食藥用真菌-蓮花菌菌絲體及多醣體發酵產成之研究

王懿丞、徐泰浩

E-mail: 9300033@mail.dyu.edu.tw

摘 要

摘 要 蓮花菌(Grifola frondosa)又稱灰樹花、舞茸等，屬於非褶菌目、多孔菌科、豬苓屬，是木生腐敗菌種的好氣性真菌，其多醣體具有抗腫瘤、降血壓、降血脂、治療肝炎、減肥、糖尿病等功效，以具有β-1,6支鏈之β-1,3葡聚糖為其生物活性主要成份，在其子實體及菌絲體均有此結構的多醣體。一般人工栽培由接種到生成子實體需要3個月以上的時間，應用發酵技術生產菌絲體及生物多糖體，具有發酵時間短、品質穩定等優點。本研究主要探討：（一）、篩選不同品系之最適產醣蛋白質之菌株，並分析其游離胺基酸、總胺基酸、主要元素（C, N, O）含量及酵素群活性；（二）、探討蓮花菌在搖瓶與靜置培養時，在不同培養條件下對生物質量、胞內與胞外粗多醣蛋白質複合物之影響；（三）、比較合成、半合成培養基培養前後之粗多醣蛋白質複合物與游離胺基酸差異；（四）、以搖瓶培養探討產程變化，並再擴展到5L、20L發酵槽階段。 研究結果顯示：不同品系蓮花菌株比較，在PDA培養時以CCRC 36434生長直徑對大，以PDB與基礎培養基培養以CCRC 36355胞內多糖體產量最高，以PDB培養胞外多糖體以CCRC 36357最高；以游離胺基酸含量，顯示CCRC 36355含量最高。總胺基酸以CCRC 36434含量最高；酵素群譜分析，以胞內酵素群酵素含量最高，不同品系蓮花菌株酵素含量和種類不相同。振盪培養時，胞內多糖體以碳源為4%葡萄糖、氮源為0.1%草酸胺、其它添加物為0.15%磷酸氫鉀能有最高產量；游離胺基酸含量在碳源為3%蔗糖、氮源為0.2%草酸銨、其它添加物為0.45%磷酸氫鉀最佳。半化學合成培養基氮源含量，以胞內多糖體以0.2%蛋白?、胞外多糖體以0.4%酵母萃出物、菌絲體生物質量以0.8%胰蛋白最佳。靜置培養時，胞內多糖體以碳源為3%果糖與氮源為0.2%硝酸銨最佳；游離胺基酸含量在碳源為4%甘露糖醇、氮源為0.4%草酸銨最高。探討產程，以搖瓶培養EPS在第11天最高，以5公升、 20公升發酵槽皆在第5天達最高量。在5公升發酵槽培養之游離胺基酸含量以第2天最高，隨時間延長，胺基酸含量減少。胞外多糖體與胞內多糖體在經陰離子交換樹脂可得正電荷之多糖體蛋白質複合物；胞內多糖體有少許負電荷之多糖體蛋白質複合物

關鍵詞 : 藥用真菌 ; 蓮花菌 ; 多醣體 ; 菌絲體 ; 浸液發酵 ; 培養基 ; 分離純化

目錄

ABSTRACT Grifola frondosa in Japan as maitake （dancing mushroom）, in China as gray tree flower, it is a Basidiomycete fungus belonging to the order Aphyllopherales, and family Polyporaceae, as a white-rot and acreobe fungus. It is polysaccharides have been reported in many research articles include antitumor, immunological enhanc- ement, antidiabetic and anti-HIV, etc. A β-(1-3)-linked glucan with branches of β-(1-6)-D-glucose showing the main of pharma- cological activity has been isolated from fruit bodies and mycelium. By synthetic-log cultivation, when the young mycelium grown to fruit body need of three months.according to the related studies, employing mycelium of the submerged culture to grow the fungus has the advantages of the shorter growth time, better product quality, and lower cost. This study investigates the process of the growth of Grifola frondosa in terms of the following issues：（1）to screen different strains producing polysaccharides and analysis of free amino acid、total amino acid、main element (C、N及O)、enzyme activity；（2）studies biomass、extracellular polysaccharide and intracellular polysaccharide under shaking and static bottles；（3）to compare of the free amino acid and polysaccharides by the chemical and semi-chemical medium；（4） effect of submerged fermentation in shaking bottles, and to expand of 5 and 20L fermentor. The study shows that, in PDA culture, the CCRC 36434 have the best growing speed of the colony, in PDB and base medium culture, the CCRC 36355 have best yields of intracellular poly- saccharides, CCRC 36357 which yields the highest content of extracellular polysaccharide；in content of free amino acid, shows the higher of CCRC 36355；in content of total amino acid by mycelium, the higher of CCRC 36434；in api-ZYM system, intracellular enzyme have higher activity；in shaking culture by chemical medium , the highest intracellular polysaccharides is achieved under the condition of 4％glucose, 0.1％ammonium oxalate, 0.15％potassium phosphate, the highest the free amino acid is achieved under the condition of 3％sucrose, 0.2％ammonium oxalate, 0.45％potassium phosphate；in shaking culture by semi- chemical medium , the highest intracellular polysaccharides is achieved under the condition of 0.2％peptone, the highest extra- cellular polysaccharides is 0.4％yeast extract, the highest the mycelium biomass is 0.8％tryptone；in static culture, the highest intracellular polysaccharides is achieved under the condition of 3％fructose, 0.2％ammonium nitrate, the highest the free amino acid is achieved under the condition of 4％mannose, 0.4％ammonium oxalate ；studies submerged fermentation, the higher extracellular polysaccharides on day 11 by shaking culture and 5、20L fermentor on day 5；in 5L fermentor, free amino acid have best yield on day 2, the free amino acid follow time to decreased. extracellular

polysaccharides and intracellular polysaccharides contain acidic glucan by ion exchange chromatography and intracellular polysaccharides contain less basic glucan.

參考文獻

參考文獻 1. 王伯徹、邱世浩及黃仁彰。1998。食用菇保健食品專輯。食 品工業月刊 30（5）:1-35。 2. 王 謙、李育岳、冀 宏、汪 虹、汪 麟、楊小仙。1998。三 種大型真菌深層培養產物的開發應用及有關檢測。河北省 科學院學報 16（1）:10-14。 3. 王培銘。2002。食藥用菇液態培養製程之開發。食品工業 34 （5）:31-35。 4. 王伯徹、黃仁彰。2002。靈芝與樟芝之研發與市場面面觀。 食品工業 34（5）:3-17。 5. 王增池、孔德平和田曉亮。1995。灰樹花的生態條件與藥用 價值。中國食用菌15（3）:31-33。 6. 水野卓和川和正允編著，賴慶亮譯。1996。舞茸。菇類的化 學、生化學。國立編譯館。台灣，台北。pp265-278。 7. 方維明、魯茂林、汪志君。1999。營養因子對灰樹花多醣發 酵的影響。江蘇農業研究20（4）:65-68。 8. 李幸儒和陳勁初。1999。舞茸-溫帶森林中的舞仙子。鄉間 小路 25（4）:32-33。 9. 李?冰、馮夢醒、徐繼組。1999。海藻醣在灰樹花深層發酵 的績累及多醣的提取。食品與發酵工業26（2）:11-15。 10. 李建雄;黃淑姿;端木梁 ;翁郁嘉。1995。生物化學〈上冊〉。 藝軒出版社。台灣，台北。 11. 汪維云、朱金華及吳守一。1999。香菇菌絲體在氣升式生物 反應器中的培養條件。中國食用菌 18（2）:11-13。 12. 汪維云、朱金華。1998。食用菌液體深層發酵技術研究進展 及展望。中國食用菌17（2）:11-12。 13. 汪維云、吳守一和朱金華。1999。灰樹花液體深層培養工藝 研究。生物工程學報15（3）378-382. 14. 吳經綸和林金枝。1997。灰樹花栽培馴化。食用菌學報 4 (2) : 33-39。 15. 林勝傑和謝堂州。1991。香菇菌株品系鑑定之研究-API-ZYM酵素分析系統之應用。林業試驗所研究報告季刊6 (1):21-26。 16. 林錫杰和王伯徹。2002。菇類之藥效功用及其生產研究。食 品工業 34（5）:18-30。 17. 宋愛榮、郭立忠及段方猛。1999。pH 對灰樹花液體深層發 酵的影響。中國食用菌 18 (3) : 29-31。 18. 宋愛榮、郭立忠、劉作亭及畢建水。1999。七個白色金珍菇 菌株發酵液中四種胞外活性的測定與分析。中國食用菌 18 （4）:31-34。 19. 紀天保。1995。灰樹花生態環境考察。中國食用菌 14（1）: 37。 20. 徐錦堂。1997。灰樹花。中國藥用真菌學707-716。 21. 徐還清。1999。基質pH值對灰樹花菌絲體增值的影響。孝 感師專學報。19（4）:64-67。 22. 徐洁、陳体強、朱培根、李開本、林章余。2000。灰樹花浸 膏粉的產生及其他成分分析。江西農業大學學報22（3）: 428-430。 23. 許瑞祥和王西華。(1986)。利用API- ZYM酵素分析系統進行靈芝屬菌株分類之研究。中國農業化學會誌24 (2):103-109。 24. 陶雪娟、徐崇敬、宋鳳菊、張建敏、陳建華。1999。蕈菌液 體生物發酵技術的研究現狀與進展。上海農學院學報 17 （2）:141-147。 25. 陳隆鐘。1997。舞菇生物學之研究(一) 舞菇菌絲生理、生 態之研究。台灣洋菇雜誌。 26. 張彥與郭倩。2002。灰樹花菌絲體與子實體的營養功能成 分。食品科學23（1）:137-139。 27. 張雅雯。2002。化學合成與天然浸液培養基及培養溫度對蓮花菌菌株發酵產裎對菌絲體及多醣體之影響。 28. 孫希雯和朱明光。1999。灰樹花深層培養基的最優化及一種 胞外粗多糖快速測定方法的建立。天津輕工業學院學報 15 （3）: 24-28。 29. 馮慧琴、楊慶堯、糜可、沈涌。2000。灰樹花液體培養菌絲 體產多醣的研究。食用菌學報7（2）:5-10。 30. 黃幸紓。1994。灰樹花多糖及其抗癌作用。中國食用菌 13（1）:41-43。 31. 黃仁彰。1999。從本草典籍記載和現代科學驗證的觀點探索 菇類的食療價值。食品工業月刊 31（8）:48-58。 32. 黃仁彰。2000。菇類多糖體製劑的研發與應用。菌種保存研 究中心簡訊:1-10。 33. 黃仕政、毛正倫。1997。洋菇菌絲體之品質評估。食品科學 24（1）:44-55。 34. 勞準均、關三弟、藏珍娣及宋士良。1997。灰樹花多糖的抗 腫瘤作用及對巨噬細胞、自然殺傷細胞的影響。上海農業 學報 13（1）:25-30。 35. 雷德柱、高大維、于淑娟。2001。糖質溶液中發酵產生灰樹 花胞外多糖體的研究。微生物學學報28（3）:15-19。 36. 裘娟萍、孫培龍、鍾衛鴻。1997。灰樹花高產栽培及營養分 析。中國食用菌17（3）:31-33。 37. 楊芳鏘和蔣明哲。2001。菌絲狀真菌之深層培養技術。化工 技術 9（2）:176-186。 38. 劉松青和武成榮。1998。灰樹花栽培技術研究。中國食用菌 18 (1) : 16。 39. 賴進此。2002。菇類機能性成分的分離與純化。食品工業 34（5）:36-48。 40. Adachi, Y., Ohno, N., Ohsawa, M., Oikawa, M. and Yadomae, T. 1990. Macropgage activation in vito by chemically cross-linkred (1→3)- β -D-Glucans. Chem. Pharm. Bull. 38(4) : 988-992. 41. Adachi, Y., Ohno, N. and Yadomae, T. 1998. Activation of murine kupffer cell by administration with gel-forming (1→ 3)- β -D-Glucan from Grifola frondosa. Biol. Pharm. Bull. 21(3) : 278-283. 42. Adachi, Y., Okazaki, M., Ohno, N. and Yadomae, T. 1994. Enhancement of Cytokine Production by Macrophage Stimnlated with (1→3)- β -D- Glucan, grifola (GRN), Isolated Grifola Frondosa. Biol. Pharm. Bull. 17(12) : 1554-1560. 43. Buchalo, A. S., Wasser, S. p., Reshetnikov, S. V. and Grigansky A. P. 1999. Studies on microstructures of vegetative mycelium in the medicinal mushrooms Hericium erinaceus （Bull.:Fr.） pers. and Grifola frondosa (Dick .:Fr) S. F. Gray (Aphyllophoromycetideae). Int. J. Med.l Mushrooms. 1 :235-241. 44. Chen, A. W. 1999. A Practical Guide for Synthetic-Log cultivation of Medicinal Mushroom Grifola frondosa (Dick .:Fr) S.F.Gray (Maitake). Int. J. Med. Mushrooms. 1 : 153-167. 45. Chen, A. W., Stamets, P., Cooper, R. B., Huang, N. and Han, S. 2000. Ecology, morphology, and morphogenesis in nature of edible and medicinal mushroom Grifola frondosa (Dicks. : Fr) S.F.Gray — Maitake (Aphyllophoromycetideae). Int. J. Med. Mushrooms. 2 : 221-228. 46. Choi, H. S., Cho, H. Y., Yang, H. C., Ra, K. S. and Suh, H. J. 2001. Angiotensin I-converting enzyme inhibitor from Grifola frondosa. Food Res. Int. 34 : 177-182. 47. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. Colorimetric Method for Determination of Sugars and Related Substances. Analysis Biochemistry. 28(3) : 350-356. 48. Fujihara, S., Atsuko K, Yasuo A, and Tatsuyuki S. 1995. Nitrogen-to-Protein conversion factors for some common edible mushrooms. Journal of Food Science. 60(5) : 1045-1047. 49. Hansson, G. and Seifert, G. 1987. Effects of cultivation techniques and media on yield and morphology of the basidiomycete Armillaria mellea. App. Microcil. Biotechnol. 26:468-473. 50. Hiruta, O., Futamura, T., Takebe, H., Satoh., A., Kamisaka, Y., Yokochi, T., Nakahara, T. and Suzuki, O. 1996. Optimization and scale-up of γ -linolenic acid production by Mortierella ramanniana MM 15-1 a High γ -linolenic acid producing mutant. J. Frem. Bioeng. 82:366-370. 51. Hishida, I., Nanba, H. and Kuroda, H. 1988. Antitumor activity exhibited by orally administered extract from fruit body of Grifola frondosa (Maitake). Chem. Pharm. Bull. 36(5) : 1819-1827. 52. Horio, H. and Ohtsuru, M.

2001. Maitake (Grifola frondosa) improve glucan tolerance of experimental diabetic rats. Nutr. Sci. Vitaminol. 47 : 57-63. 53. Hiroshi Y., Hiroko S., Suiseki F. and Tatsuyuki S., 1996, The chemical components of the vegetative mycelia of basidiomycetes, Nippom shokuhin jagaku kaishi, 43(6):748-755 54. Hatvani, N. 2001. Antibacterial effect of the culture fluid of Lentinus edodes mycelium grown in submerged liquid culture. International Journal of Antimicrobial Agent. 17 : 71-74. 55. Kawagishi, H., Nomura, A., Mizuno, T., Kimura, A. and Chiba, S. 1990. Isolation and characterization of a lectin from Grifola frondosa fruiting bodies. Biochimica Biophysica Acta. 1034 : 247-252. 56. Kubo, K. and Nanba, H. 1998. Modification of cellular immune responses in experimental autoimmune hepatitis in mice by maitake (Grifola frondosa). Mycoscience. 39 : 351-360 57. Kubo, K., Aoki, H. and Nanba, H. 1994. Anti-diabetic activity present in the fruit body of Grifola frondosa (Maitake). J. Biol. Pharm. Bull. 17(8) : 1106-1110. 58. Kubo, K. and Nanba, H. 1997. Anti-hyperliposis effect of Maitake fruit body (Grifola frondosa). J. Biol. Pharm. Bull. 20(7) : 781-785. 59. Lee, E. W., He, P., Kawagishi, H. and Sugiyama, K. 2000. Suppres- sion of D-Galactosamine-induced liver injury by mushrooms in rats. Biosci Biotech. Biochem. 64(9) : 2001-2004. 60. Leung, M. Y. K., Fung, K. P. and Choy, Y. M. 1997. The isolation and characterization of an immunomodulatory and anti-tumor poly- saccharide preparation from Flammulina velutipes. Immunopharma- cology 35 : 255-263. 61. Litchfield, J. H., 1967. Submerged culture of mushroom mycelium. In:Micobial Technology (Peppier, H. J., ed.), 107-114. Reinhold, New York. 62. Litchfield, J. H., 1979. Production of single cell protein for use in food and feed. In:Micobial Technology, 2nd ed. (Peppier, H. J. and Pertman, D., eds), 93-145. Academic Press, New York. 63. Lopez-barajas, M., Lopez-tamames, E. and Buxaderas, S. 1998. Improved size-exclusion high-performance liquid chromatographic method for the sample analysis of grape juice and wine polysaccha- rides . J. Chromato. A. 823 : 339-347. 64. Leatham G. F. 1983. A chemically defined medium for the fruiting of Lentinus edodes. Mycologia. 75(5) : 905-908. 65. Mayell, M. 2001. Maitake extracts and their therapeutic potential. Altern. Med. Rev. 6(1) : 48-60. 66. Mau, J.L., Lin, H. C., and Song, S.F. 2002. Antioxidand properties of several specialty mushrooms. Food. Rev. Int. （35）:519-526 67. Mau, J., Lin, H., Ma, J. and Song, Si-Fu. 2001. Non-volatile taste components of several speciality mushrooms. Food Chem.. 73 : 461-466. 68. Mizuno, T. and Zhuang, C. 1995. Maitake, Grifola frondosa : Pharmacological effects. Food Rev. Int.. 11(1) : 135-149. 69. Mizuno, T., Ohsawa, K., Hagiwara, Ni. and Kuboyama, R. 1986. Fractionation and characterization of antitumor polysaccharides from Maitake, Grifola frondosa. Agric. Biol. Chem. 50(7) : 1679-1688. 70. Nanba, H., Kodama, N., Schar, D. and Turner, D. 2000. Effects of Maitake (Grifola frondosa). glucan in HIV-infected patients. Mycoscience 41 : 293-295. 71. Nanba, H., hamaguchi, A. and Kuroda, H. 1987. The chemical structure of an antitumor polysaccharide in fruit bodies of Grifola frondosa (Maitake). Chem. Pharm. Bull. 35(3) : 1162-1168. 72. Miura, N. N., Ohno, N., Aketagawa, J., Tamure, H., Tanaka, S., Yadomae, T. 1996. Blood clearance of (1→3)- β -D-Glucan in MRL lpr/lpr mice. FEMS Immunology and Medical Microbiology 13 : 51-57. 73. Nakai, R., Masui, H., Horio, H. and Ohtsuru, M. 1999. Effect of Maitake (Grifola frondosa) water extract on inhibition of adipocyte conversion of C3H10T1/2B2C1 cells. J. Nutr. Sci. Vitaminol. 45 : 385-389. 74. Nakanishi, I., Kimura, K., Suzuki, T., Ishikawa, M., Banno, I. and Sakane, T. 1976. Demonstration of curdlan-type polysaccharide and some other β - (1→3)- D-Glucan in microorganisms with aniline blue. J. Gen. Appl. Microbiol. 22 : 1-11. 75. Nishiwaki, T. and Hayashi, K. 2001. Purification and Characterization of a Aminopeptidase from the Edible Basidiomycete Grifola frondosa. Biosci. Biotechnol. Biochem. 65(2) : 424-427. 76. Nonaka, T., Hashimoto, Y. and Takio, K. 1998. Kinetic Characterization of Lysine-Specific Metalloendopeptidases from Grifola frondosa and Pleurotus ostreatus Fruiting Bodies. J. Biochem. 124 : 157-162. 77. Nonaka, T., Ishikawa, H., Tsumuraya, Y., Hashimoto, Y., Dohmae, N. and Takio, K. 1995. Characterization of a Thermostable lysine-Specific Metalloendopeptidase from the Fruiting Bodirs of a Basidiomycete, Grifola frondosa. J. Biochem. 118 : 1014-1020. 78. Nora, H. 2001.Antibacterial effect of the culture fluid of Lentinus edodes mycelium grown in submerged liquid culture. Int. J. of antimicrobial. （17）:71-74. 79. Ohno, N., Adachi, Y., Suzuki, I., Sato, K., Oikawa, S. and Yadomae, T. 1986. Characterization of antitumor glucan obtained from liquid-cultured Grifola frondosa. Chem. Pharm. Bull. 34(4) : 1709-1715. 80. Ohno, N., Hayashi, M., Suzuki, I., Oikawa, S., Sato, K., Suzuki, Y. and Yadomae, T. 1986. Effect of activation or blockade of the phagocytic sustem on the antitumor activity of Grifolan. Chem. Pharm. Bull. 34(10) : 4377-4381. 81. Ohno, N., Egawa, Y., Hashimoto, T., Adachi, Y. and Yadomae, T. 1996. Effect of β -Glucans on the Nitric Oxide Synthesis by Peritoneal Macrophage in Mice. Biol. Pharm. Bull. 19(4) : 608-612. 82. Ohno, N., Iino, K., Suzuki, I., Oikawa, S., Sato, K., Mitazaki, T. and Yadomae, T. 1985. Neutral and acidic antitumor polysaccharides extracted from cultured fruit bodies of Grifola frondosa. Chem. Pharm. Bull. 33(3) : 1181-1186. 83. Ohno, N., Suzuki, I., Oikawa, S., Sato, K., Mitazaki, T. and Yadomae, T. 1984. Antitumor activity and structural characteriza- tion of glucan extracted from cultured fruit bodies of Grifola frondosa. Chem. Pharm. Bull. 32(3) : 1142-1151. 84. Ohno, N., Adachi, Y., Ohsaea, M., Sato, K., Oikawa, S. and Yadomae, T. 1987. Conformational Changes of the Two Different Conformers of Grifolan in Sodium Hydroxide, Urea or Dimethysulfoxide Solution. Chem. Pharm. Bull. 35(5) : 2108-2113. 85. Papagianni, M., Mattey, M. and Kristiansen, B. 1999. Hyphal vacuolation and fragmentation in batch and fed-batch culture of Aspergillus niger and its Relation to citric acid production. Process. Biochem. 35:359-366. 86. Pecina, R. and Bone, G. 1984. High-performance liquid chromato- graphic elution behavior of alcohols, aldehydes, ketones, organic, and carbohydrates on a strong cationexchange stationary phase. J. Chromat.. 287 : 245-258. 87. Rao, P. and Pattabiraman, T. 1989. Reevaluation of the phenol- sulfuric acid reaction for the estimation of hexoses and pentoses. Anal. Biochem.. 181 : 18-22. 88. Riley, G. L., K. G. Tucker, G. C. Paul, C. R. Thomas. 2000. Effect of biomass concentration and mycelial morphology on fermentation broth rheology. Biotechnology and bioengineering. 68(2) : 160-172. 89. Roukas, T., Maria L.-K. 1999. Production of pullulan from beet molasses by Aureobasidium pullulans in a stirred tank fermentor. Journal of Food Enginerring. 40 : 89-94. 90. Shen, Q. 2001. Molecular phylogenetic analysis of Grifola frondosa（Maitake）and related species and the influence of selected nutrient supplements on mushroom yield.The pennsulvania state university. 91. Shu, C. H., Chen. Y. C., and Hsu. Y. C. 2002. Effects of citric acid on cell growth and schizophyllan formation in the submerged culture of Schizopgyllum commune. J. Chin. Inst. Chem. Engrs. 33

(3):315-320. 92. Saito, K., Yamazaki, H., Ohnishi, Y., Fujimoto, S., Takahashi, K and Hjorinouchi, S. 1998. Production of trehalose synthaes from a Basidiomycete, Grifola frondosa, in Escherichia coli. Microbiol. Biotech.. 50 : 193-198. 93. Seto, M., Nishibori, K.,Masai, E., Fukuda, M.& Ohdaira,Y. 1999. Degradation of polychlorinated biphenyla by a ‘ Maitake ’ mushroom, Grifola frondosa. Biotechnology letter . 21 : 27-31. 94. Saito, K., Kase, T., Takahashi, E., Takahashi, E. and Horinouchi, S. 1998. Purification and Characterization of a Trehalose Synthase from the Basidiomycete Grifola frondosa. Microbiology, Nov : 4340-4345. 95. Seguchi, M., Morimoto, N., Abe, M. and Yoshino, Y. 2001. Effect of Maitake (Grifola frondosa) Mushroom Powder on Bread Properties. Journal Of Food Science. 66 (2) : 261-264. 96. Smith, M. D. and Ho, C. S. 1985. The effect of dissolved carbon dioxide on Penicillin production:Mycelial Morphology. J. Biotechnol. 2:347-363. 97. Suzuki, I., Hashimoto, K., Oikawa, S., Sato, K., Osaea, M. and Yadomae, T. 1989.Antitnmor and immunomodulating activities of a β-Glucan obtained from liquid-cultured Grifola frondosa. Chem. Pharm. Bull. 37(2) : 410-413. 98. Sugiyama, K., Saeki, S., Tanaka, A., Yoshida, S., Sakamoto, H. and Ishiguro, Y. 1992. Hypocholesterolemic activity of Ningyotake (Polyporus confluens) mushroom in rats. J. Jpn. Soc. Nutr. Food. Sci. 45(3) : 265-270. 99. Seto, M., Nishibori, K., Masai, E., Fukuda, M.& Ohdaira, Y. 1999. Degradation of polychlorinated biphenyla by a ‘ Maitake ’ mushroom, Grifola frondosa. Biotechnology letter . 21 : 27-31. 100. Shimaoka, I., Kodama, J., Nishino, K. and Itokawa, Y. 1993. Purification of a copper binding peptide from the mushroom Grifola frondosa and its effect on copper absorption. J. Nutr. Biochem. 4 : 3338. 101. Takashi, M. and Cun, Z. 1995. Maitake, Grifola frondosa : Pharmacological Effects. Food Rev. Int.. 11(1):135-149. 102. Tan, Y. H., David, M. 1992. Convenient and effective methods for in vitro cultivation of mycelium and fruiting bodies of Lentinus edodes. 96(12) : 1077-1084. 103. Young, S. and Jacobs, R. 1998. Sodium hydroxide-induced confor- mational chang in schizophyllan detected by the fluorescence dye, aniline blue. Carbohydr. Res. 310 : 91-99. 104. Zhuang, C., Mizuno, T., Ito, H., Shimura, K., Sumita, T. and Kawade, M. 1994. Fractionation and antitumor activity of polysa-ccharides from Grifola frondosa mycelium. Biosci Biotech. Biochem. 58(1) : 188-188. 105. Zhuang, C., Mizuno, T., Ito, H. and Shimura, K. S. 1994. Chemical modifiction and antitumor activity of polysaccharides from the mycelium of liquid-cultured Grifola frondosa. Nippon Shokuhin Kogyo Gakkaishi. 41（10）:733-740. 106. Zhuang, C. and Mizuno, T. 1999. Biological responses from Grifola frondosa (Disk . : Fr.) S. F. Gray - Maitake (Aphyllophoromyce-tideae). Int. J. Med. Mushr. 1 : 317-324.