Scale up of submerged fermentation and effect of medium compositions on chemical characteristics of

黃思齊、徐泰浩、林芳儀

E-mail: 345329@mail.dyu.edu.tw

ABSTRACT

Trametes versicolor syn. Coriolus versicolor is a valuable medicinal fungi, it ' s polysaccharide peptide has been demonstrated having several biological functions, such as anti-tumor, anti-cancer, liver protection, antioxidant power and immune-regulation. At present, the production of batch fermentation of polysaccharide peptide of T. versicolor, the fermentation technological handles the optimum growing condition. In addition, it reduces manpower, space and shortens production time. Base on the technology, the amount is similar to the fruiting bodies. The technology avoids the contamination from heavy metals. T. versicolor mycelium and polysaccharides of the production activity due to the use of media types, concentration varies and biological activity. It will be a result by the differences. In general, the cost is higher by chemical culture medium, therefore, this study to improve higher production of polysaccharide peptide and lower the cost of the medium under the premise, agricultural products alternative chemical medium, to fit develop the natural carbon and nitrogen source of agricultural product to increase polysaccharide peptide production of T. versicolor LH1. Shake flask culture to explore the optimal carbon and nitrogen to increase the natural production of polysaccharide peptide. The result suggests using 4% sucrose and 0.3% peanut flour as carbon and nitrogen sources, which is available to higher biomass and extracellular polysaccharide peptide. In 5L fermenter culture conditions of optimum culture growth conditions, at 25 °C, 100 rpm, 1.5vvm, that ' s the most suitable conditions to the growth of mycelia T. versicolor and polysaccharide peptide with the production. To plant one ton fermentation tank training. The sterile mycelia have more loose ball, biomass and extracellular polysaccharide peptide is significantly increased. To compare the agricultural culture medium and chemical culture medium on the extracellular polysaccharide peptide composition differences. The result shows that produced in different medium are different characteristics of extracellular polysaccharide in the general composition, molecular weight, monosaccharide composition, heat resistance, functional groups, composition and proportion. The results showed that extracellular polysaccharide peptide in both culture media produced are a β-1, 3 glucan groups for the bonding with Fourier Transform Infrared Spectroscopy. Are biologically active and therefore promote the activity of the immune characteristics. The result can provide relevant health product development and application of reference in the future.

Keywords : Trametes versicolor、polysaccharide peptide、agricultural medium、chemical medium

Table of Contents

封面內頁 簽名頁 中文摘要 iii 英文摘要 v 誌謝 vii 目錄 viii 圖目錄 xii 表目錄 xiv 1. 前言 1 2. 文獻回顧 2 2.1雲芝簡介 2 2.1.1雲芝的分類 2 2.1.2雲芝的型態特徵 2 2.1.3分佈及生態環境 4 2.2雲芝多醣? 6 2.2.1雲芝多醣?化學特性 7 2.3雲芝菌的培養 8 2.3.1發酵工程 8 2.3.2液態培養 11 2.3.2雲芝菌菌絲體液態培養之培養基 13 2.4雲芝菌液態培養條件 14 2.5雲芝菌菌絲形態之影響因子 15 2.6胞外多醣?的回收 18 2.7花生的營養成分 19 2.8試藥級蔗糖、冰糖、白砂糖以及紅砂糖之差別 20 3. 材料與方法 24 3.1實驗材料 24 3.1.1實驗菌種 24 3.1.2實驗藥品 24 3.1.3儀器設備 25 3.2實驗流程與研究目的 26 3.3雲芝菌株培養 27 3.3.1菌種保存與更新 27 3.3.2母株培養 27 3.3.3液態培養 27 3.4不同培養基組成對Trametes versicolor LH1生產胞外多醣?之探討 31 3.4.1不同碳源對胞外多醣?生產之影響 31 3.4.2不同天然氮源對胞外多醣?生產之影響 31 3.5五公升發酵槽液態發酵最適生產條件之探討 31 3.5.1不同溫度對雲芝胞外多醣?之影響 31 3.5.2不同攪拌速率對雲芝胞外多醣?之影響 32 3.5.3不同通氣量對雲芝胞外多醣?之影響 32 3.6二十公升發酵槽液態發酵探討蔗糖與紅砂糖對於胞外多醣?產量之影響 32 3.7由三角錐形瓶放大培養至1頓發酵槽之產程探討 33 3.8分析方法 33 3.8.1菌絲生物質量之製備與測定 33 3.8.2胞外多醣?之製備與分析 33 3.8.3酚-硫酸法分析總醣 33 3.9基本成分分析 34 3.9.1含水量測定 34 3.9.2灰分測定 34 3.9.3粗蛋白測定 35 3.9.4粗脂肪測定 35 3.10胞外多醣?分子量分佈測定 36 3.10.1標準曲線配置 36 3.11胞外多醣?之單糖組成分析 37 3.11.1標準曲線配置 38 3.12發酵液中胞外多醣?耐熱性試驗 38 3.13傅立葉紅外線光譜儀試驗(Fourier Transform Infrared Spectroscopy，FTIR) 38 4. 結果與討論 39 4.1三角錐形瓶最適培養碳源及天然氮源之探討 39 4.1.1添加不同碳源對雲芝菌絲體、胞外多醣?產量之影響 39 4.1.2添加不同天然氮源對雲芝菌絲體、胞外多醣?之影響 41 4.2五公升發酵槽最適培養條件之探討 42 4.2.1不同培養溫度對雲芝菌絲體與胞外多醣?之影響 42 4.2.2不同攪拌速率對菌絲體與胞外多醣?之探討 49 4.2.3不同進氣量對雲芝菌絲體與胞外多醣?產量之探討 55 4.3試藥級蔗糖、冰糖、白砂糖以及紅砂糖成本之差異性 61 4.4二十公升發酵槽液態發酵探討蔗糖與紅砂糖對胞外多醣?產量之影響 61 4.5由三角錐形瓶放大培養至1噸發酵槽之產程探討 64 4.6農產品培養基與半化合培養基

所產胞外多醣?成分組成上之差異性 68 4.6.1半化合培養基與農產品培養基所產之胞外多醣?基本成分分析之比較 68 4.6.2半化合培養基與農產品培養基所產之胞外多醣?分子量分佈 70 4.6.3半化合培養基與農產品培養基所產之胞外多醣?單糖組成分析 71 4.7發酵液中胞外多醣?耐熱性試驗 79 4.8以FTIR分析PSK、PSP及農產品培養基與半化合培養基所產胞外多醣?之官能基 81 5. 結論. 84 參考文獻 85 圖目錄 圖2.1 雲芝子實體 5 圖2.2 菇類多醣?(polysaccharide-peptides)的分子結構 9 圖2.3 雲芝胞外多醣?的化學結構 10 圖2.4 蔗糖結構 22 圖3.1 實驗流程圖1 28 圖3.2 實驗流程圖2 29 圖4.1 添加不同碳源於雲芝LH1培養基中對菌絲體、胞外多醣?之影響 40 圖4.2 添加不同天然氮源於雲芝LH1培養基中對菌絲體、胞外多醣?之影響 43 圖4.3 以5L發酵槽在25°C下培養雲芝LH1之發酵產程 44 圖4.4 以5L發酵槽在28°C下培養雲芝LH1之發酵產程 45 圖4.5 以5L發酵槽在30°C下培養雲芝LH1之發酵產程 46 圖4.6 不同培養溫度對雲芝LH1生質量之影響 47 圖4.7 不同培養溫度對雲芝LH1胞外多醣?之影響 48 圖4.8 以5L發酵槽在50rpm下培養雲芝LH1之發酵產程 50 圖4.9 以5L發酵槽在100rpm下培養雲芝LH1之發酵產程 51 圖4.10 以5L發酵槽在150rpm下培養雲芝LH1之發酵產程 52 圖4.11 在不同轉速培養下對雲芝LH1生質量之影響 53 圖4.12 在不同轉速培養下對雲芝LH1胞外多醣?之影響 54 圖4.13 以5L發酵槽在0.5vvm下培養雲芝LH1之發酵產程 56 圖4.14 以5L發酵槽在1vvm下培養雲芝LH1之發酵產程 57 圖4.15 以5L發酵槽在1.5vvm下培養雲芝LH1之發酵產程 58 圖4.16 在不同通氣量培養下對雲芝LH1生質量之影響 59 圖4.17 在不同通氣量培養下對雲芝LH1胞外多醣?之影響 60 圖4.19 以試藥級蔗糖與紅砂糖為碳源對雲芝LH1胞外多醣?之影響 63 圖4.20 雲芝LH1放大產程生質量之變化 65 圖4.21 雲芝LH1放大產程胞外多醣?之變化 66 圖4.22 雲芝LH1發酵液低溫濃縮後之結果 67 圖4.23 分子量標準曲線 72 圖4.24 以半化合培養基培養雲芝LH1所產胞外多醣?(PS-EPS)分子量 73 圖4.25 以農產品培養基培養雲芝LH1所產胞外多醣?(PB-EPS)分子量 75 圖4.26 商業滅菌對不同培養基培養雲芝LH1所產胞外多醣?之影響 80 圖4.27 β-1,3-Glucan、PSK、PB-EPS、PS-EPS之FTIR圖譜分析 83 表目錄 表2.1 花生營養成分表 21 表2.2 不同精製程度之蔗糖 23 表3.1 液態培養基組成 30 表4.1 雲芝LH1胞外多醣?一般組成 69 表4.2 以半化合培養基培養雲芝菌所產胞外多醣?(PS-EPS)分子量 74 表4.3 以農產品培養基培養雲芝LH1所產胞外多醣?(PB-EPS)分子量 76 表4.4 PS-EPS與PB-EPS之單糖組成含量 78

REFERENCES

1.丁湖廣。2004。雲芝的特性及人工栽培技術。特種經濟動植物7(7) 。 2.于清偉、王明才、薛會麗、孔怡、許慶國。2009。雲芝菌絲體液體培養條件的研究。山東農業科學 11:80-82。 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.卯曉嵐。1989。中國的食用和葯用大型真菌。微生物學通報16:290-297。 5.余曉斌、繆靜、胡衛珍、陳坤、濮文林。2004。液態發酵法生產雲芝胞內糖?。食品與發酵工業30(11)。 6.吳文劍。2005。以蔗糖為原料制取高果糖漿工藝的研究:66。昆明理工大學碩士論文。昆明。 7.宋萬杰。2008。雲芝液體發酵制備多?的研究:72。江蘇大學碩士論文。江蘇。 8.李如光。1991。吉林省真菌志. 東北師範大學出版社:193-194。 9.李俊峰。2003。雲芝的生物學特性、藥理作用及應用前景。安徽農業科學31(3):509-510。 10.李會娟。2007。甘蔗制糖脫色新方法的研究:64。廣西大學碩士論文。廣西。 11.周選圍。2000。雲芝細胞核研究。實用菌學報 7(2):55-57。 12.林芳儀。2009。中草藥萃取液對雲芝胞外多醣?產量、化學特性及免疫活性之影響:105。大葉大學博士論文。彰化。 13.林曉霞、熊強、陸利霞、熊曉輝。2006。雲芝糖?的液體發酵培養基的研究。生物加工過程4(2)。 14.范文霞、蔡友華、劉學銘、肖更生、張名位、陳衛東、徐玉娟、吳娛明。2008。毛雲芝菌產漆?液體培養條件的優化。食品與生物技術學報27(3)。 15.徐錦堂。1996。中國藥用真菌學。北京醫科大學、中國協和醫科大學聯合出版社475-495。 16.秦文。2004。食用蕈菌生物技術研究進展。食品科學 25(9)。 17.翁卓。2006。黃砂糖工藝的探討。甘蔗糖業(2)。 18.高躍、袁萍、茅仁剛、陳浙江、田翠。2008。雲芝菌絲體原生質體制備與再生條件的研究。食用菌學報 15(1)。 19.張忠信、朱松、劉莉娜、吳艷榮。2007。花生的營養成分與食療方劑。中國食物與營養(11)。 20.梅娜、周文明、胡曉玉、李玲玲。2007。花生粕營養成分分析。西北農業學報16(3)。 21.陳怡倩。2001。利用批式液態培養來探討檸檬酸對裂褶菌生長及其多醣體生成影響之研究:98。中央大學碩士論文。桃園。 22.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。 23.黃志立。2009。雲芝菌絲體產SOD的深層發酵培養條件的優化。食用菌31(4)。 24.黃鈺中。2010。雲芝多醣之分離純化研究:61。大葉大學碩士論文。彰化。 25.黃麗娜。1998。菇類菌絲體深層發酵在食品工業上之應用。食藥用菇類培養與應用144-150。 26.楊士賢。2006。影響雲芝菌絲體與多醣體批次饋料發酵產程控制參數因子之探討:116。大葉大學碩士論文。彰化。 27.楊芳鏘、蔣明哲。2001。菌絲狀真菌之深層培養技術。化工技術9(2):176-186。 28.萬書波。2004。花生營養成分綜合評價與產業化發展戰略研究。花生學報33(2) 。 29.劉祖同、羅信昌。2002。食用蕈菌生物技術及應用。166-205。 30.劉德海。2006。提高蔗糖結晶效率的研究進展。廣東化學33(7) 。 31.賴佩君。2008。雲芝(Trametes versicolor)在不同發酵槽及不同培養條件對菌絲體及多醣體之影響:119。東海大學碩士論文。台中。 32.蘇慶華。1991。靈芝之分類學及生理活性物質。北醫學報20:1-16。 33.龔全、許國煥、付天璽、吳月嫦、王小玉、朱毅菲。2008。雲芝多醣對奧尼羅非魚生長、血清溶菌?活性和補體活性的影響。淡水魚業38(1):16-19。 34.Tavares, A. P., A. MSMA, M. A. M. Coelho, J. A. Lopes da Silva, A. Barros-Timmons, J. A. J. Coutinho and A. M. R. B. Xavier. 2005. Selection and Optimization of Culture Medium for Exopolysaccharide Production by Coriolus (Trametes) Versicolor World Journal of Microbiology and Biotechnology. 21:1499-1507. 35.Association of official analytical chemisit (AOAC). 1995. Official methods of analysis (16th ed.). Arlington, VA:AOAC. 36.Aytar P, Gedikli S, ?am M, Unal A, Cabuk A, Kolankaya N, and Yurum A. 2011. Desulphurization of some low-rank Turkish lignites with crude laccase produced from Trametes versicolor ATCC 200801. Fuel Processing Technology. 92(1):71-76. 37.B. Metz NWFK. 1977. The growth of molds in the form of pellets – a literature review. Biotechnology and Bioengineering. 19(6):781-799. 38.Barker, S. A., Bourne, E. J., Stacey, M., and Whiffen, D. H. 1954. Infra-red spectra of carbohydrates. Part I. Some derivatives of D-glucopyranose. Journal of Chemical Society. p:171 – 176. 39.Borras

E, Blanquez P, Sarra M, Caminal G, and Vicent T. 2008. Trametes versicolor pellets production: Low-cost medium and scale-up. Biochemical Engineering Journal. 42(1):61-66. 40.Chaplin, M. F., and Kennedy, J. F. 1994. Carbohydrate analysis: A practical approach. Oxford, UK: IRL Press Ltd.. 41.Cheng G-Y WG-R, Zhou Y-Z, Cheng S-L. 1998. Extraction and characterization of proteoglycan from the mycelium of Polystictus versicolor (L.) Fr. by submerged culture. Zhiwu Ziyuan Yu Huanjing. 7(4):19-23. 42.Collins RA, and Ng TB. 1997. Polysaccharopeptide from Coriolus versicolor has potential for use against human immunodeficiency virus type 1 infection. Life Sciences. 60(25):383-387. 43.Cui J, Goh KKT, Archer R, and Singh H. 2007. Characterisation and bioactivity of protein-bound polysaccharides from submerged-culture fermentation of Coriolus versicolor Wr-74 and ATCC-20545 strains. Journal of Industrial Microbiology & Biotechnology. 34(5):393-402. 44.Cui J. 2003. Polysaccharopeptides of Coriolus versicolor: physiological activity, uses, and production. Biotechnology Advances. 21(2):109-122. 45.Eduard Borrasa, Paqui Blanqueza, Montserrat Sarra, Gloria Caminal, Teresa Vicent. 2008. Trametes versicolor pellets production: Low-cost medium and scale-up. Biochemical Engineering Journal. 42:61-66. 46.H. X. WANG TBN, W. K. LIU ,V. E. C. OOI ,S. T. CHANG. 1996. Polysaccharide—peptide complexes from the cultured mycelia of the mushroom Coriolus versicolor and their culture medium activate mouse lymphocytes and macrophages. Int J Biochem Cell Biol. 28(5):601-607. 47.Hawksworth PMK, B. C. Sutton, and D. N. Pegler. 1996. Dictionary of the Fungi. Fungal Genetics and Biology. 20(173). 48.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). 49.Ho CY, Lau CBS, Kim CF, Leung KN, Fung KP, Tse TF, Chan HHL, and Chow MSS. 2004. Differential effect of Coriolus versicolor (Yunzhi) extract on cytokine production by murine lymphocytes in vitro. International Immunopharmacology. 4(12):1549-1557. 50.Ho MDSaCS. 1985. The effect of dissolved carbon dioxide on Penicillin production:Mycelial Morphology. Biotechnology Advances 2(6):347-363. 51.Hotta T, Enomoto, A., Yoshikumi, C., Ohara, M. and Ueno, S. 1981. Protein-bound polysaccharides. US Patent. 4(271):151. 52.Jong-Pil Park S-WK, Hye-Jin Hwang, Youn-Jeung Cho, Jong-Won Yun. 2002. Stimulatory effect of plant oils and fatty acids on the exo-biopolymer production in Cordyceps militaris. Enzyme and Microbial Technology. 31(250-255). 53.JUN TAB BAE JPP, CHI HYUN SONG, CHOON BAL YU, MOON KI PARK, AND JONG WON YUN. 2001. Effect of Carbon Source on the Mycelial Growth and Exo-Biopolymer Production by Submerged Culture of Paecilomyces japonica. BIOSCIENCEA ND BIOENCWEERING. 91(5):522-524. 54.Lee BC, Bae JT, Pyo HB, Choe TB, Kim SW, Hwang HJ, and Yun JW. 2004. Submerged culture conditions for the production of mycelial biomass and exopolysaccharides by the edible Basidiomycete Grifola frondosa. Enzyme and Microbial Technology. 35(5):369-376. 55.Lee C, Yang X, and Wan J. 2006. The culture duration affects the immunomodulatory and anticancer effect of polysaccharopeptide derived from Coriolus versicolor. Enzyme and Microbial Technology. 38(1-2):14-21. 56.Lin F, Lai Y, Yu H, Chen N, Chang C, Lo H, and Hsu T. 2008. Effects of Lycium barbarum extract on production and immunomodulatory activity of the extracellular polysaccharopeptides from submerged fermentation culture of Coriolus versicolor. Food Chemistry. 110(2):446-453. 57.Litchfield, J. 1967. Submerged culture of mushroom mycelium. In:Micobial Technology, 2nd ed. (Peppier, H. J. and Pertman, D., eds). p: 93-145. Genetic engineering. New York. 58.Litchfield, J. 1979. Producion of sigle cell proein for use in food and feed. In:Micobial Technology, 2nd ed. (Peppier, H. J. and Pertman, D., eds). p: 93-145. Academic Press. New York. 59.Moradali M-F, Mostafavi H, Ghods S, and Hedjaroude G-A. 2007. Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). International Immunopharmacology. 7(6):701-724. 60.Ng TB, and Chan WY. 1997. Polysaccharopeptide from the mushroom Coriolus versicolor possesses analgesic activity but does not produce adverse effects on female reproductive or embryonic development in mice. General Pharmacology: The Vascular System. 29(2):269-273. 61.Ng TB. 1998. A review of research on the protein-bound polysaccharide (polysaccharopeptide, PSP) from the mushroom Coriolus versicolor (basidiomycetes: Polyporaceae). General Pharmacology: The Vascular System. 30(1):1-4. 62.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 Biochemistry. 35(3-4):359-366. 63.Park J.P., Kim Y.M., Kim S. W., Hwang H.J., Cho Y.J., Lee Y.S., Song C.H., and Yun J.W. 2002. Effect of aeration rate on the mycelia morphology and exo-biopolymer production in Cordyceps militaris. Process Biochem. 37:1257-1262. 64.Rau U, Kuenz A, Wray V, Nimtz M, Wrenger J, and Cicek H. 2009. Production and structural analysis of the polysaccharide secreted by Trametes (Coriolus) versicolor ATCC 200801. Applied Microbiology and Biotechnology. 81(5):827-837. 65.Sandula J, Kogan G, Kacurakova M, and Machova E. 1999. Microbial (1-->3)-[beta]--glucans, their preparation, physico-chemical characterization and immunomodulatory activity. Carbohydrate Polymers. 38(3):247-253. 66.Shah V, Dobiasova P, Baldrian P, Nerud F, Kumar A, and Seal S. 2010. Influence of iron and copper nanoparticle powder on the production of lignocellulose degrading enzymes in the fungus Trametes versicolor. Journal of Hazardous Materials. 178(1-3):1141-1145. 67.Soothill EaF, A. 1987. The new field guide to fungi. Michael JosephLondonEngland. 68.Takashi Mizuno KO, Naomi Hagiwara and Reiko Kuboyama. 1986. Fractionation and characterization of antitumor polysaccharides from Maitae, Grifola frondosa. Agric Bio Chem. 50(7):1679-1688. 69.Tavares AP, Coelho, M. A., Agapito, M. S., Coutinho, J. A. and Xavier, A.M. 2006. Optimization and modeling of laccase production by Trametes versicolor in a bioreactor using statistical experimental design. Appl Biochem Biotechnol. 134(3):233-248. 70.U. Rau EG, E. Olszewski and F. Wagner. 1992. Enhanced glucan formation of filamentous fungi by effective mixing, oxygen limitation and fed-batch processing. Industrial Microbiology & Biotechnology. 9(1):19-25. 71.Ueno S, Yoshikumi, C., Hirose, F., Omura, Y., Wada, T. and Fujii, T. 1980a. Method of producing nitrogen-containing polysaccharides. US Patent 4,229,969. 72.Wang HX, Ng TB, Liu WK, Ooi VEC, and Chang ST. 1996. Polysaccharide--peptide complexes from the cultured mycelia of the mushroom Coriolus versicolor and their culture medium activate mouse lymphocytes and macrophages. The International Journal of Biochemistry & Cell Biology. 28(5):601-607. 73.Yang QY JS, Li XY, Zhou JX, Chen RT, Xu LZ. 1992. Antitumor and immunomodulating activities of the polysaccharide – peptide (PSP) of Coriolus versicolor. J Immunol Immunopharmacol. 12:29-34. 74.Yang QYaZ, Y. F. 1993. A protein bound polysaccharide-PSP. In Proceedings of PSP International Symposium (Edited by Yang, Q. Y. and Kwok, C. Y.). 22-34. Fundan Univeersity Press, Shanghai, China.