

不同培養條件對舞菇多醣生產之探討

曾美華、謝建元；陳明造

E-mail: 9318442@mail.dyu.edu.tw

摘要

中文摘要 本研究主要探討不同培養條件對舞菇(*Grifola frondosa*)多醣生產之影響，包括：(一)以多次在斜面繼代培養之菌絲體進行固態發酵，經57天培養出完整子實體，其子實體胞內多醣 $0.018 \pm 0.002 \text{ g/g}$ 。(二)不同氣體交換速率以不同瓶塞控制，以棉花塞菌體濃度最高 2.8 g/L 。(三)在5-L發酵槽培養時，以通入21% 氧氣下培養，比在使用40% 氧氣培養有較佳的菌體生長。上述培養基質中添加1% 橄欖油分別使用21% 氧氣與40% 氧氣培養下，菌體濃度分別從 9.29 g/L 增高至 10.05 g/L 和 6.34 g/L 增高至 14.89 g/L ，在多醣生產方面也分別從 0.92 g/L 增至 2.39 g/L 和 0.8 g/L 增至 3.00 g/L 。(四)餌料批次發酵中，葡萄糖濃度控制在5~10 g/L之間，基質同時添加1% 橄欖油進行餌料批次培養，比先前的研究，胞外多醣產量提高一倍從 2.04 g/L 增至 4.31 g/L ，天數從第13天提早至第9天而菌體濃度則無明顯差異。使用GPC進行胞內多醣分子量測定，其分子量大小介於 $17,272 \text{ KDa} \sim 948 \text{ KDa}$ 。關鍵字：舞菇、胞外多醣、餌料批次發酵、分子量

關鍵詞：舞菇；胞外多醣；餌料批次發酵；分子量

目錄

簽名頁 授權書.....	iii 中文摘要.....
.....iv 英文摘要.....	v 誌謝.....
.....vi 目錄.....	vii 圖目錄.....
.....xii 表目錄.....	xv 附錄.....
.....xvi 第一章 前言.....	1 第二章 文獻回顧.....
.....3.2.1 舞菇的由來.....	3.2.2 舞菇簡介.....
.....3.2.2.1 舞菇的分類.....	3.2.2.2 舞菇的形狀特徵.....
.....4.2.3 舞菇的生理功能.....	5.2.3.1 抗癌作用.....
.....5.2.3.2 降血壓作用.....	5.2.3.3 免疫調節作用.....
2.3.4 治療肝炎作用.....	6.2.3.5 抗愛滋作用.....
作用.....	6.2.3.6 抗氧化作用.....
.....9.2.3.7 其他生體機能.....	9.2.4 舞菇生活特性.....
.....9.2.4.1 人工栽培.....	10.2.4.2 舞菇於液態培養之優點.....
.....10.2.4.3 液態發酵培養舞菇的環境影響因子.....	11.2.5 菌絲球的形成與構造.....
.....15.2.6 多醣之定義.....	16.2.6.1 多醣的分類.....
2.6.2 多醣之回收方法.....	16.2.6.2.1 酯類提取法.....
多醣之測定方法.....	17.2.6.3 水溶性粗
析法.....	17.2.6.3.1 酚硫酸法.....
.....18.2.7 餌料批次式操作.....	17.2.6.3.2 分子篩高效能液相層
.....19 第三章 材料與方法.....	18.2.7.1 餌料批次式發酵的優點.....
.....21.3.1.1 菌株.....	21.3.1 實驗材料.....
.....21.3.1.3 實驗器材.....	21.3.1.2 實驗藥品.....
平板培養.....	22.3.2 實驗方法.....
.....24.3.2.3.1 G. frondosa 子實體栽培試驗.....	23.3.2.1 23.3.2.3 瓶裝固態發酵...
.....24.3.2.4.1 G. frondosa 在液態搖瓶培養階段菌體濃度與多醣產量之變化.....	24.3.2.4.2 發酵期間
.....25.3.2.4.3 橄欖油的添加與發酵期間通氣量	24.3.2.4.3 橄欖油的添加與發酵期間通氣量
對G. frondosa 在搖瓶中生長之影響.....	25.3.2.4.3 橄欖油的添加與發酵期間通氣量
對G. frondosa 在搖瓶中生長之影響.....	25.3.2.5.1 不
同通氣量對G. frondosa 於發酵槽中批次發酵之影響.....	26.3.2.5.2 不同氧氣濃度對G. frondosa 於
發酵槽中批次發酵之影響.....	26.3.2.5.3 不同氧氣濃度與橄欖油的添加對G. frondosa 於發酵槽中
.....27.3.2.6 發酵槽餌料批次式發酵.....	27.3.2.6.1 探討橄欖油對G. frondosa 於
.....27.3.2.6.2 改變通氣量對G. frondosa 於發酵槽中餌料批次發酵之	27.3.2.6.2 改變通氣量對G. frondosa 於發酵槽中餌料批次發酵之
變化.....	27.3.2.6.3 不同氧氣濃度對G. frondosa 於發酵槽中餌料批次發酵之影響.....
.....28.3.3 分析方法.....	28.3.3.1 pH.....
.....28.3.3.2 功率.....	29.3.3.3 菌體濃度.....
	29.3.3.4 基

質殘醣量.....	29	3.3.5 發酵液黏度.....	30	3.3.6 舞菇胞外多醣.....	
.....30	3.3.6.1 酚硫酸法.....	30	3.3.6.1.1 標準曲線製作步驟.....		
.....30	3.3.6.1.2 胞外多醣濃度.....	31	3.3.6.2 胞外多醣分子量分佈.....	31	3.3.7 舞 菇胞內多醣.....
.....32	3.3.7.1 胞內多醣濃度.....	32	3.3.8 動力參數命名法.....		
.....33	第四章 結果與討論.....	34	4.1 瓶裝固態發酵.....		
.....34	4.1.1 <i>G. frondosa</i> 子實體栽培試驗.....	34	4.2 搖瓶液態發酵.....		
.....38	4.2.1 <i>G. frondosa</i> 在液態搖瓶培養階段菌體濃度與多醣產量之變化.....	38	4.2.2 發酵期間通氣量對 <i>G. frondosa</i> 在搖瓶中生長之影響.....	40	4.2.3 添加橄欖 油與發酵期間不同通氣量對 <i>G. frondosa</i> 在搖瓶中生長之影響.....
.....43	4.3 發酵槽批次式液態發酵.....	43	4.3.1 不同通氣量對 <i>G. frondosa</i> 於發酵槽中批次發酵之影響.....	47	
.....53	4.3.2 不同氧氣濃度對 <i>G. frondosa</i> 於發酵槽中批次發酵之影響.....	53	4.3.3 不同氧氣濃度與 橄欖油的添加對 <i>G. frondosa</i> 於發酵槽中批次發酵之影響.....		
.....59	4.4 發酵槽餌料批次式發酵.....	68	4.4.1 變更通氣量對 <i>G. frondosa</i> 於發酵槽中餌料批次發酵之影響.....	68	
.....74	4.4.2 探討 添加橄欖油對 <i>G. frondosa</i> 於發酵槽中餌料批次發酵之影響.....	74	4.4.3 不同氧氣濃度對 <i>G. frondosa</i> 於發酵槽中餌料批次發酵之影響.....		
.....78	第五章 結論.....				
.....86	參考文獻.....	87			

參考文獻

參考文獻 1.水野卓、川合正允(賴慶亮譯) (1997) 菇類的化學、生化學，國立編譯館，台北。 2.尤新 (2001) 機能性發酵製品，藝軒圖書出版社。 3.王伯徹 (1990) 藥用真菌系列報導(一)靈芝，食品工業，22(1):23-33。 4.王伯澈 (2001) 保健用菇的生理活性物質，農業世界雜誌，(210):56-59。 5.王伯徹、陳啟楨、華傑 (1998) 食藥用菇類的培養與應用，財團法人食品工業發展研究所報告-第M87-019號，187頁。 6.王進琦、賴敏男 (1998) 猴頭菇以液態浸漬培養產製水溶性多醣類之探討，食品科學，26(5):714-726。 7.王懿丞 (2003) 食藥用真菌 - 蓮花菌菌絲體及多醣體發酵產程之研究，大葉大學食品工程所碩士論文。 8.全漢霖、楊芳鏘 (1994) Fed-Batch培養技術在生化製程上之應用，製酒科技專論彙編，16:151-166。 9.汪維云、朱金華、吳守一 (1999) 香菇菌絲體在氣升式生物反應器中培養條件，中國食用菌，18(2):11-13。 10.李幸嫻、陳勁初 (1999) 食藥用菇類:舞茸，鄉間小路，25(4):32-33。 11.宋細福、許玲卿 (1996) 舞菇之開發與研究(一)，台灣省農業試驗所技術服務，25:14-17。 12.宋細福、許玲卿 (1996) 舞菇之開發與研究(二)，台灣省農業試驗所技術服務，26:5-7。 14.呂淑芳 (2002) 靈芝之機能性與其分析方法，食品工業，34(11):3-10。 15.杜雙田 (2002) 蟠蟲草灰樹花天麻高栽培新技術，中國農業出版社，北京，81-96。 16.吳國暉 (2003) 以餌料批次式發酵進行蟲草菌多醣生產之研究，大葉大學食品工程所碩士論文。 17.林佳滿 (2002) 以餌料批次式發酵行本土靈芝菌之菌體與多醣生產，大葉大學食品工程所碩士論文。 18.周碧溫 (2003) 以餌料批次式發酵行本土靈芝菌之菌體與多醣生產，大葉大學食品工程所碩士論文。 19.俞國平 (1997) 光散射與膠體滲透層析儀的聯結與應用，科儀新知，18(5):44-53。 20.紀天保 (1995) 灰樹花生態環境考察，中國食用菌，14(1):37。 21.徐泰浩、謝建元 (2001) 靈芝生物活性成分與生物活性之療養品觀，生物產業，12(2):117-135。 22.陳國朱、陳玉先 (1995) 灰樹花馴化栽培與研究，中國食用菌，14(4):24。 23.郭美娟 (1999) 天然植物--(1)舞菇、(2)大麥嫩草，新營養，24:75-78。 24.舒志遠 (1995) 姬菇栽培與加工技術，中國食用菌，14(1):36。 25.黃麗娜 (1996) 靈芝液體培養及氣舉式生化反應器應用之研究，東海大學化工所碩士論文。 26.黃麗娜 (1996) 食用菇菌絲體深層培養在食品工業上之應用，食品工業，28(9):20-26。 27.傅偉光 (1998) 高效能液相層析在食品方面之應用，科儀新知，19(4):86-95。 28.傅偉光、呂淑芳、宮昭雲 (2001) 靈芝中水溶性粗多醣分析方法之研究，台灣農業化學與食品科學，39(2):153-161。 29.黃惠琴 (2000) 檀芝菌絲體深層培養之研究，東海大學化工所碩士論文。 30.張淑芬 (2001) 食藥用菇類搖瓶液態培養條件之探討，科學與技術，33(7):39-46。 31.張志堂、楊方鏘 (2002) 不同培養條件對靈芝多醣性質之影響，第七屆生化工程研討會論文集，617-622。 32.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(*Grifola frondosa*)菌株間發酵產程菌絲體及多醣之影響，大葉大學食品工程所碩士論文。 33.賴進此 (2003) 菇類活性物質的分離及其應用，食品工業，35(5):29-38。 34.Exopolysaccharides of Some Medicinal Mushroom:Production and Composition. Internation Journal Medical Mushrooms. 2:55-61. 35.Delavega, M. G., Cejudo, F. J., and Panque, A. 1990. Product of Exocellular Polysaccharide by *Azotobacter chroococcum*. Applied Biochemistry and Biotechnology. 30:273-284. 36.Dubois, M., Gilles, K. A., Hamilton, J. K., Reber, P. A. and Smith F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 28(3):350-356. 37.Fang, Q. H., and Zhong, J. J., 2002. Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. Process Biochemistry. 37:769-774. 38.Fang, Q. H., Tang, Y. J., and Zhong, J. J., 2002. Significance of inoculation density control in production of polysaccharide and ganoderic acid by submerged culture of *Ganoderma lucidum*. Process Biochemistry. 37:1375-1379. 39.Fang, Q. H., and Zhong, J. J., 2002. Submerged fermentation of higher fungus *Ganoderma lucidum* for production of valuable bioactive metabolites-ganoderic acid and polysaccharide. Biochemical Engineering. 10:61-65. 40.Gunter, E. A., and Ovodov, Y. S. 2002. An alternate carbon source for enhancing production of polysaccharides by *Silene vulgaris* callus. Carbohydrate Research. 337:1641-1645. 41.Hsieh, C. H., and Yang, F. C., 2004. Reusing soy residue for the solid-state fermentation of *Ganoderma lucidum*. Bioresource Technology. 91:105-109. 42.Inoue, A., Kodama, N., and Nanba, H., 2002. Effect of Maitake(*Grifola*

frondosa)D-Fraction on the control of the T Lymph Node Th-1/Th-2 Proportion. *Biological & Pharmaceutical Bulletin*. 25(4):536-540. 42.Jayus, McDougall, B. M., and Seviour, R.J., 2002. Factors affecting the synthesis of (1 3) and (1 6) - β -glucanases by the fungus *Acremonium* sp. IMI 383068 grown in batch culture. *Enzyme and Microbial Technology*. 31:289-299. 43.Kim, H. J., Kim, J. H., Oh, H. J., and Shin, C. S. 2002. Morphology control of *Monascus* cells and scale-up of pigment fermentation. *Process Biochemistry*. 38:649-655. 44.Kodama, N., Komuta, K., Sakai, N. and Nanba, H. 2002. Effect of D-fraction, a polysaccharide from *Grifola frondosa* on tumor growth involve activation of NK cells. *Biological & Pharmaceutical Bulletin*. 25(12):1647-1650 45.Kubo,K.and Nanba,H.1998.Modification of cellar immune responses in experimental autoimmune hepatitis in mice by maitake (*Grifola frondosa*). *Mycoscience* 39:351-360. 46.Lee, K. M., Lee, S. Y., and Lee, H. Y. 1999. Bistage Control of pH for Improving Exopolysacchride Production from Mycelia of *Ganoderma lucidum* in an Air-Lift fermentor. *Bioscience and Bioengineering*. 88 (6) :646-650. 47.Lee, B. C., Bae, J. T., Pyo, B. H., Choe, T. B., Kim, S. W., Hwang, H. J. and Yun, J. W. 2003. Biological activities of the poltsaccharides produced from submerged culture of the edible Basidiomycete *Grifola frondosa*. *Enzyme and Microbial Technology*. 32:574-581. 48.Litchfield, J. H. 1967. Submerged culture of mushroom mycelium. In : *Microbial Technology*(Peppler, H. J., ed), Reinhold, New York, 107-144. 49.Litchfield, J. H. 1979. Production of single cell protein for use in food and feed. In: *Microbial Technology*, 2nd ed. (Peppier, H.J. and Perlman, D., eds):93-145,Academic Press, New York. 50.Liu, X., Yuan, J. P., Chung, C. K., and Chen, X. J. 2002. Antitumor activity of the sporoderm-broken germinating spores of *Ganoderma lucidum*. *Cancer Letters*. 182:155-161. 51.Matsui, K., Kodama, N., and Nanba, H., 2001. Effect of Maitake(*Grifola frondosa*)D-Fraction on the carcinoma angiogenesis. *Cancer Letters*. 172:193-198. 52.Nanba, H., Kodama, N., Schar, D, and Turner,D. 2000. Effects of maitake (*Grifola frondosa*).glucan in HIV-infected patients. *Mycoscience*. 41:293-295. 53.Park, E.Y., Koike, Y., Higashiyama K., Fujikawa, S., and Okabe, M. 1999. Effect of Nitrogen Source on Mycelial Morphology and Arachidonic Acid Production in Culture of *Mortierella alpine*. *Bioscience and Bioengineering*. 88 (1) :61-67. 54.Park,J. P., Kim, S. W., Hwang, H. J., Cho, Y. J., and Yum, J. W. 2002. Stimulatory effect of plant oils and fatty acids on the exo-biopolymer production in *Cordyceps militaris*. *Enzyme and Microbial Technology*. 31:250-255. 55.Pavlova, K., and Grigorova, D. 1999. Production and properties of exopolysaccharide by *Rhodotorula acheniorum* MC. *Food Research International*. 32:473-477. 56.Saito, K., Yamazaki, H., Ohnishi, T., Fujimoto, S., and Takahashi, S. 1998. Production of trehalose synthase from a basidiomycete, *Grifola frondosa*,in *Escherichia coli*. *Applied Microbiology and Biotechnology*. 50:193-198. 57.Sarra, M., Ison, A. P., and Lilly, M. D. 1996. The relationships between biomass concentration,determined by a capacitance-based probe,rheology and morphology of *Saccharopolyspora erythraea* culture. *Biotechnology*. 51:157-165. 58.Shen, Q., and Royse, D. J. 2001. Effect of nutrient supplements on biological efficiency, quality and crop cycle time of maitake (*Grifola frondosa*). *Applied Microbiology and Biotechnology*. 57:74-78. 59.Shen, Q., and Royse, D. J. 2002. Effect of genotypes of maitake (*Grifola frondosa*) on biological efficiency, quality and crop cycle time. *Applied Microbiology and Biotechnology*. 58:178-182. 60.Singh, S., and Fett, W. F., 1995. Stimulation of exopolysacchride production by fluorescent pseudomonads in sucrose media due to dehydration and increased osmolarity. *FEMS Microbiology Letters*. 130:301-306. 61.Sinha, J., Bae, J. T., Park, J. P., Song C. H., and Yun, J. W. 2001. Effect of substrate concentration on broth rheology and fungal morphology during exo-biopolymer production by *Paecilomyces japonica* in a batch bioreactor. *Process Biochemistry*. 29:392-399. 62.Smith, I. H. and Pace, G. W. 1982. Recovery of microbial polysaccharides. *J. of Chem. Journal of Chemical Technology and Biotechnology*. 32:119-129. 63.Stasinopoulos, S. J., and Seviour, R. J. 1990. Stimulation of exopolysacch avide production in the fungus *Acremonium persicinum* with fatty acids. *Biotechnology and Bioengineering*. 36:778-782. 64.Stasinopoulos, S. I. and Seviour, R. J. 1992. Exopolysaccharide production by *Acremonium persicinum* in stirred-tank and air-lift fermentors. *Applied Microbiology and Biotechnology*. 36:465-468. 65.Stejskal, J. and Potucek, F. 1985. Oxygen transfer in liquids. *Biotechnology and Bioengineering*. 27:503-508. 66.Tang, Y. J., and Zhong, J. J. 2002. Fed-batch fermentation of *Ganoderma lucidum* for hyperproduction of polysaccharide and ganoderma acid. *Enzyme and Microbial Technology*. 31:20-28. 67.Yang, F. C. and Liau, C. B. 1998a. Effects of cultivating conditions on the mycelial growth of *Ganoderma lucidum* in submerged flask cultures. *Bioprocess Engineering*. 19:233-236. 68.Yang, F. C. and Liau, C. B. 1998b. The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged cultures. *Process Biochemistry*. 33(5):547-553. 69.Yang, F. C. and Hwang S. Y. 1998c. Nutritional studies on submerged culture of *Ganoderma lucidum*. *Tunghai J.* 39:1-10. 70.Yang, F. C., Ke, Y. F., and Kuo, S. S. 2000. Effect of fatty acids on the mycelial growth and polysaccharide formation by *Ganoderma lucidum* in shake flask cultures. *Enzyme and Microbial Technology*. 27(3-5):295-301. 71.Young, C. S., Young, H. K., Hyun, S. L., Young, N. K. and Si, M. B. 1987. Production of Pullulan by a Fed-batch Fermentation. *Biotechnology Letters*. 9(9):621-624. 72.Umesaki, Y., Kawai, Y., and Mutai, M. 1977. Effect of Tween 80 on Glucosyltransferase Production in *Streptococcus mutans*. *Applied and Environmental Microbiology*. 34 (2) :115-119. 73.Zacchi, L., Burla, G., Zuolong, D., and Harvey, P.J. 2000. Metabolism of cellulose by *Phanerochaete chrysosporium* in continuously agitated culture is associated with enhanced production of lignin peroxidase. *Journal of Biotechnology* 78:185-192.