica Meigen: Fungus Gnat
Scaphisoma convexum Say: Beetle
Scaphisoma stephani Leschen and Lobl, 1990: Beetle
Sciara fenestralis Zetterstedt: Fungus Gnat
Silvicola cinctus (Fabricius, 1787):Fly

Pleurotus

Bleptina sp.: Moth, Cutworms, Armyworms
Cyllodes ater (Herbst, 1792): Beetle
Cyllodes literatus (Reitter): Beetle
Dasyses barbata (Christoph): Fungus Moth
Dasyses rugosella Stainton: Fungus Moth
Heteropezina cathistes Pritchard: Gall Midge
Hydnobioides pubescens Sen Gupta and Crowson: Beetle
Megaselia chaetoneura (Malloch): Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Megaselia frameata Schmitz: Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Megaselia giraudii (Egger): Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Megaselia plurispinulosa (Zetterstedt, 1960): Mushroom Phorid, Scuttle Fly, Humpbacked Fly

Megaselia sylvatica (Wood, 1910): Mushroom Phorid, Scuttle Fly, Humpbacked Fly

Mycomya duplicata Edwards, 1925: Fungus Gnats

Mycetophila ruficollis Meigen: Fungus Gnat

Mycomya marginata (Meigen, 1818): Fungus Gnats

Onthophagus villaneuvai Delgado-Castillo and Deloya, 1990: Scarab Beetle

Phanerota dissimilis (Erichson): Rove Beetle

Phanerota fasciata (Say): Rove Beetle

Pleurotobia tristigmata (Erichson): Rove Beetle

Rondaniella sp.: Fungus Gnat

Sciophila lutea Macquart, 1826: Fungus Gnat

Symbiotes spp.: Beetle

Ulodes spp.: Beetle

7.4 Nematodes

Pleurotus ostreatus

Species name not given: Gill Knot Disease

Aphelenchoides composticola Franklin (1957): Mycophagous Nematode

Ditylenchus myceliophagus Goodey (1958): Mycophagous Nematode

Paraphalenchus myceliophthorus Goodey (1958): Mycophagous Nematode

Rhabditis axei (Cobbold) Dougherty (1955): Bacterial Feeding Nematode

Rhabditis spp.: Bacterial Feeding Nematode

7.5 Molluscs

Pleurotus ostreatus

Meghimatium striatum van Hasselt (1823): Slug

7.6 Mites

Pleurotus ostreatus

Acarus immobilis Griffith, 1964: Acarid Mite

Histiostoma feroniarum (Dufour, 1839): Bacterial Feeding Mite

Proctolaelaps spp.: Ascid Mite

Rhizoglyphus echinopus (Fumouze et Robin, 1868): Bulb Mite

Rhizoglyphus spp.: Acarid Mite

Sancassania spp. indet: Acarid Mite

Tarsonemus spp.: Tarsonemid Mite

Tyrophagus longior (Gervais, 1844): Seed Mite

7.7 Viruses

Pleurotus ostreatus

Partitiviruses and Totiviruses

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