

Effects of Media and Culture Conditions on Production of Bioactive Ingredients by *Wolfiporia cocos* and *Lactobacillus acidophilus*

呂志豪、徐泰浩、林芳儀

E-mail: 360007@mail.dyu.edu.tw

ABSTRACT

Many studies had reported that medicinal fungi and lactic acid bacteria possess a number of different bioactive properties, including anti-oxidant, immunomodulatory, and anti-tumor bioactive functions. For the two species, co-culture systems using fermentation studies were conducted less frequently. Therefore, this study was conducted employing a co-culture system of the medicinal mushroom - *Wolfiporia cocos* and lactic acid bacteria- *Lactobacillus acidophilus*. Natural carbon and nitrogen sources were used instead of a half-compound medium. Effects of culture conditions on the production of bio-active ingredients in a 5L stirred tank fermenter(PTF). Further, to assess of antibacterial activity and antioxidant activity. The results: Optimal medium carbon source 4% food grade- sucrose and nitrogen source 1% peanut powder. Add to 4% of *Lactobacillus acidophilus* co-cultured, the best production by polysaccharides and triterpenoid. In 5L fermenter culture conditions of optimum culture growth conditions, at 25 °C, 150 rpm, 1vvm, initial pH=5.5, that was the most suitable conditions to bioactive ingredients with the production. Under the same conditions (1vvm, 25 °C, 150rpm, initial pH5.5), employing single strain cultures and double strain co-cultures it was found that there was significantly greater extracellular polysaccharide (EPS), intracellular polysaccharide (IPS), triterpenoid, ergosterol and polyphenol improvement in the double strain co-culture than the single strain culture (10.7mg/mL, 1.5mg/mL, 0.7mg/mL, 0.6mg/mL, 2.73 mg/g, 31.08 mg/g, respectively). The antibacterial activity results: Six strains of pathogenic, liquid antibacterial test, including *Staphylococcus aureus* and *Escherichia coli* were better, the zone of inhibition were 8.5mm and 7.4mm. Minimum inhibitory concentration (MIC) of the 10% test, including *Staphylococcus aureus* and *Bacillus cereus* were better. The antibacterial stability test, including *Staphylococcus aureus* and *Escherichia coli* were better.

Keywords : *Wolfiporia cocos*、*Lactobacillus acidophilus*、bioactive ingredients、co-culture

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	xiii	表目錄	xv	1.前言	1	2.文獻回顧	3	2.1茯苓之生物學特性	3	2.2茯苓生物活性成分	4	2.2.1茯苓多醣	5	2.2.2三萜類化合物	6	2.3.3甾醇類化合物	11	2.3.4羊毛固醇	12	2.4乳酸菌簡介	13	2.5共培養系統	15	2.6影響發酵產物之環境因子	17	2.6.1碳源	17	2.6.2氮源	17	2.6.3無機鹽類	18	2.6.4脂肪酸	19	2.6.5溫度	19	2.6.6 pH值	20	2.6.6進氣量	21	2.6.7攪拌速度	21	3.材料與方法	23	3.1實驗架構	23	3.2實驗材料	23	3.2.1實驗菌株	24	3.2.2實驗藥品	24	3.2.3儀器設備	26	3.3培養基組成	27	3.4菌株培養	28	3.4.1茯苓菌株培養	28	3.4.2嗜酸乳桿菌菌株培養	28	3.5液態培養	29	3.6搖瓶培養試驗	29	3.6.1碳源對於共培養產生多醣體及其二次代謝物產量之影響	29	3.6.2氮源對於共培養產生多醣體及其二次代謝物產量之影響	30	3.6.3氮源濃度對於共培養產生多醣體及其二次代謝物產量之影響	30	3.6.4添加不同接菌量嗜酸乳桿菌對茯苓多醣體及其二次代謝物產量之影響	30	3.7 5L發酵槽液態培養最適培養條件之探討	31	3.7.1不同進氣量對共培養之影響	31	3.7.2不同溫度對共培養之影響	31	3.7.3不同攪拌速率對共培養之影響	31	3.7.4不同初始pH對共培養之影響	32	3.8.5不同培養時間對共培養之影響	32	3.8分析方法	33	3.8.1菌絲體生物質量測定與製備	33	3.8.2胞內、外多醣含量測定	33	3.8.3粗三萜含量測定	35	3.8.4麥角固醇含量測定	36	3.8.5總多酚含量之測定	36	3.8.6抗氧化活性測定	38	3.8.7抑菌活性測定	39	4.結果與討論	41	4.1共培養之搖瓶培養試驗	41	4.1.1共培養於搖瓶液態培養之型態變化	41	4.1.2碳源對於共培養產生多醣體及其二次代謝物產量之影響	43	4.1.3氮源對於共培養產生多醣體及其二次代謝物產量之影響	46	4.1.4氮源濃度對於共培養產生多醣體及其二次代謝物產量之影響	49	4.1.5接菌量對於共培養產生多醣體及其二次代謝物產量之影響	52	4.2共培養之5L攪拌式發酵槽試驗	54	4.2.1共培養於5L攪拌式發酵槽液態培養之型態變化	55	4.2.2進氣量對於共培養產生多醣體及其二次代謝物產量之影響	57	4.2.3溫度對於共培養產生多醣體及其二次代謝物產量之影響	65	4.2.4轉速對於共培養產生多醣體及其二次代謝物產量之影響	73	4.2.5初始pH值對於共培養產生多醣體及其二次代謝物產量之影響	80	4.2.5培養時間對於共培養產生多醣體及其二次代謝物產量之影響	89	4.3相同條件下單一培養與共培養之影響	92	4.4抗氧化能力試驗	95	4.4.1亞鐵離子螯合能力	95	4.4.2清除DPPH能力	96	4.4.3還原力能力	97	4.5抑菌試驗	100	4.5.1發酵液與萃取液對不同菌株之抑菌能力	100	4.5.2發酵液與萃取液對不同菌株之最小抑菌濃度試驗	100	4.5.3發酵液與萃取液對不同菌株之穩定性試驗	101	5.結論	106	參考文獻	108	圖目錄	圖2.1茯苓菌核上附著子實體	4	圖2.2茯苓菌核	4	圖2.3茯苓多醣基本結構	6	圖2.4三萜化合物合成路徑	9	圖2.5茯苓酸的結構	11	圖2.6麥角固醇的結構	13	圖2.7羊毛固醇的結構	13	圖4.1(a-d) <i>Wolfiporia cocos</i> 、 <i>Lactobacillus acidophilus</i> 共培養之搖瓶生長型態變化	42	圖4.2碳源對共培養產生菌絲體與生物活性之影響	46	圖4.3碳源對共培養產生	
------	-----	------	-----	------	---	----	-----	----	------	-----	------	-----	----	------	---	--------	---	-------------	---	-------------	---	-----------	---	-------------	---	-------------	----	-----------	----	----------	----	----------	----	----------------	----	---------	----	---------	----	-----------	----	----------	----	---------	----	-----------	----	----------	----	-----------	----	---------	----	---------	----	---------	----	-----------	----	-----------	----	-----------	----	----------	----	---------	----	-------------	----	----------------	----	---------	----	-----------	----	-------------------------------	----	-------------------------------	----	---------------------------------	----	-------------------------------------	----	------------------------	----	-------------------	----	------------------	----	--------------------	----	--------------------	----	--------------------	----	---------	----	-------------------	----	-----------------	----	--------------	----	---------------	----	---------------	----	--------------	----	-------------	----	---------	----	---------------	----	----------------------	----	-------------------------------	----	-------------------------------	----	---------------------------------	----	--------------------------------	----	-------------------	----	----------------------------	----	--------------------------------	----	-------------------------------	----	-------------------------------	----	----------------------------------	----	---------------------------------	----	---------------------	----	------------	----	---------------	----	---------------	----	------------	----	---------	-----	------------------------	-----	----------------------------	-----	-------------------------	-----	------	-----	------	-----	-----	----------------	---	----------	---	--------------	---	---------------	---	------------	----	-------------	----	-------------	----	---	----	-------------------------	----	--------------	--

生菌絲體與生物活性之影響 49 圖4.4氮源濃度對共培養產生菌絲體與生物活性之影響 52 圖4.5接菌量對共培養產生菌絲體與生物活性之影響 56 圖4.6a-d為*Wolfiporia cocos*、*Lactobacillus acidophilus*共培養之5L-發酵槽生長型態變化 56 圖4.75L-攪拌式發酵槽探討不同進氣量下，共培養對pH、*Lactobacillus acidophilus*、胞外多醣變化 62 圖4.85L-攪拌式發酵槽探討進氣量對共培養之生物質量、胞外多醣、胞內多醣、總三萜、總多酚、麥角固醇之影響 63 圖4.95L-攪拌式發酵槽探討不同溫度下，共培養對pH、*Lactobacillus acidophilus*、胞外多醣變化 70 圖4.105L-攪拌式發酵槽探討溫度對共培養之生物質量、胞外多醣、胞內多醣、總三萜、總多酚、麥角固醇之影響 71 圖4.115L-攪拌式發酵槽探討轉速下，共培養對pH、*Lactobacillus acidophilus*、胞外多醣變化 77 圖4.125L-攪拌式發酵槽探討轉速對共培養之生物質量、胞外多醣、胞內多醣、總三萜、總多酚、麥角固醇之影響 78 圖4.135L-攪拌式發酵槽探討初始pH下，共培養對pH、*Lactobacillus acidophilus*、胞外多醣變化 86 圖4.145L-攪拌式發酵槽探討初始pH對共培養之生物質量、胞外多醣、胞內多醣、總三萜、總多酚、麥角固醇之影響 87 圖4.155L-攪拌式發酵槽探討培養時間對共培養之生物質量、胞外多醣、胞內多醣、總三萜、總多酚、麥角固醇之影響 91 圖4.16發酵液與萃取液對螯合亞鐵離子、清除DPPH、還原力能力之影響 98 圖4.17以發酵液與萃取液對抑制致病菌生長之影響 103 圖4.18以發酵液與萃取液對致病菌最小液菌濃度(MIC)試驗 104 圖4.19以發酵液與萃取液對抑菌穩定性試驗 105 表目錄 表4.1進氣量對共培養產生菌絲體與生物活性成分之影響 64 表4.2溫度對共培養產生菌絲體與生物活性成分之影響 72 表4.3轉速對共培養產生菌絲體與生物活性成分之影響 79 表4.4初始pH對共培養產生菌絲體與生物活性成分之影響 88 表4.5相同條件單一培養與共培養產生菌絲體與生物活性成分之影響 94

REFERENCES

- 1.丁瓊、張俐娜、張志強。2000。茯苓菌絲體多醣的分離與結構的分析。高分子學報2:224-227。
- 2.卜永士、郭本桓。乳酸菌對膽固醇降低作用的研究。上海水產大學學報13(4)。
- 3.王傳霞、李福后、陳立國。2006。茯苓菌絲體液態培養條件的探討。食用菌(2)。
- 4.王文梅、許麗、王秋菊。2009。乳酸菌對動物腸道的黏附作用研究進展。東北大學農業學報40(12)。
- 5.王謙、蘭蕾蕾、王超、冀宏、丁萬杰、劉傳斌、刑志華、李寶佳。2003。茯苓異體培養條件研究及其營養濃縮液的相關檢測。食用菌學報10(2):17-20。
- 6.中醫藥大辭典上、中、下。新文豐出版社
- 7.水野卓?川合正允(賴慶亮)。1997。菇類的化學、生化學。國立編譯館。
- 8.江宏文、張毅、林曉珊。2008。麥角固醇高產量菌株選育方法的新進展。科技創新與食品產業可持續發展。
- 9.邵祥龍、蘇日娜、李傳立。2009。茯苓多醣抗腫瘤作用研究進展。北京中醫藥28(4)。
- 10.李冀、萬德光、斐璟、陳新。2005。茯苓液態發酵條件的研究。成都中醫藥大學學報28(1):52-55。
- 11.李順來。2010。牛樟芝三萜類的結構與生化活性。南台科技大學碩士論文。
- 12.李慧、常景玲。2006。茯苓多糖發酵工藝的優化。安徽農業科學34(5):920-921。
- 13.李微萱。2007。液態培養條件對舞菇菌絲體及多醣體生產之影響。東海大學碩士論文。
- 14.沈思。2008。茯苓皮中三萜的提取、分離純化及其美白皮膚活性基礎性研究。華中農業大學碩士論文。
- 15.林家如。2002。浸液發酵培養基與培養條件對藥用真菌茯苓菌絲體及胞外多醣生成之影響。大葉大學碩士論文。
- 16.林克融。2002。探討培養基之pH值與Xanthan gum的添加對巴西蘑菇多醣體生產之影響。中央大學碩士論文。
- 17.林欣儀。2008。以固態發酵製備舞菇小麥及其品質與抗氧化特性。中興大學食碩士論文。
- 18.易華西、張蘭威、杜明、韓雪。2010。乳酸菌細菌素抗菌潛力挖掘研究進展。中國食品添加劑。
- 19.周立、張璋、許津。1994。茯苓素誘生腫瘤壞死因子的作用。中國抗生素雜誌19(5):367-380。
- 20.周曉芳、葉蕊芳、儲成量、馬立新。2011。林可霉素生物和成培養基之優化。工業微生物 41(2):21-25。
- 21.胡國元、游慧珍、董蘭蘭、戴欣鵬、李傳傳、胡靖、李有國。2010。茯苓菌絲體液態培養條件研究。武漢工程大學學報32(7)。
- 22.洪松虎、吳祖芳。乳酸菌抗氧化作用研究進展。寧波大學學報(理工版)23(2)。
- 23.姚廣印、張雙鳳、謝宗良。2007。酵母細胞麥角固醇的提取。河南大學學報27(2)。
- 24.陳怡倩。2001。利用批示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。中央大學碩士論文。
- 25.陳明造。2007。機能性食品。富林出版社。
- 26.陳怡君。2002。乳酸發酵綜合蔬果汁之試製與儲藏時間變化。中興大學碩士論文。
- 27.陳宏慧。2004。液態培養環境對茯苓(*Wolfiporia cocos*)菌絲體生長及其多醣體成分之影響。東海大學碩士論文。
- 28.陳慶源、林富美。2004。益生菌之保健功效。食品工業36(3):1-3。
- 29.陳崇凱。2010。探討共培養對*Chlorella sp.*的生長與生產活性多醣的影響。中央大學碩士論文。
- 30.陳南吟。2008。浸液培養條件對黃金銀耳菌型態、多醣體生成及其生物活性之影響。大葉大學博士論文。
- 31.陳書豪。2006。探討樟芝的溫度變化對液態發酵與固態發酵生產三萜類與多醣體之影響。中央大學碩士論文。
- 32.高遠。2009。樺褐孔菌甾類化合物的分析及深層液態發酵條件的優化。江南大學碩士論文。
- 33.陸震鳴。2009。樟芝深層液態發酵及其三萜類化合物的研究。江南大學博士論文。
- 34.徐胤桓。2011。不同蟲草菌株親源分析、生物活性成分含量及抗氧化特性探討。大葉大學研究所碩士論文。
- 35.許淳均。2004。探討培養基組成對巴西洋菇發酵生產活性多醣及其特性之影響。中央大學博士論文。
- 36.張敏、高曉紅、徐嘉瞳、李香豔、史衍杰、范新田。2008。茯苓的藥理作用及研究進展。北華大學學報9(1)。
- 37.張照明、譚天傳。2002。麥角固醇的市場前景。精細與專用化學22:9-10。
- 38.張思訪、劉靜涵、蔣建勤、周?梅。2005。茯苓的化學成分和藥理作用及開發利用。中華實用中西醫18(2)。
- 39.張淑芬。2001。食藥用菇類搖瓶液態培養條件之探討。食品工業33:37-46。
- 40.張志豐。2004。深層培養松茸(*Tricholoma matsutake*)之最適化培養液組成。大同大學碩士論文。
- 41.張明堯。2001。液態培養生產靈芝菌絲體與靈芝多醣最適化之研究。國立海洋大學碩士論文。
- 42.黃兵。2010。放線菌共培養效應級活性產物誘導的研究。北京化工大學碩士論文。
- 43.黃爾竺。2003。數株益生菌之4-nitroquinoline-N-oxide之抗致突變性。台灣大學碩士論文。
- 44.黃思齊。2011。發酵產程擴大化級不同培養基對雲芝胞外醣?化學特性之影響。大葉大學碩士論文。
- 45.黃小龍、黃東益、周雙清、吳繁花、陶思宇。2009。粘質沙雷氏菌產靈芝紅素培養基的篩選。生物技術 19(5):65-67。
- 46.黃崇凱。2007。探討pH值對*Agaricus blazei*液態發酵生產一次及二次代謝產物之影響。中央大學碩士論文。
- 47.鄧剛民、許津。1992。茯苓素:一種潛在的醣固酮拮抗劑。17(1):34-37。
- 48.曹宇、高文遠、張黎明、王娟、張強。2009。液態發酵茯苓胞外多醣的研究。現代食品科技25(12):1438-1442。
- 49.程建新、周艾琳、高麗彩、李秀娟。乳酸菌抗腫瘤作用的概況與應用進展

。Journal of Practical Oncology14(1)。50.楊政儒。2005。生長溫度與pH值對乳酸菌胞外多醣生成影響之研究。中國文化大學碩士論文。

51.梁清樂、王秋穎、曾念開、王懷凱。2006。不同茯苓菌株深層培養比較試驗。食用菌(4):6-7。52.閻三弟。1996。真菌的藥用價值。食用菌學報3(4):55-64。53.趙繼鼎主編。1998。中國真菌誌(第三卷)。科學出版社。54.蔡介中。2011。探討光品質和漁業廢棄物對*Chlorella sp.* 和*Saccharomyces cerevisiae* 共培養生產油脂之影響。中央大學碩士論文 55.曾馨誼、許瑞田、盧訓。2009。國內保健食品產值技產業概況分析精要。中國穀類食品工業技術研究所。56.熊杰、林芳燦、王克勤、蘇璋、傅杰。2006。茯苓基本生物學特性研究。菌物學報25(3):446~453。57.廖啟成。1998。乳酸菌之分類與應用。食品工業2:1-10。58.廖召聖。2005。乳酸菌發酵萃取物對植物促進植物生長之研究。東華大學碩士論文。59.劉佳茹。2005。油脂與介面活性劑隊舞菇生長與多醣生產之影響。大葉大學碩士論文。60.劉忠義、曾虹燕。2002。茯苓液體培養研究初探。湘潭大學食品科學與工程系，湖南湘潭411105。61.劉晶、黃珊珊、趙征。乳酸菌抗氧化能力研究進展。中國乳品工業，2010。62.薛正連、歐陽明、王嵐嵐。2006。茯苓菌液態培養條件的優化及其多醣的提取。工業微生物地36卷第2期44-47。63.蘇紅旭、徐凱、江國金。2011。用HPLC測定麥芽及大麥中的麥角固醇含量。康迪日用化工有限公司。64.葉昇達。2003。厭氧發酵產氫菌與光合產氫菌共培養之產氫效率比較研究。台中師範學院碩士論文。65.詹宏宜。2006。優勢產氫菌共培養之特性分析。高雄第一科技大學碩士論文。66.趙金賢。2010。結合纖維素分解菌與固定化產醇菌之共培養系統以提升乙醇產量之研究。大葉大學碩士論文。67.Cho, E. J., Oh, J. Y., Chang, H. Y. and Yun, J. W. 2006. Production of exo-polysaccharides by submerged mycelia culture of a mushroom *Tremella fuciformis*. *Journal Biotechnol.* 127(1):129-140. 68.Cuellar, M, J., Giner, R. M., Recio, M. C., Just, M. J., Manze, S. and Rios, J. L. 1997. Two fungal lanostane derivatives as phospholipaseA2 Inhibitors.*J.Natl.Product.*59:977-979. 69.Czub, J. and Baginski, M. 2006. Comparative Molecular Dynamics Study of Lipid Membranes Containing Cholesterol and Ergosterol. *Biophophysical Journal* .90:2368-2382. 70.Desmond, T., Fergus, J. L. and Brian, O. S. 1990. Synthesis, structure and properties of stereochemically non-rigid molybdenum pyrazolylborato complexes containing a dihapto-thiocarboxamido ligand. *Jurnal of organo metallic chemistry.* 381:33-37. 71.Fang, Q. H. and Zhong, J. J. 2002a. Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. *Bioprocess Biochemistry.* 37:769-774. 72.Fang, Q. H and Zhong, J. J. 2002b. Submerged fermentation of higher fungus *Ganoderma lucidum* for production of valuable bioactive metabolites-ganoderic acid polysaccharide. *Biochem. Eng. J.*, 10(1):61-65. 73.Forage, R. G., Harrison, D. E. F. and Pitt, D. E. 1985. Effect of environment on microbial activity. *Comprehensive Biotechnology*1:253-279. 74.Fooks, L. J. and Gibson, G. R. 2002. Proiotics as modulators of the gut flora. *Br.J. Nutr.* 88:39-49. 75.Gapter, L., Wang, Z., Glinski, J and Ng, K. 2005. Induction of apoptosis in prostate cancer cells by pachymic acid from *Poria cocos*. *Biochemical and Biophysical Research Communication.* 332:1553-1161. 76.Houng J. Y., Chang M. Y. and Tsai G. J. 2006. Optimization of the medium composition for the submerged culture of *Ganoderma lucidum* by Taguchi array design and steepest ascent method. *Enzyme Micro.Tech.* 38:407-414. 77.Huang, Y. C., Chang, W. L., Huang, S. F., Lin, C. Y., Lin, H.C. and Chang, T. C. 2010. Pachymic acid stimulates glucose uptake through enhanced GLUT4 expression and translocation. *European Journal of Pharmacology.* 648:39-49. 78.Hsieh, C., Liu, C. J., Tseng, M.H., Lo, C. T. and Yang, Y. C. 2005. Effect of olive oil on the production of mycelia biomass and polysaccharides of *Grifola frondosa* under high oxygen concentration aeration. *Enzyme and Microbial Technology.* 39:434-439. Huang, Q., Jin, Y., Zheung, L., Cheung, P. G. and Kennedy, J. F. 2007. Structure, molecular size and antitumor activities of polysaccharides from *Poria cocos* mycelia produced in fermenter. *Carbohydrate Polymers.* 70:324-333. 79.Hsieh, C., Tseng, M. H. and Liu, C. J. 2006. Production of polysaccharides from *Ganoderma lucidum* (CCRC 36041) under limitations of nutrients. *Enzyme Micro. Tech.* 38:109-117. 80.Jin, Y., Zhang, L., Chen, L., Cheung, P. C. K. and Chen, Ligu. 2003. Effect of culture media on the chemical and physical characteristics of polysaccharides isolated from *Poria cocos* mycelia. *Carbohydrate Research*338:1507-1515. 81.Ke, R. D., Lin, S. F., Chen, Y., Ji, C. R. and Shu, Q. G. 2010. Analysis of chemical composition of polysaccharides from *Poria cocos* Wolf and its anti-tumor activity by NMR spectroscopy. *Carbohydrate Polymers.*80:31-34. 82.Kim, S. W., Hwang, H. J., Xu, C. P., Sung, J. M., Choi, J. W. and Yun, J. W. 2003. Optimization of submerged culture process for the production of mycelial biomass and exo-polysaccharides by *Cordyceps militaris* C738. *Journal of Applied Microbiology.* 94:120-126. 83.Lee, K. Y. and Jen, Y. J. 2003. Polysaccharide isolated from *Poria cocos* sclerotium induces NF-Kb/Rel activation and iNOS expression in murine macrophages. *International Immunopharmacology* 3: 1353-1362. 84.Lee, W. Y., Youngki, P., Ahn, J. K., Ka, K. H. and Park, S. Y. 2007. Factors influencing the production of endopolysaccharides and exo-polysaccharides from *Ganoderma applanatum*. *Enzyme Microb. Technol.*, 40(2):249-254. 85.Lee, K. Y., You, H. J., Jeong, H. G., Kang, J. S., Kim, H. M., Rhee, S. D. and Jeon, Y. J. 2004. Polysaccharide isolated from *Poria cocos* sclerotium induces NF-Nb/Rel activation and iNOS expression through the activation of p38 kinase in murine macrophages. *International Immunopharmacology*4: 1029-1038. 86.Li, G. Y., Huang, L. H., Hse, C. Y. and Qin, T. F. 2011. Chemical compositions, infrared spectroscopy, and X-ray diffractometry study on brown-rotted woods. *Carbohydrate Polymers.* 87.Ling, H., Zhou, L., Jia, X., Gapter, L., Agarwal, R and Ng, K. 2009. Polyporenic Acid C Induces Caspase-8-Mediated Apoptosis in Human Lung Cancer A549 Cells. *Molecular Carcinogenesis.*48:498-507. 88.Matsuzaki, T. and Chin, J. 2000. Modulating immune responses with probiotic bacteria. *Immunol.Cell Biol.* 78:67-73. 89.Mau, J. L., Lin, H. C. and Song, S. F. 2002. Antioxidant properties of several specially mushroom. *Food Research International* 35:519-526. 90.Mizuo, T. 1999. The extraction and development of antitumor-active polysaccharides from medicinal mushroom in Japan (Review). *Inter. J. Medicinal mushroom.*1:9-29. 91.Niemenmaa, Q., Galkin, S. and Hatakka, A. 2008. Ergosterol contents of some wood-rotting basidiomycete fungi grown in liquid and solid culture conditions. *International Biodeterioration & Biodegradation* 62:125-134. 92.Ool, V. E. C. and Fan, L. 2000. Immunomodulation and Anti-Cancer Activity of Polysaccharide-Protein Complexes. *Curr.Med.Chem*7:715-729. 93.Park, J. P., Kim, S. W., Hwang, H. J., Cho, Y. J. and Yun, J. W. 2002. Stimulatory effect of plant oils and fatty acid on the exo-biopolymer production in *Cordyceps militaris*. *Enzyme and Microbial Technology*, 31:250-255. 94.Rhee, S. D., Cho, S. M. and Park, J. S. 1999. Chemical composition and

biological activities of immunostimulants purified from alkali extract of *Poria cocos sclerotium*. The Korean journal of mycology. 27(4):293-298.

95.Proma, K. E., Aidoo, R. F., 2002.Sugar profile of extracellular polysaccharides from different *Tremella* species. International Journal of Food Microbiology 79:121-129. 96.Shih, I. L., Pan, K and Hsieh, C. 2006. Influence of nutritional components and oxygen supply on the mycelia growth and bioactive metabolites production in submerged culture of *Antrodia cinnamomea*. Process Biochemistry 41:1129-1135. 97.St.Onge, M. P., Farnworth, E. R., Jones, P. J. H. 2000. Consumption of fermented and metabolism. Am. J. Clin. Nutr. 71:674-681. 98.Tsai, S. Y., Huang, S. J. and Mau, J. L., 2002b. Antioxidant properties of hot-water extracts from *Agrocybe cylindracea*. Food Chemistry 98:670-677. 99.Tran, H. T. M., Cheirsilp, B., Hodgson, Brian and Umakul, K. 2010. Potential use of *Bacillus subtilis* in a co-culture with *Clostridium butylicum* for acetone – butanol – ethanol production from cassava starch. Biochemical Engineering Journal 48:260 – 267. 100.Ukiya, M., Akihisa, T., Hirano, M., Oshikubo, M., Nobukuni, Y., Tai, T., Kondo, S. and Nishino, H. 2002. Inhibition of tumor-promoting effect by poricoic acids G and H and other lanostane-type triterpenes and cytotoxic activity of poricoic acids A and G from *Poria cocos*. J. Nat. Prod. 65:462-465. 101.Vercruyse, L., Gelman, D., Van de Velde, S., Raes, E., H. B., Vermeirssen, V., Van, C. J and Smagghe, G. 2005. Inhibitor Captopril Reduces Ecdysteroids and Oviposition in Moths. Ann NY Acad sci, 1040:498-500. 102.Xi, M., Hai, C., Tang, H., Chen, M., Fang, K. and Liang, X. Antioxidant and antiglycation properties of total Saponins extracted from traditional chinese medicine used to treat Diabetes Mellitus. Phytotherapy research .22:228-237. 103.Yang, F. C., Ke, Y. F. and Kuo, S. S. 2000. Effect of fatty acids on the mycelia growth and polysaccharide formation by *Ganoderma lucidum* in shake flask culture. Enzyme and microbial technology. 27: 295-301. 104.Yasukawa, K., Kaminaga, T., Kitanaka, S., Tai, T., Nunoura, Y., Natori, S. and Takido, M. 1998. 3-*p*-Hydroxycinnoyl-*o*-hydroxybenzoic acid from *Poria cocos*, and its anti-inflammatory effect. Phytochemistry. 48:1357-1360. 105.Yang, J. H., Lin, H. C. and Mau, J. L. 2002. Antioxidant properties of several commercial mushroom. Food Chemistry. 77(2):229-235. 106.Yang, F. C. and Liao, C.B. 1998a. Effect of cultivating conditions on the mycelia growth of *Ganoderma lucidum* in submerged flask cultures. Bioprocess Engineering. 19:233-236. 107.Yun J. W., Hwang H. J., Kim S. W., Hwang H. J. and Choi J. W. 2003. Production and characterization of exo-polysaccharides from submerged culture of *Phellinus linteus* KCTC 6190. Enzyme Micro. Tech. 33:309-319. 108.Yun J. W., Lee B. C., Bae J. T., Pyo H. B., Choe T. B., Kim S. W. and Hwang J. H. 2004. Submerged culture conditions for the production of mycelial biomass and exopolysaccharides by the edible Basidiomycete *Grifola frondosa*. Enzyme Micro. Tech. 35:369-376. 109.Zhong, J. J., Fang, Q. H. and Tang, Y. J. 2002. Enhanced production of valuable bioactive metabolites in submerged cultures of medicinal mushroom *Ganoderma lucidum* by manipulation of oxygen supply. J. Plant Biotechnol. 4:109-115.