REFERENCES

Alexopoulos, C. J., Mims, C. W. and Blackwell, M. 1996. Introductory mycology.New York : John Wiley and Sons, 869 pp.

Ainsworth, G. C., Sparrow, F. K. and Sussman, A. S. 1973. Basidiomycetes and lower Fungi. The fungi an advanced treatise (vol.IV B). N. Y. & London: Academic Press.

American Health Association. 1987, May. The health part of shiitake: Mushroom and health. http://www.shiitakemall.com/shiitake.html

Ban Bruranachonabot. 1992. Hed-Hom. second edition. n.p.

Berry, M. J. and Samuel ,C. E. 1982. Detection of subnanogram amounts of RNA in polyacrylamide gels in presence and absence of protein by staining with silver. Analytical Biochemistry. 124 : 180-184.

Blaiseau, P. -L., Kunz, C., Grison,R.,Bertheau, Y. and Brygoo, Y. 1992. Current Genetics. 21: 61-66.

Caetano-Anolles, G., Bassam, B. J. and Gresshof, P. M. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primer. BioTechnology. 9: 553-557.

Changtragoon, S., Szmidt, A. E. and Boontawee, B. 1993. The study of genetic diversity of *Azadirachta* spp. By using isoenzyme analysis. In: The proceedings of the third Asia-Pacific conference on agricultural biotechnology. 10-15 November, Melia Hotel, Thailand. p.41.

Chaparro, J. X., Werner, D. J., Malley, D. O., Sederoff, R. R.1994. Targeted mapping and linkage analysis of mosphological isozyme, and RAPD markers in peach. Theoretical and Applied Genetics. 87 : 805-815.

Chinese Darr Moo Qigong Sanatorium. 1997. Shiitake. http://totooine.fortunecity.com/herbert/306/10-05.html

Cherdshewasart, W. 1991. Total DNA extraction – alternative protocols. In I. Negrutiu; and G.B. Gharti – Chhertri (eds.). A laboratory guide for cellular and molecular plant biology. 4: 161-167. Switzerland: Brikhauser Verlag.

Chulee Chalsrisook. 1994. Random amplified polymorphic DNA (RAPD) technique: the rapid PCR application for genome analysis. Kasetsart University Science Journal. 12(1): 47-53.

Dayhoff, M. O. and Eck, R. V. 1968. Restriction analyses. In Avise, J. C. 1993. Molecular Tools: Molecular Markers, Natural History and Evolution, pp.57-78. Great Britain: Chapman and Hall.

Delpech, P., Olivier, J. M. 1991. Cultivation of shiitake on straw based pasteurised substrates. In Maher(ed.). 1991. Science and cultivation of edible fungi. Balkerma, Rotterdam. 523-528.

Demeke, T., Adams, R. P. and Chibbar, R. 1992. Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in Brassica. Theoretical and Applied genetics. 84 : 990-994.

Demeke, T., Adams, R. P. 1994. The use of PCR-RAPD Analysis in plant taxonomy and evolution. In Griffin, H. G. and Griffin, A. M. (eds.), PCR Technology: Current Innovations. pp. 179-189. Boca Raton: CRC Press.

DeMoreno, M. R.; Smith, J. F. and Smith, R. V. 1985. Silver staining of proteins in polyacrylamide gels: Increased sensitivity through a combined coomassie blue-silver stain procedure. Analytical Biochemistry. 151: 466-470.

Diehle, D. A. and Royse, D. J. 1991. Effect of substrate heat treatment on biological efficiency (BE) and size of a selected line of *Lentinula edodes*. In Maher (ed.). 1991. Science and cultivation of edible fungi. Balkema, Rotterdam. 517-521.

Do, N. and Adams, R. P. 1991. Biotechniques. 10 : 162-166.

Edwards, K. , Johnstone, C. and Thompson, C. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Research. 19(6) : p. 1349. Erlich, A., Gelfand, D. and Sninsky, J. J. 1991. Recent advances in the polymerase chain reaction. Science. 252 : 1643, cited in Demeke, T., Adams, R. P. 1994. The use of PCR-RAPD analysis in plant taxonomy and evolution. In Griffin, H. G. and Griffin, A. M. (eds.), PCR Technology : Current Innovations. pp.179-191. Boca Raton : CRC Press.

Fernández, F. A. and Hanlin, R. T. 1996. Morphological and RAPD analyses of *Diaporthe phaseolorum* from soybean. Mycologia. 8(3) : 425-440.

Freifelder, D. 1935. Study guide for Molecular biology, second edition (Boston: Jones and Bartlett Publishers), p. 8.

Fritsch, P.; Hanson, M. A.; Spore, C. D.; Pack, P. E. and Rieseberg, L. H. 1993. Consistency of RAPD primer amplification strength among distantly related taxa of flowering plants. Plant Molecular Biology Reporter. 11(1) : 10-20.

Fujian Piangnan Dachang Foodstuff Co. 1997. Shiitakemal : About Shiitake. http://www.shiitakemall.com/shiitake.html

Fukuda, M., Nakai, Y. F., Hibbett, D. S., Matsumoto, T. and Hayashi, Y. 1994. Mitochodrial DNA restriction fragment length polymorphisms in natural populations of *Lentinula edodes*. Mycology Research. 98(2) : 169-175.

Garber, R. C. and Yoder, O. C. 1983. Isolation of DNA from filamentous fungi and separation into nuclear, mitochondria, ribosomal and plasmid components Analytical Biochemistry. 135 : 416-422.

Goodwin, P. H. and Annis, S. L. 1991. Rapid identification of genetic variation and pathotype of *Leptoshaeria maculans* by random amplified polymorphic DNA assay. Applied and Environmental Microbiology. 57 (9) : 2482-2486.

Hansen, B. M. and Winding, A. 1997. Detection of *Pseudomonas Putida* B in the rhizosphere by RAPD. Letters in Applied Microbiology. 24 : 249-252.

Hashizume, T., Sato, T. and Hirai, M. 1993. Determination of genetic purify of hybrid seed in watermelon *(Cilrullus lanatus)* and tomato (*Lycopersicon esculentum*) using random amplified polymorphic DNA (RAPD). Japan J. Breeding. 43 : 367-375, cited in Somsak Apisitwanish, Sumon Masuthon, Pradir Pongtongkam, Saowanee Suputtitada and Surin Peyachoknagul. 1995. Phylogenetic relationship among *Oryza* species as determined by random amplified polymorphic DNA (RAPD). Kasetsart Nat. Scien. 29 : 454-461.

Helentjaris, T., Slocum, M., Wright, S., Schaefer, A. and Neienhuis, J. 1986. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphism. Theoretical and Applied Genetics. 72 : 761, cited in Yu, L.–X. and Nguyen, H. T. 1994. Genetic variation detected with

RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theoretical and Applied Genetics. 87: 668-672.

Hilton, A. C., Banks,J. G. and Penn, C. W. 1997. Optimization of RAPD for fingerprinting *Salmonella*. Letters in Applied Microbiology. 24 : 243-248.

Hiromoto, B. T. 1991. Comparative analysis of shiitake culture systems. In Maher (ed.). 1991. Science and cultivation of edible fungi. Balkema, Rotterdam. 489-496.

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. Bull Soc Vaud Sci Nat. 44 : 223-270.

Jittra Kanchanaprayudh, 1996. Improving growth of pine seedling (*Pinus kesiya*) by ectomycorrhizal fungi isolated from *Astraeus hygrometricus* and *Bolytus edulis* mushrooms. Master's Thesis, Program of Biotechnology, Graduate school, Chulalongkorn University.

Khana, P. and Garcha, H. S. 1985. Physiological studies on *Pleurotus* spp. II carbon utilization. Mushroom Newsletter for the tropics. 6(1) : 9-14.

Kenneth, J. (n.d.). Shiitake possesses amazing healing properties. http://jum.com/health/shiitake.html

Khush, R. S., Becker , E. and Wach, M. 1992. DNA amplification polymorphisms of the cultivated mushroom *Agaricus bisporus*. Applied and Environmental microbilogy. 58(9) : 2971-2977.

Kirchhoff, B. and Lelley, J. 1991. Investigations of Shiitake (*Lentinus edodes* (Berk.) Sing) bag-log cultivation to increase the yield in Germany. In Maher (ed.). 1991. Science and cultivation of edible fungi, Balkema, Rotterdam.

Kulkarni,R. K. 1991. DNA polymorphisms in *Lentinula edodes*, the shiitake mushroom. Applied and Environmental Microbiology. 57(6) : 1735-1739.

Landry, B. S., Hubert, N., Etoh, T., Harada, J. J., and Lincoln, S. E. 1991. A genetic map for *Brassica napus* based on restriction fragment length polymorphisms detected with expressed DNA sequences. Genome. 34: 543, cited in Yu, K. F., Deynze, A. V. and Pauls, K. P. 1993. Random amplified polymorphic DNA (RAPD) analysis. In Glick, B. R. and Thompson, J. E., Methods in Plant

Molecular Biology and Biotechnology. pp. 287-301. Boca Raton, Florida: CRC Press.

Lee, D., Turner, L., Dvies, D. R., and Ellis, T. H. N. 1988. An RAPD marker for r_b in pea. Theoretical and Applied Genetics. 75 : 784, cited in Yu, K. F., Deynze, A. V. and Pauls, K. P. 1993. Random amplified polymorphic DNA (RAPD) analysis. In Glick, B. R. and Thompson, J. E., Methods in Plant Molecular Biology and Biotechnology. pp. 287-301. Boca Raton, Florida: CRC Press.

Lee, S. B. and Taylor, J. W. 1990. Isolation of DNA from fungal mycelia and single spores. In Innis, M. A., Gelfand, D. H., Sninsky , J. J. and white, T. J. (eds.), PCR Protocals : A Guide to methods and applications. pp.282-287. San Diego : Academic Press.

Lin, J. J., Kuo , J., Ma, J., Saunders, J. A., Beard , H. S., Mac Donald, M. H., Kenworthy, W., Ude, G. N. and Matthews, B. 1996. Identification of molecular markers in Soybean comparing RFLP, RAPD and AFLP DNA Mapping techniques. Plant Molecular Biology Reporter. 14(2) : 156-169.

Lowe , A. J., Hanotte, O. and Guarino, L. 1996. Standardization of molecular genetic techniques for the characterization of germplasm collections: the case of random amplified polymorphic DNA (RAPD). Plant Genetic Resources Newsletter, No.107 : 50-54.

McGrath, J. M., Quiros, C. F., Harada , J. J. and Landry , B. S. 1990. Identification of *Brassica oleracea* monosomic alien chromosome addition lines with molecular markers reveals extensive gene duplication. MGG. 223 : 198, cited in Yu, K. F., Deynze, A. V. and Pauls, K. P. 1993. Random amplified polymorphic DNA (RAPD) analysis. In Glick, B. R. and Thompson, J. E., Methods in Plant Molecular Biology and Biotechnology. pp. 287-301. Boca Raton, Florida: CRC Press.

Mileham, A. J. 1997. Protocol : Identification of microorganisms using random primed PCR. Molecular biotechnology. 8 : 139-145.

M.L. Saovaros Svasti. DNA detection of 4 varieties of *Lentinus edodes* (Berk.) Sing. Bachelor's Senior Project, Department of Botany, Chulalongkorn University.

Möller, E. M., Bahnweg, G., Sandermann, H. and Geiger, H. H. 1992. A simple and efficient protocol for isolate of high molecular weight DNA from filamentous fungi, fruit bodies and infected plant tissue. Nucleic Acids Research. 20(22) : 6115-6116.

Mullis, K. B. 1990. The unusual origin of the polymerase chain reaction. Scien. Am. p. 56, cited in Demeke, T., Adams, R. P. 1994. The use of PCR-RAPD analysis in plant taxonomy and evolution. In Griffin, H. G. and Griffin, A. M. (eds.), PCR Technology: Current Innovations. pp. 179-189. Boca Raton: CRC Press.

Nicholson, M. S., Bunyard, B. A. and Royse, D. J. 1997. Phylogeny of the genus *Lentinula* based on ribosomal DNA restriction fragment length polymorphism analysis. Mycologia. 89 (3) : 400-407.

Onhealth Network. 1998. September. Taking control of your health : Guide to alternative and conventional medicine.
http ://onhealth.com/ch1/resource/herbs/item,16057.asp

Osborn, T. C., Alexander, D. C. and Fobes, J. F. 1987. Identification of restriction fragment length polymorphisms linkage to genes controlling soluble solids content in tomato fruit, Theoretical and Applied Genetics. 73 : 350. Cited in Yu, K. F., Deynze, A. V. and Pauls, K. P. 1993. Random amplified polymorphic DNA (RAPD) analysis. In Glick, B. R. and Thompson, J. E., Methods in Plant Molecular Biology and Biotechnology. pp. 287-301. Boca Raton, Florida: CRC Press.

Penner, G. A., Bush, A., Wise, R., Kim, W., Domier, L., Kasha, K., Laroche, A., Scoles, G. Molnar, S. J. and Fedak, G. 1993. Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Methods and Applications. Cold Spring Habor Laboratory Press. 2 : 341-345.

Przybylowicz, P. and Donoghue, J. 1988. Shiitake Growers Handbook (The Art and Science of Mushroom Cultivation). Kenda II/Hunt ; USA. p 217.

Reader, U. and Broda, P. 1985. Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology. 1 : 17-20.

Rodriguez, R. J. and Yoder, O. C. 1991. A family of conserved repetitive DNA elements from the fungal plant pathogen *Glomerella cingulata* (*Colletotrichum lindemuthlanum*). Experimental Mycology. 15 : 232-242.

Royse, D. J. Spear, M. C. and May, B. 1983 b. Single and joint segregation of marker loci in the shiitake mushroom, *Lentinus edodes*. Journal of Genetic Applied Microbiology. 29 : 217-222.

San Antonio, J. P. 1981. Cultivation of the shiitake mushroom. Hort Science. 16 : 151-156.

Sambrook, J.; Maniatis, T. and Frisch, E. F. 1989. Molecular cloning : A laboratory manual. 2nd ed. New york : Cold Spring Habor Laboratory Press.

Schneiderbauer, A., Sandermann, H. and Ernst, D. 1991. Isolation of functional RNA from plant tissue rich in phenolic compounds. Analytical Biochemistry. 197 : 91-95.

Shiitake Center. 1998. Shiitake health benefits. http:www.shiitakecenter.comhealth/health.html

Solaya Suksa-Ard. 1995. Variation of DNA in shiitake *Lentinus edodes* (Berk.) Pegler. Master' s Thesis, Program of Biotechnology, Graduate School, Chulalongkorn University.

Somsak Apisitwanish, Sumon Masuthon, Pradir Pongtongkam, Saowanee Suputtitada and Surin Peyachoknagul. 1995. Phylogenetic relationship among *Oryza* species as determined by random amplified polymorphic DNA (RAPD). Kasetsart Nat. Scien. 29 : 454-461.

Song, K. M., Osborn, T. C. and Williams, P. H. 1988 a. Brassica taxonomy based on nuclear restriction fragment length polymorphisms (RELPs). 1. Genome evolution of diploid and amphidipliod species. Theoretical and Applied Genetics. 75 : 784, cited in Yu, K. F., Deynze, A. V. and Pauls, K. P. 1993. Random amplified polymorphic DNA (RAPD) analysis. In Glick, B. R. and Thompson, J. E., Methods in Plant Molecular Biology and Biotechnology. pp. 287-301. Boca Raton, Florida: CRC Press.

Song,K. M., Osborn, T. C. and Williams, P. H. 1988b. Brassica taxonomy based on nuclear restriction fragment length polymorphisms (RELPs). 2. Preliminery

analysis of subspecies within *B. rapa* (syn. *Campestris*) and *B. oleracea* . Theoretical and Applied Genetics. 76 : 593, cited in Yu, K. F., Deynze, A. V. and Pauls, K. P. 1993. Random amplified polymorphic DNA (RAPD) analysis. In Glick, B. R. and Thompson, J. E., Methods in Plant Molecular Biology and Biotechnology. pp. 287-301. Boca Raton, Florida: CRC Press.

Specht, C. A., DiRusso, C. C., Novotny, C. P. and Ullirich, R. C. 1981. A method for extracting high-molecular weight deoxynucleic acid from fungi. Analytical Biochemistry. 119 : 158-163.

Suzuki, S. and Oshima, S. 1976. Influence of shiitake (*Lentinus edodes*) on human serum cholesterol. Mushroom science. 9 (part I) : 463.

Tanksley, S. D., Young, N. D., Paterson, A. H. and Bonierbale, M. W. 1989. RFLP mapping in plant breeding : new tools for an old science. Bitechnology. 7 : 257-264.

Tingey, S. V. and delTufo, J. P. 1993. Genetic analysis with random amplified polymorphic DNA markers. Plant Physiology. 101 : 349-352.

Vaccino. P., Accerbi, N. and Corbellini, N. 1993. Cultivar identification in *Triticum aestivum* using highly polymorphic RFLP probes. Theoritical Applied of Genetic. 86 : 883-836.

Waiblinger, H. U., Wurz, A., Feyer, R. and Pietsch, K. 1999. Spezifischer Nachweis von gentechnisch verändertem Raps in Honing. Deutsche Lebensmittel-Rundschau I 95. Jahrgang, Heft 5 : 192-195.

Walsh, P. S., Metzger, D. A. and Higuchi, R. 1991. Chelex® 100 as a Medium for simple extraction of DNA for PCR-based typing from forensic material. BoiTechniques. 10(4): 506-513.

Welsh, J. and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic acid Research. 18: 7213-7218.

Wilkie, S. E., Issac, P. G. and Slater, R. J. 1993. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*. Threoretical and Applied genetics. 86 : 497-501.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research. 18: 6531-6535.

Wolff, K., Schoen, E. D. and Van Rijn, J. P. 1993. Optimizing the generation of random amplified polymorphic DNAs in *chrysanthemum*. Threoretical And Applied Genetics. 86: 1033-1037.

Yu, K. and Pauls, K. P. 1992. Optimization of the PCR program for RAPD analysis. Nucleic Acids Research. 20(10): p. 2606.

Yu, K. F., Deynze, A. V. and Pauls, K. P. 1993. Random amplified polymorphic DNA (RAPD) analysis. In Glick, B. R. and Thompson, J. E., Methods in Plant Molecular Biology and Biotechnology. pp. 287-301. Boca Raton, Florida: CRC Press.

Yu, L. –X. and Nguyen, H. T. 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theoretical and Applied Genetics. 87: 668-672.

Yu, Y. L. and Lin, T. Y. 1997. Construction of phylogenetic tree for *Nicotiana* species based on RAPD markers. Journal of Plant Research. 110: 187-193.

APPENDICES

APPENDIX I

I-1 : Imports and exports values of dried mushrooms between 1995 to 1999

Year	Imports		Exports	
	Quantity (kg)	Value (baht)	Quantity (kg)	Value (baht)
1995	383,972	73,922,774	47,307	5,627,893
1996	415,673	75,301,446	54,484	10,393,762
1997	295,618	48,298,362	47,872	7,614,091
1998	137,312	14,543,838	38,094	9,442,565
1999(Jan-Sep)	101,175	8,626,828	314,502	18,767,195

Source : Trade statistics and Economic indicates of Thailand, Department of Business Economics, Ministry of Commerce with cooperation of the Custom Department, Thailand.

I-2 : Nutritional value of low calories count, having all amino acids, rich in protein (18%), fiber, vitamins and minerals of 100 g black mushroom contains

Nutrition	**Quantity**
Calories	39
Protein	15-35%
Fat	Less than 1 g
Carbohydrate	7.3 g
Crude fiber	0.8 g
Thiamine	0.8 g
Riboflavin	0.5 mg
Niacin	5.5 mg (27.5%)
Vitamin D2, B2 and B12	Rich in (50%)

Source : American Health Association (1987).

I-3 : All amino acids, rich in protein (18%) of 100 g shiitake mushroom

Protein	Quantity (mg)
Isoleucine	21.8
Leucine	348
Lysine	174
Methionine	87
Cystine	ND*
Phynylalanine	261
Tyrosine	174
Treonine	261
Tryptophan	ND*
Valine	261
Arginine	348
Histidine	87
Alanine	305
Aspartic acid	392
Glutamic acid	349
Glycine	219
Proline	218
Serine	261
Total amino acid	3,762.8
Essential amino acid	1,784

Source : Ban Bruranachonabot (1992).

* No data

I-4 : Biologically active compounds found in *Lentinus edodes* (Berk.) Sing.

Compound	Effects	Type of compound	Activity
Eritadinine	lowers cholesterol antiviral	adenine derivative	accelerates cholesterol metabolism and excretion
Ac 2P	antiviral	polysaccharide	inhibits viral replication
Virus-like particles	antiviral antitumor	double stranded RNA	induces interferon production
KS-2	antiviral antitumor	polysaccharide	induces interferon production
Lentinan	antitumor	polysaccharide	stimulates T-helper cells in immune system
LAP-1	antitumor	polysaccharide	immune system modulator
Polyphenol oxidase	antitumor	protein	unknown
Unknown	reduces blood coagulation	possibly nucleosides or nucleotides	inhibits pletlet aggregation
Cortinellin	antibacterial	unknown	broad spectrum antibiotic
Unknown	antifungal	disulfide	unknown
FBP	antiviral	protein	inhibits viral infection in plant

I-5 : The characteristic of *Lentinus edodes* used in this study (Solaya Suksa-Ard, 1995)

Code	Sources	morphology			
		fruiting behavior	size	color	morphology
MuL2	Taiwan	consistency	Medium-large	Brown	High productivity
MuL4	Japan	consistency	Medium	Brown	High productivity
MuL5	Japan	consistency	Medium	Dark brown	High productivity
MuL12	Wild type	Non-consistency	Small Long-stalked	Light brown	Heat tolerant
Japan *	Japanese cultivar	consistency	medium	Brown	High productivity

MuL represents Mushroom Research Unit *Lentinus edodes*

Source : - Mushroom Research Unit , Department of Botany Chulalongkorn University

- *gifted from Dr.Piyasak Chaumpluk Department of Botany Chulalongkorn University

I-6 : Ten differences arbitrary oligonucleotide sequences

Primer	Sequence (5' → 3')	% GC content
University of British Columbia (UBC)		
101	GCGCCTGGAG	80
174	AACGGGCAGC	70
228	GCTGGGCCGA	80
268	AGGCCGCTTA	60
273	AATGTCGCCA	50
299	TGTCAGCGGT	60
428	GGCTGCGGTA	70
456	GCGGAGGTCC	80
457	CGACGCCCTG	80
459	GCGTCGAGGG	80

I-7 : The different liquid medium formulations.

Liquid medium condition (%)	**1**	**MEB**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**MYG**	**11**	**PDB**
Malt extract	2	2	2	2	2	2	2	2	2	2	-	-
Yeast extract	-	-	-	-	-	-	-	-	-	0.2	2	
Glucose	-	-	-	1	1	1	2	2	2	2	2	
Peptone	-	0.5	1	-	0.5	1	-	0.5	1	-	0.1	

APPENDIX II

Statistical Analysis

Statistical analysis by IRRISTAT programming as following:

1. Input data for statistical analysis into computer program
2. In this experimental is using Split plot design
3. Analysis of variance and comparison means by Ducan's New Multiple-Range Test (DMRT) and show data as :

II-1 : ANALYSIS OF VARIANCE FOR GROWTH RATE OF DRY WEIGHT

SV	DF	SS	MS	F
REP (R)	3	0.00531758	0.00177253	4.04 *
DAY (D)	3	0.06772413	0.02257471	51.42 **
ERROR (a)	9	0.00395109	0.00043901	
MEDIA (M)	11	0.05974734	0.00543158	14.09 **
DxM	33	0.02680331	0.00081222	2.12 **
ERROR (b)	132	0.05054201	0.00038289	
TOTAL	191	0.21408546		

cv (a) = 3.5% ; cv (b) = 3.2 %

** = significant at 1% level ; * = significant at 5% level

Comparison means value and dry weight of *L. edodes* on 12 different liquid culture formulations

Liquid culture	Dry weight of mycelia (g)*			
	5 days	10 days	15 days	20 days
1	0.56200 ab	0.56845 c	0.57260 e	0.58000 f
MEB	0.57877 a	0.59345 bc	0.58560 e	0.61497 cde
3	0.56967 ab	0.58655 bc	0.59545 de	0.60297 ef
4	0.57380 a	0.60255 b	0.62130 bcd	0.64445 abc
5	0.57490 a	0.59912 bc	0.59953 de	0.60210 ef
6	0.54295 b	0.58977 bc	0.60063 de	0.63933 bcd
7	0.58827 a	0.61735 ab	0.60338 cde	0.60987 def
8	0.56410 ab	0.59880 bc	0.63580 ab	0.63335 bcd
9	0.59213 a	0.60795 ab	0.64898 ab	0.66180 ab
MYG	0.58438 a	0.59405 bc	0.63168 abc	0.64260 bc
11	0.58797 a	0.63518 a	0.65925 a	0.67280 a
12	0.57468 a	0.60102 b	0.58830 e	0.60173 ef

* In a column , means followed by a common letter are not significantly different at the 5% level by DMRT.

Average of 4 replications

II-2 : ANALYSIS OF VARIANCE FOR GROWTH RATE OF FRESH WEIGHT

SV	DF	SS	MS	F
REP (R)	3	0.11706767	0.03902256	8.87 **
DAY (D)	3	5.15376789	1.71792263	390.49 **
ERROR (a)	9	0.03959446	0.00439938	
MEDIA (M)	11	02.15305899	0.1953264	34.50 **
DxM	33	0.97915962	0.02967150	5.23 **
ERROR (b)	132	0.74890145	0.00567350	
TOTAL	191	9.19155008		

cv (a) = 5.6% ; cv (b) = 6.4 %

** = significant at 1% level ; * = significant at 5% level

Comparison means value and fresh weight of *L. edodes* on 12 different liquid culture formulations

Liquid culture	Fresh weight of mycelia (g)*			
	5 days	10 days	15 days	20 days
1	0.80080 e	1.02103 d	0.97543 g	1.13953 f
MEB	0.93503 a-d	1.13658 bcd	1.07123 fg	1.33575 de
3	0.84453 de	1.09090 cd	1.17660 ef	1.18853 f
4	0.86300 cde	1.16390 bc	1.29743 bcd	1.46725 bc
5	0.93988 a-d	1.17970 bc	1.19480 de	1.19980 f
6	0.86298 cde	1.13553 bcd	1.24445 cde	1.39405 cd
7	0.97908 abc	1.15753 bc	1.16600 ef	1.41795 cd
8	1.00508 ab	1.22613 b	1.35073 abc	1.43370 cd
9	0.90460 b-e	1.23815 b	1.39503 ab	1.73158 a
MYG	1.02720 a	1.14528 bc	1.24175 cde	1.57678 b
11	1.04503 a	1.48438 a	1.45165 a	1.56450 b
12	0.98470 ab	1.08568 cd	1.08063 fg	1.24183 ef

* In a column , means followed by a common letter are not significantly different at the 5% level by DMRT.

Average of 4 replications

APPENDIX III

Classification of shiitake mushroom

Shiitake mushroom also known as Haerg-Ko in Chinese, Pyoko in Korea, a scientific name as *Lentinus edodes* (Berk.) Sing, is accepted as a formal taxonomic Kingdom as follows:

Kingdom Fungi
Division Eumycota
Subdivision Basidiomycotina
Class Basidiomycetes
Subclass Hymenomycetes
Order Agaricales
Family Tricholomataceae
Genus *Lentinus*
Species *Lentinus edodes*

Modified from Ainsworth (1973) ; Przybylowicz and Donoghue (1988) ; Alexopoulos (1996).

Morphology of shiitake mushroom (Chinese Darr Moo Qigong Sanitorium, 1997 ; Fujian Pingnan Dachang Foodstuff, 1998 ; Health Network, 1988).

Either shiitake mushroom is grown usually on logs in the forests or on farm by heavy watering, shading and manipulating of growing environments, also have a morphology as following.

Cap/Pileus spheroidal ; wide about 5-12 cm., the top flat or slightly convex (umbrella shape caps), cuticle color is brownish or tan to purple brown, has crackle

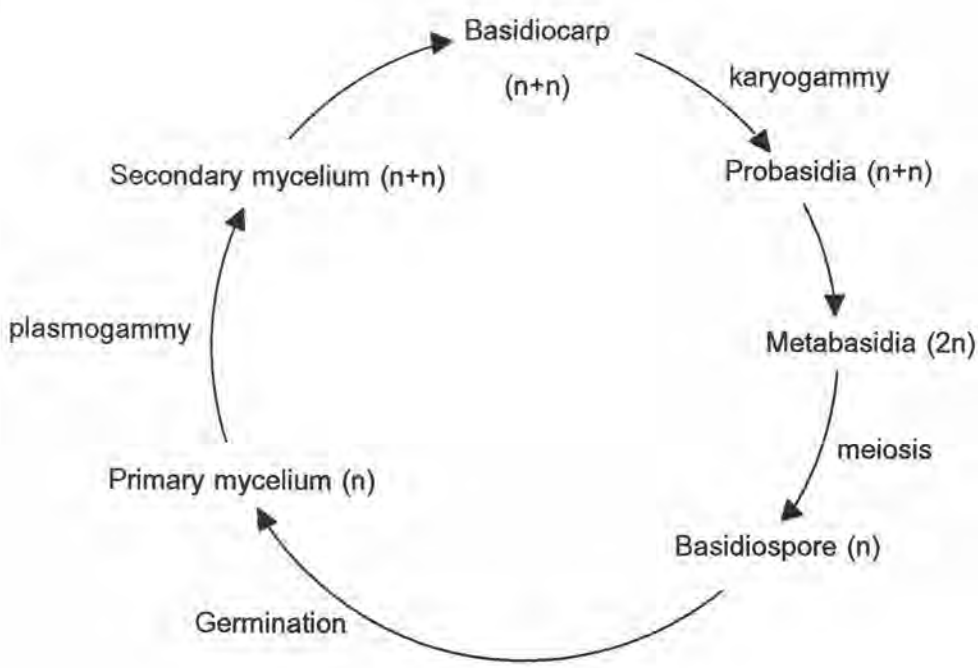
on its cap which is broken irregularly with white gill exposed, tasty, thick and meaty cap, mild in nature and fragrant.

Gill/Lamellae white gill, gill trama is a colorless , thick wall mycelia, size 5-7 μm, edge of gill is serrate to denticulate.

Stipe size 3-5 x 8-13 mm., hard and sticky stalk

Spore size 3.0-3.5 x 5.5-6.5 μm, subcylindric nonamyloid shape, 1 basidia consists of 4 basidiospores, no cystidia on hymenium.

Life cycle of shiitake mushroom



Life cycle of shiitake mushroom have an important stages (Solaya Suksa-Ard, 1995) as following :

1. Mature of basidiospores were released from pileus by wind dispersing and drop on a suitable of germinating environments.

2. Primary or monokaryotic mycelium were grown rapidly which have no clamp connection of haploid nuclear (n).

3. When have a suitable genetics factor, cytoplasm and nucleus of primary mycelium will fuse together and become a secondary mycelium which is dikaryon or heterokaryon (n+n). In this manner, clamp connection was produced.

4. When having a suitable of growing environments, e.g. temperature, shading, air, pH and nutrients; secondary mycelium will compact as primordium or pin and then grow tube fruiting body later. When mature fruiting body, 2 nuclei of each cells on the gill were fused divided to 4 nuclei by meiotic division nuclear and become basidiospore.

APPENDIX IV

Chemical Preparation of solutions and buffers used in DNA extraction.

- 1M Tris-HCl, pH 8.0

Recept. : per litre

Ref : Sambrook *et al.* (1989).

Tris 121.1 g are dissolved in 800 ml of DW Adjust the pH to 8.0 with conc. HCl. Adjust the volume of the solution to 1.0 litre and sterilize by autoclaving at 121°C for 20 min.

- 0.5 M EDTA, pH 8.0

Recept. : per litre

Ref : Sambrook *et al.* (1989).

1M Tris-HCl, pH 8.0	10	ml
0.5 M EDTA	1	ml

These chemicals are dissolved in DW Adjust the volume of the solution to 1.0 litre and sterilize by autoclaving at 121 °C for 20 min.

- Phenol

Ref : Sambrook *et al.* (1989).

Remark : Before use, phenol must be equilibrated to pH > 7.8 because DNA will partition into organic phase at acid pH.

Allow phenol to warm to room temperature, and then melt it at 68 °C. Add 8-hydroxyquinoline to a final concentration of 0.1%. To the melt phenol, add an equal volume of 1 M Tris-HCl (pH 8.0) at room temperature. Stir the mixture on a magnetic

stirrer for 15 min, and when the two phases have separated, aspirate as much as possible of the upper (aqueous) phase using a glass pipette. Add an equal volume of 0.1 M Tris-HCl (pH 8.0) to the phenol. Stir the mixture on a magnetic stirrer for 15 min, and remove the upper aqueous phase. Repeat the extractions several times until the pH of the phenol phase is > 7.8 (as measured with pH paper). After the phenol is equilibrated and the final aqueous phase has been removed, add 0.1 volume of 0.1 M Tris-HCl (pH 8.0) containing 0.2% β - mercaptoethanol. The phenol solution may be stored in this from under 100 mM Tris-HCl (pH 8.0) in a light-tight bottle at 4 °C for a periods of up to one month.

- Phenol : Chloroform (1:1, v/v)

Ref : Sambrook *et al.* (1989).

Mix an equal amount of phenol and chloroform. Equilibrate the mixture by extracting several times with 0.1 M Tris-HCl (pH 7.6). Store the equilibrated mixture under an equal volume of 0.1 M Tris-HCl (pH 7.6) in dark glass bottles.

- Chloroform : Isoamylalcohol (24:1, v/v)

Ref : Sambrook *et al.* (1989).

A mixture consists of chloroform and isoamylalcohol (24:1, v/v). The mixture is stable and may be stored in a closed light-tight bottle at room temperature.

- 2 N NaOH

Recept. : per 100 ml

Ref : Sambrook *et al.* (1989).

NaOH 8.0 g are dissolved in DW. Adjust the volume of the solution to 100 ml. Sterilize by autoclaving at 121 °C for 20 min.

- 5 M NaCl

Recept. : per 500 ml

Ref : Sambrook *et al.* (1989).

NaCl 146.1 g are dissolved in DW. Adjust the volume of the solution to 500 ml. Sterilize by autoclaving at 121 °C for 20 min.

- 10% SDS

Recept. : per 100 ml

Ref : Sambrook *et al.* (1989).

SDS 10 g are dissolved in 80 ml of sterile DW. Then heat to 68°C to assist dissolution. Adjust the volume of the solution to 100 ml with sterile DW. There is no need to sterilize SDS.

- 3 M Sodium acetate, pH 5.2

Recept. : per 100 ml

Ref : Sambrook *et al.* (1989).

$CH_3COONa.3H_2O$ 40.827 g are dissolved in 80 ml of DW. Adjust the pH to 5.2 with glacial acetic acid. Adjust the volume of the solution to 100 ml. Sterilize by autoclaving at 121°C for 20 min.

- 0.5 M Na_2EDTA, pH 8.0

Recept. : per litre

Ref : Sambrook *et al.* (1989).

Na_2EDTA 186.1g are dissolved in 800 ml of DW. Adjust the pH to 8.0 with NaOH (≈ 20 g of NaOH pellets). Adjust the volume of the solution to 1.0 litre. Sterilize by autoclaving at 121°C for 20 min.

- 20% sarkosine

Recept. : per 5.0 ml

Ref : Sambrook *et al.* (1989).

N-Lauryl sarkosyl 1.0 g are dissolved in DDW. Adjust the volume of the solution to 5.0 ml.

- DDW

Recept. : per litre

Ref : Sambrook *et al.* (1989).

DPEC 1 ml are dissolved in DW. Adjust the volume of the solution to 1.0 litre and stored overnight. Sterilize by autoclaving at 121°C for 60 min.

-50 mM EDTA

Recept. : per 1.0 ml

0.5M EDTA 100 μl are dissolved in sterile DW. Adjust the volume of the solution to 1.0 ml.

- DNA extraction buffer

Recept. : per 100 ml

Ref : Sambrook *et al.* (1989).

1M Tris-HCl, pH7.6	5 ml
5M NaCl	1 ml
0.5M EDTA	1 ml
1% SDS	1 g
3% Bentonite	3.3 ml

Dissolved 1M Tris-HCl, pH7.6, 5M NaCl and 0.5M EDTA in 95.7ml of DDW. Sterilize by autoclaving at 121°C for 20 min. Then add 1% SDS and 3% bentonite in the solution. There is no need to sterilize SDS and bentonite.

- Urea extraction buffer

Recept. : per 50 ml

Ref : Cherdshewasart (1991).

Urea	21 g
5M NaCl	3.125 ml
1M Tris-HCl, pH 8.0	2.5 ml
0.5M Na_2EDTA, pH 8.0	2.0 ml
20% Sakosine	2.5 ml

These chemicals are dissolved in sterile DDW. Adjust the volume of the solution to 50 ml. Do not autoclave (autoclave the bottle before use).

- SDS lysis buffer

Recept. : per 1.0 ml

Ref : Lee and Taylor (1990).

50mM Tris-HCl, pH 7.2

50mM EDTA

3% SDS

1% 2-mercaptoethanol

These chemicals are dissolved in sterile DW. Adjust the volume of the solution to 1.0 ml.

- 2x CTAB extraction buffer

Recept. : per 200 ml

Ref : Sambrook *et al.* (1989).

CTAB	4 g
1M Tris-HCl, pH	20 ml
0.5M EDTA	8 ml
5M NaCl	56 ml
PVP	2 g

These chemicals are dissolved in sterile DW. Adjust the volume of the solution to 200 ml. There is no need to sterilize CTAB.

-1x CTAB extraction buffer

Ref : Sambrook *et al.* (1989).

Mix an equal amount of 2xCTAB extraction buffer and sterilize DW (1:1). Then heat the solution to assist dissolution. There is no need to sterilize CTAB.

- 10% CTAB precipitation buffer

Recept. : per 20 ml

Ref : Sambrook *et al.* (1989).

These chemical are dissolved in sterilize DW. Adjust the volume of the solution to 20 ml. There is no need to sterilize CTAB.

- 1% CTAB precipitation buffer

Recept. : per 100 ml.

CTAB	1 g
1M Tris.HCl, pH 8.0	5 ml
0.5M EDTA	2 ml

These chemicals are dissolved in sterile DW. Adjust the volume of the solution to 100 ml. There is no need to autoclave CTAB.

- 1M NaCl-TE

Recept. : per 50 ml

Ref : Sambrook *et al.* (1989).

5M NaCl	10 ml
1M Tris.HCl, pH 8.0	500 ml
0.5M EDTA	100 μl

These chemicals are dissloved in sterile DW. Adjust the volume of the solution to 50 ml.

BIOGRAPHY

Miss Vachiraphorn was born on March 20, 1973 in Petchaburi Province, Thailand. She finished her primary education from Benchamathepulit School in 1987 and secondary education from Prommanusorn School in 1990. She entered Faculty of Science, Silpakorn University in 1991, and graduated with a Bachelor of Science in Biology in 1994. She began working on Master's degree in Genetics at Department of Botany, Faculty of Science, Chulalongkorn University since 1996. She was a recipient of scholarships from NSTDA in 1997 and UDC in 1998.