CHAPTER 4

PHYSIOLOGY, CYTOLOGY AND GENETICS OF MUSHROOMS

Siu Wai Chiu

Department of Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

1. INTRODUCTION

Mushrooms are familiar macrofungi being well-known of or having potential for nutritive, medical and industrial importance. Regardless of this, emphasis is in most cases placed on the cultivation technology of these mushrooms (Chang & Hayes, 1978; Chang & Quimio, 1982; Chiu & Chang, 1992) with scarce information on other aspects which are fundamental and basic. In this way, unlike other crops, breeding in edible mushrooms is handicapped, and conventional means, such as single spore isolation, mass pairing and screening, is employed to hunt for a good hybrid (Chang *et al.*, 1993). It is not the intention to say that such conventional method does not work. Yet with our better knowledge on mushrooms, we can explain the phenomena encountered in handling mushrooms, and it hopefully enables us to devise a better and more directed strategy for improving strains, obtaining higher yield of mushrooms or mushroom products. Thus, mushroom biology serves us.

For *Agaricus bisporus*, the most popular cultivated mushroom in the world, researches fall on various aspects including genetics, cytology, physiology and cultivation (Flegg *et al.*, 1985; Moore *et al.*, 1985; Wuest *et al.*, 1987; Kerrigan *et al.*, 1993), with the result that commerical strains are patented using molecular markers, and a computerized system has been developed to control composting to guarantee good yield (Leendertse, 1988). However, a protocol of directed breeding of a strain with desirable traits is not available. In comparison, less attention and research activities are paid to other mushrooms. I, therefore, utilize this opportunity to review some information on various fundamental aspects of these so-called specialty mushrooms and their significance and possible impacts on applied field.

2. DEVELOPMENTAL PLASTICITY - A CHALLENGE TO TAXONOMISTS

Developmental plasticity is an inborn property of an organism to adapt to the fluctuating environment and is enabled by the differential expression of the genome according to the immediate

needs. Spontaneous fruitbody plasticity is widely observed during mushroom cultivation although mushroom farmers usually regard the variant form as abnormal. Unlike abnormal forms caused by infections (Burden, 1992), the variant forms just reflect the response towards a certain stimulus, e.g. the uprolling of the *Pleurotus* caps exposing the gills or the premature exposure of the fruitbody proper (hymenophore) from a basal bulb of *Volariella* to the stagnant air, and the tumor outgrowth on the caps of *Agaricus bisporus* induced by the fuming oil in the mushroom farm. Once when the environmental conditions change or become better, normal fruitbodies appear again. In *Volvariella bombycina*, spontaneous variants in the forms of exposed cap, morchelloid forms, supernumerary gills, inverted caps, enclosed form (gasteromycetoid form) and even the agaric form without basal volva which is a characteristic feature of genus *Volvariella* were found together with the normal agaric form (Chiu *et al.*, 1989). All of these normal and variant fruibodies except the morchelloid form matured with spore formation. Yet there were some irregularities: gills were heavily covered with mucilage, asynchronous maturation of spores borne on the same basidium, the number of spores/ sterigmata borne on a basidium ranged from 2 to 4, and the basidiospore shape which is also of taxonomic value was not egg-shaped but heart-shaped (Chiu *et al.*, 1989). By *in vitro* and *in vivo* studies using gills of *Coprinus cinereus*, basidial maturation can be disturbed by exogenous factors leading to arrestment of development without spore formation, a basidium with fewer than 4 spores/ sterigmata, a mosaic basidium bearing both spore and hyphal tips and giant spherical spores (Chiu & Moore, 1988 & 1990a). By carrying out a developmental study with *V. bombycina*, some of the variants were found to be actually the embryonic form which under favourable conditions will transit into the normal agaric form (Chiu & Moore, 1990b). Therefore, care is needed to differentiate this developmental plasticity with the disease symptoms caused by pathogens. The latter requires an environmental study isolating the causative agent. In addition, it is common to find the different morphological forms when a fungus is subcultured onto different media, the same old phenomenon named plasticity. Therefore, when variant fruitbodies are encountered, the critical thing to do is to check for any unfavourable environmental conditions and contaminants present.

Taxonomists in the old days stressed on morphological features (phenotype in genetic term) for classification purpose but a developmental biologist regards the phenotype which is the product of interaction between environment and genotype. No wonder why nowadays, taxonomists are collecting information at the DNA level in addition to the morphological and physio-chemical features of organisms. These information collected in addition to the strains preserved in a culture collection centre would surely reflect and conserve the fungal biodiversity on earth and provide a rich resource for human goodness.

3. CYTOGENETICS - A CHALLENGE TO MUSHROOM BREEDERS

Today, breeding through protoplast fusion technology is available although transformation system using protoplasts has not been well established for edible mushrooms.

3.1. Karyotype Analysis

Fungal chromosomes are minute and at the limit of resolution by conventional light microscopes. With the development of pulsed field gel electrophoresis (PFGE) (Lai *et al.*, 1989), the karyotypes of various mushroom species showing molecular size and numbers of chromosomes have been worked out. We successfully devised a freeze-grinding method to prepare the intact chromosomal DNAs of the yeast *Saccharomyces cerevisiae* which is commonly employed as the size marker

(Kwan *et al.*, 1991) but such method is found causing too much mechanical damage to most mushroom chromosomal DNAs of size greater than 2Mb (million base pairs).

The importance of karyotype for identification of species is not justified as chromosomal polymorphism among strains is now known to be a widespread phenomenon. Yet electrophoretic karyotype is a tool to reveal chromosomal aberration during growth and development if any in fungi. Strain instability and sectoring are commonly encountered especially for old cultures but no one has looked at this phenomenon from this aspect, perhaps simply because of the lack of funding for basic research.

The fungal karyotype can also be revealed by chromosome spread at the meiotic prophase stage. Up to now such technique first developed in plant system has only been applied to the mushroom *Coprinus cinereus* (Pukkila & Lu, 1985). This protocol requires good preparation of chromosome spread and the equipment scanning electron microscope for best resolution. In comparison, electrophoretic karyotype requires high yield of protoplasts which are then embedded in agarose, *in-situ* lysis of protoplasts to release the chromosomal DNAs which are protected from mechanical shear by the agarose and an equipment to do PFGE. PFGE is an important tool for gene mapping and for gene cloning especially when using vectors such as yeast artifical chromosome which can harbour DNA up to 1Mb. Therefore, the latter technique should have more widely uses. All these in future will build up into a transformation system involving a particular gene or chromosome for strain improvement.

3.2. Molecular Markers

Although a particular agaric is by convention characterized by the specific morphological properties of the fruitbodies (Buchanan, this proceedings), it is impractical to always fruit different strains in order to distinguish among them, especially with our poor knowledge on the fruiting initiation mechanism and the encounter of sterile strains. Therefore, DNA fingerprinting techniques were attempted. Currently, by using polymerase chain reaction, different strains (no matter it is of the same or different species) can be differentiated by arbitrarily-primed polymerase chain reaction (Williams *et al.*, 1990; Kwan *et al.*, 1992; Chiu *et al.*, 1993). When 2 or more primers are used, the differences can be revealed definitely. The isozyme pattern, another molecular marker commonly used (Royse, this proceedings), has a drawback that enzyme expression and so the detection is in some cases dependent on the physiological conditions of the strains. i.e. Before an enzyme can be used, the isozyme pattern of a particular strain should not show temporal or spatial specificity. Also, isozyme pattern only samples the expression of the genome while DNA markers reveal indiscriminately various sequences of the genome. In comparison, there are fewer isozyme markers than DNA markers.

Similar DNA fingerprinting technique using RAPD (random amplified polymorphic DNA markers) (Welsh & McClelland, 1990; Kerrigan *et al.*, 1993) is commerically employed to patent *Agaricus bisporus* strains. This way of strain characterization can be routinely employed for culture collection centre and can have great potential for being incorporated for any hybridization/breeding experiments for labelling the parental strains and characterizing hybrid formation. In addition, when the segregation pattern of the amplified markers are followed in the progeny, some of them will be found to be genetic markers (Chiu *et al.*, 1993; Kerrigan *et al.*, 1993). Linkage relationship will then be established between phenotypic traits and DNA markers. These information will facilitate the direct cloning of genes.

3.3. Sexuality & Life History

Chang in this proceedings gives a general grouping of sexuality patterns found in mushrooms. For self-sterile cross-fertile species such as *Lentinus edodes*, *Pleurotus sajor-caju*, strain improvement can be done by mating with a good homokaryon, hoping for hybrid vigor to occur. Yet when two homokaryons are of the same mating types or different species are involved, only protoplast fusion can bring the two genomes into the same cytoplasm. Although many research groups succeed in performing protoplast fusion, commercialization of the hybrid is rare. The reasons may be as follows: auxotrophic mutants instead of the cultivated strains are used; protoplast fusion does not guarantee genetic recombination in the way desired by an experimenter. With the availability of DNA markers in addition to other biochemical and morphological markers, hybridization using wild type strains is now made possible to be traced and is in progress in many laboratories. However, no one study follows what has happened after protoplast fusion in terms of the genetic makeup and the nuclear-cytoplasm compatibility, indicating our poor knowledge on the selection process. Therefore, protoplast fusion has not yet been a directed strategy. Heterologous gene expression is possible for a foreign gene from one organism be transformed and expressed in another organism. Perhaps this is the way to which breeding can de directed.

For self-sterile species such as *Volvariella volvacea* and *V. bombycina* (Chang & Yau, 1971; Li & Chang, 1978; Chang *et al.*, 1981; Chiu & Chang, 1987a), heterokaryon formation is possible by pairing two homokaryons as demonstrated by isozyme markers (Royse *et al.*, 1987). Based on morphological features, fruiting ability and growth rate, variation is observed in the progenies of the two species. Recently, microspectrophotometric study on the DNA content and nuclear behaviour of *V. bombycina* has shown that a primary homothallic species bears a uninucleate not binucleate hymenial cell initial which if develops into a basidium, will synthesize its DNA content at prophase I instead of prekaryogamy stage contrasting with those of heterothallic species (Chiu & Moore, 1993). Although we now realize more differences between heterothallic and homothallic species in terms of the nuclear cycle, we still do not know how *Volvariella* species create variation.

4. MODULATING MUSHROOM GROWTH & DEVELOPMENT - A CHALLENGE TO FARMERS

Both spores and vegetative mycelium are commonly used for seeding mushroom cultures. Also, in conventional breeding programmes, single spore isolates are commonly used. Basidiospores of some mushrooms germinate readily when plated onto nutrient media but some, such as *Agaricus bisporus*, *Volvariella volvacea* and *V. bombycina* (Chiu & Chang, 1987b), require a heat soak treatment and the presence of an actively growing mycelium for stimulation. Once when a spore germinates, and it establishes into a mycelial mat. Various attempts have been made to heighten the mycelial/metabolite yields using solid-state-fermentation and submerged fermentation technologies. In addition to the classical method by searching for the best C- and N-sources, nutrient supplementation is another common method used (Batterley, 1989). Addition of lipid/waste tea leaves was found to enhance biomass gain (Nair *et al.*, 1990; Li *et al.*, 1992). Lipid and waste tea leaves not only add more nutrients to the medium but also promote production of substrate-utilizing enzymes (Nair *et al.*, 1990; Chiu *et al.*, unpublished result). Supplementation with waste tea leaves and slow-release nutrients were found to increase fruiting yield (Batterley, 1989; Tan & Chang, 1989). However, the triggering factors for fruiting remain obscure. In *Coprinus cinereus*, the monokaryotic mutant with an elevated level of endogenous cAMP produced fruitbodies but exogenous cAMP did not induce fruiting in

Schizophyllum commune and other mushrooms (Uno & Ishikawa, 1971; Schwalb & Miles, 1978). Although in many mushrooms, endogenous glycogen elevated at time of fruiting (Moore *et al.*, 1985; Chiu & Cheng, 1991), at least in *C. cinereus* and *Pleurotus sajor-caju*, by using differnt approaches, endogenous glycogen was found not a trigger for fruiting (Chiu & To, 1993; Ji & Moore, 1993). We really are in great need for financial support and research activities to examine the fruiting pathway in details.

5. CONCLUSION

A brief account on some current aspects of mushroom biology is presented. From it we can find that:

i. Financial support is urgently needed for mushroom research and development;
ii. Basic studies using model mushrooms, such as *Coprinus cinereus* and *Schizophyllum commune* can provide us good and relevant information for cultivated mushrooms although specificity does exist in some cases; and most of all,
iii. Basic and fundamental research is necessary and essential for mushroom application.

ACKNOWLEDGEMENT

The author thanks UPGC for a grant to perform some of the studies mentioned. This article is dedicated to my graduate supervisors, Dr. David Moore, Prof. Shu-ting Chang and Dr. Grace Shui-fong Li.

REFERENCES

BATTERLEY, D.A. (1989). Supplementation of *Agaricus* and *Pleurotus* substrate - an overview. *Mushroom News* **37**, 10-16.

BURDEN, J.L. (1992). Mushroom malaise. *Mushroom Journal* **506**, 16-21.

CHANG, S.T., BUSWELL, J.A. & MILES, P.G. (1993). *Culture Collection and Breeding of Edible Fungi*. Philadelphia: Gordon & Breach Pub., Inc.

CHANG, S.T. & CHIU, S.W. (1992). Mushroom production - an economic measure in maintenance of food security. In *Microbial Technology: Economic and Social Aspects,* pp.110-141. Edited by DaSilva, E.J., Ratledge, C. & Sasson, A. Cambridge: Cambridge University Press.

CHANG, S.T. & HAYES, W.A. (1978). *The Biology and Cultivation of Edible Mushrooms*. New York: Academic Press.

CHANG, S.T., MILES, P.G. & WAI, C.C. (1981). A study of monosporous isolates of *Volvariella volvacea*. *Mushroom Science* **11**, 603-619.

CHANG, S.T. & QUIMIO, T.H. (1982). *Tropical Mushrooms: Biological Nature and Cultivation Methods*. Hong Kong: The Chinese University Press.

CHANG, S.T. & YAU, C.K. (1971). *Volvariella volvacea* and its life history. *American Journal of Botany* **58**, 552-562.

CHIU, S.W. & CHANG, S.T. (1987a). *Volvariella bombycina* and its life history. *Mushroom Journal for the Tropics* **7**, 1-12.

CHIU, S.W. & CHANG, S.T. (1987b). Activation of spore germination in *Volvariella bombycina*. *Mushroom Journal for the Tropics* **7**, 61-66.
CHIU, S.W. & CHENG, H.P. (1991). Involvement of endogenous glycogen in fruiting of *Volvariella volvacea* and *Pleurotus sajor-caju*. *Mycological Research* **95**, 632-634.
CHIU, S.W., KWAN, H.S. & CHENG, S.C. (1993). Application of arbitrarily-primed polymerase chain reaction in molecular studies of mushroom species with emphasis on *Lentinula edodes*. *Culture Collection and Breeding of Edible Fungi*, pp.265-284. Edited by Chang, S.T., Buswell, J.A. & Miles, P.G. Philadelphia: Gordon & Breach Pub., Inc.
CHIU, S.W. & MOORE, D. (1988) Ammonium ions and glutamine inhibit sporulation of *Coprinus cinereus* basidia assayed *in vitro*. *Cell Biology International Reports* **12**, 519-526.
CHIU, S.W. & MOORE, D. (1990a). Sporulation in *Coprinus cinereus*: use of *in vitro* assay to establish the major landmarks in differentiation. *Mycological Research* **94**, 249-253.
CHIU, S.W. & MOORE, D. (1990). Development of the basidiome of *Volvariella bombycina*. *Mycological Research* **94**, 327-337.
CHIU, S.W. & MOORE, D. (1993). Cell form, function and lineage in the hymenia of *Coprinus cinereus* and *Volvariella bombycina*. *Mycological Research* **97**, 221-226.
CHIU, S.W., MOORE, D. & CHANG, S.T. (1989). Basidiome polymorphism in *Volvariella bombycina*. *Mycological Research* **92**, 69-77.
CHIU, S.W. & TO, S.W. (1993). Endogenous glycogen is not a trigger for fruiting in *Pleurotus sajor-caju*. *Mycological Research* **97**, 363-366.
FLEGG, P.B., SPENCER, D.M. & WOOD, D.A. (1985). *The Biology and Technology of the Cultivated Mushrooms*. Chichester: John Wiley & Sons.
JI, J. & MOORE, D. (1993). Glycogen metabolism in relation to fruitbody maturation in *Coprinus cinereus*. *Mycological Research* **97**, 283-289.
KERRIGAN, R.W., ROYER, J.C., BALLER, L.M., KOHLI, Y., HORGEN, P.A. & ANDERSON, J.B. (1993). Meiotic behaviour and linkage relationships in the secondarily homothallic fungus, *Agaricus bisporus*. *Genetics* **133**, 225-236.
KWAN, H.S., CHIU, S.W., PANG, K.M. & CHENG, S.C. (1992). Strain typing in *Lentinula edodes* by polymerase chain reaction. *Experimental Mycology* **16**, 163-166.
KWAN, H.S., LI, C.C., CHIU, S.W. & CHENG, S.C. (1991). A simple method to prepare intact yeast chromosomal DNA for pulsed field gel electrophoresis. *Nucleic Acids Research* **19**, 1347.
LAI, E., BIRREN, B.W., CLARK, S.M., SIMON, M.I. & HOOD, L. (1989). Pulsed field gel electrophoresis. *BioTechniques* **7**, 34-42.
LEENDERTSE, I.K.D.K. (1988). The Agrisystems-Sohm indoor composting system: is this the future? *Mushroom News* **36**, 10-13.
LI, S.F. & CHANG, S.T. (1978). Variation in the homothallic basidiomycete, *Volvariella volvacea*. *Mushroom Science* **10**, 171-184.
LI, Y., CHO, K.Y., WU, Y.Z. & NAIR, N.G. (1992). The effect of lipids and temperature on the physiology and growth of *Volvariella volvacea*. *World Journal of Microbiology and Biotechnology* **8**, 621-626.
MOORE, D., CASSELTON, L.A., WOOD, D.A. & FRANKLAND, J.C. (1985). *Developmental Biology of Higher Fungi*. Cambridge: Cambridge University Press.
NAIR, N.G., HOLLEY, M.P., SONG, C.H. & CHO, K.Y. (1990). Lipid metabolism of *Pleurotus sajor-caju*. *Annual of Applied Biology* **114**, 167-176.
PUKKILA, P.J. & LU, B.C. (1985). Silver staining of meiotic chromosomes in the fungus, *Coprinus cinereus*. *Chromosoma* **91**, 108-112.

ROYSE, D.J., JODON, M.H., ANTONIO, G.G. & MAY, B.P. (1987). Confirmation of intraspecific crossing and single and joint segregation of biochemical loci of *Volvariella volvacea*. *Experimental Mycology* **11**, 11-18.
SCHWALB, M.N. & MILES, P.G. (1978). *Genetics and Morphogenesis in the Basidiomycetes*. New York: Academic Press.
TAN, Y.H. & CHANG, S.T. (1989). Effect of growth regulators, enzyme inhibitors and stimulatory additives on the vegetative development and fruitification of *Lentinus edodes*. *Mushroom Science* **12**, 267-271.
UNO, T. & ISHIKAWA, T. (1971). Chemical and genetical control of induction of monokaryotic fruiting bodies in *Coprinus macrorhizus*. *Molecular and General Genetics* **113**, 228-239.
WELSH, H. & MCCLELLAND, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* **18**, 7213-7218.
WILLIAMS, J.G.K., KUBELIK, A.R., LIVAK, K.J., RAFALSKI, J.A. & TINGEY, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**, 6531-6535.
WUEST, P.J., ROYSE, D.J., & BEELMAN, R.B. (1987). *Cultivating Edible Fungi*. Amsterdam: Elsevier.