

# Fed-batch fermentation of mycelia and polysaccharide by the local strain Ganoderma lucidum(CCRC36041)

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## ABSTRACT

ABSTRACT The objective of this study was to investigate the production of cell mycelia and polysaccharide in shake flask and 5L fermenter. In flask batch study a various of initial pH the cell dry weight was not shown the significant different from initial 4 to initial pH 6 but the higher extracellular polysaccharide (EPS) was found in pH 5 of  $937.18 \pm 38.58$  mg/L. For the nutrients of fermentation the CSP as the nitrogen source has higher cell dry weight and polysaccharide production. As the carbon source the glucose and sucrose was found better than other tested carbon sources for the production of polysaccharide and cell dry weight, respectively. The intracellular polysaccharide (IPS) was found from 24.85 mg/g-cell to 29.95 mg/g-cell in different media. The molecular weight analyzed by HPLC-SEC was in the range of 35,300 ~ 169,200 Da. and 22,200~205,800 Da. for EPS and IPS, respectively. Increasing the additive of the basal salts, glucose, and nitrogen source were able to enhance the production of cell dry weight and ESP. In flask fed-batch study 10 ml of 35% glucose, 10 ml of 25% CSP, and 10 ml of 35% glucose plus 25% CSP were feed to 100 ml fermentation broth after 7 days cultivation. The cell was harvest after 13 days and the dell dry weight were 9.21 g/L, 8.67g/L, and 9.28 g/L, respectively. The control set of experiment was using water additive and the cell dry weight was 3.81 g/L, 5.94 g/L, and 7.66g/L after 7 days, 9 days, and 13 days. The 10 ml of 25% CSP feeding the polysaccharide was found 869.69 mg/L at day 7 and it decrease to 755.13 mg/L after 9 days and increase back to 845.83 mg/L at day 13. On the other hand, the feeding of 10 ml of 35% glucose and 10 ml of 35% glucose plus 25% CSP was found the polysaccharide increase to 1317.84 mg/L and 1339.96 mg/L, respectively. The P-limiting media has the cell dry weight close to the control and the EPS was found slightly lower than control. In the media of N-limiting the specific production of polysaccharide was increase from 107.28 mg/g cell to 177.86 mg/g cell. Experiment in 5L jar fermentor, the agitation speed was from 200rpm increase to 350rpm cell dry weight was from 11.61 g/L increase to 12.22 g/L, but the production of polysaccharide was from 2231.33 mg/L decrease to 1765.88mg/L. The fed-batch experiment was conducted by feeding 1L of 1% CSP at day 3 and 1L of 4% glucose at day 7, the specific polysaccharide production was from 144.51 mg/g cell increase to 200.78 mg/g cell. However, the feeding 2L of 1% CSP only at day 3 the specific polysaccharide production was decrease to 129.28 mg/g cell.

Keywords : Ganoderma lucidum ; fed-batch ; extracellular polysaccharide ; intracellular polysaccharide

## Table of Contents

目錄 頁次 封面內頁 簽名頁 授權書.....	iii 中文摘要.....
.....iv 英文摘要.....	vi 誌謝.....
.....viii 目錄.....	ix 圖目
錄.....	xiv 表目錄.....
....xvii 第壹章 前言.....	1 第貳章 文獻回顧.....
.....3 2.1靈芝菌簡介.....	3 2.2靈芝的功效.....
.....4 2.3靈芝的藥理作用.....	6 2.3.1靈芝的一般化學成分.....
.....6 2.3.2降血壓.....	6 2.3.3降血糖與降血脂作用.....
.....6 2.3.4免疫作用作用.....	7 2.3.5保肝作用.....
2.3.6對內分泌與代謝的影響.....	8 2.3.7抗癌作用.....
多醣之種類.....	8 2.5靈芝培養.....
芝液態培養之優點.....	13 2.5.2影響靈芝菌絲生長之因子.....
.....19 2.6.1多醣的分類.....	15 2.6.多醣之定義.....
法.....	20 2.6.2多醣之回收方.....
.....20 2.6.2.1醇類提取法.....	20 2.6.2.2膜過濾法.....
.....22 2.6.3多醣之測定方法.....	23 2.6.3.1酚硫酸法.....
.....23 2.6.3.2高效能分子篩層析法 ( HPLC -SEC ) .....	24 2.6.3.黏度測定法.....
酵素法.....	26 2.6.4影響多醣產量因子.....
對多醣生成影響.....	26 2.6.4.1碳源與氮源.....
.....26 2.6.4.2 pH對多醣生成影響.....	28 2.6.4.3轉速對多醣生成影響.....

.....28 2.6.3.4溫度對多醣生成影響.....	29 2.6.3.5其他因子.....	30
2.7 饵料批次式操作.....	30 2.7.1發酵槽進料方式.....	31
2.7.2 饵料式發酵的優點.....	31 2.8 微生物的代謝作用.....	32 第參
章 材料與方法.....	36 3.1實驗材料.....	36
3.1.1 菌株.....	36 3.1.2 實驗藥品.....	36 3.1.3 實
驗器材.....	37 3.2 實驗方法.....	38 3.2.1 平板
培養.....	38 3.2.2 液態菌原培養.....	38 3.2.3 搖瓶液態發
酵.....	38 3.2.3.1 不同起始pH值對菌絲體與多醣產量之影響.....	39 3.2.3.2 不同氮源對菌絲體與多醣體產量之影響.....
多醣體產量之影響.....	39 3.2.3.3 不同碳源對菌絲體與多醣體產量之影響.....	39 3.2.3.4 培養階段之菌絲體與多醣體產量之變化.....
40 3.2.3.5 鹽類的添加對菌絲體與多醣體產量之影響.....	40 3.2.3.6 不同濃度碳源菌絲體與多醣體產量之影響.....	40 3.2.3.7 不同濃度氮源菌絲體與多醣體產量之影響.....
41 3.2.3.8 培養階段餵入不同基質之菌絲體與多醣體產量之變化.....	41 3.2.4 發酵槽批次式液態發酵.....	41 3.2.5 發酵槽餵料批次式液態發酵.....
42 3.3 分析方法.....	42 3.3.1 菌體乾重測定.....	42 3.3.2 pH測定.....
42 3.3.2 pH測定.....	42 3.3.3 靈芝胞外多醣分析.....	42 3.3.3.1 酚硫酸法.....
43 3.3.3.1 酚硫酸法.....	43 3.3.3.1.1 標準曲線製作步驟.....	43 3.3.3.1.2 胞外多醣濃度測定.....
43 3.3.3.1.2 胞外多醣濃度測定.....	43 3.3.3.2 胞外多醣分子量分佈測定.....	44 3.3.4 靈芝胞內多醣分析.....
44 3.3.4.1 胞內多醣濃度測定.....	44 3.3.4.2 胞內多醣分子量分佈測定.....	44 3.3.4.1 胞內多醣濃度測定.....
45 第肆章 結果與討論.....	46 4.1 不同起始pH值對菌絲體與多醣之探討.....	46 4.1 不同起始pH值對菌絲體與多醣之探討.....
46 4.2 不同氮源基質對菌絲體與多醣體之探討.....	54 4.3 不同碳源基質對菌絲體與多醣之探討.....	54 4.3 不同碳源基質對菌絲體與多醣之探討.....
64 4.4 G. lucidum 於液態搖瓶培養階段菌絲體與多醣體產量變化之影響.....	72 4.5 鹽類的添加對G. lucidum 生成菌絲體與多醣體產量之影響.....	72 4.5 鹽類的添加對G. lucidum 生成菌絲體與多醣體產量之影響.....
74 4.6 不同碳源濃度對G. lucidum 生成菌絲體與多醣體產量之影響.....	74 4.6 不同碳源濃度對G. lucidum 生成菌絲體與多醣體產量之影響.....	74 4.6 不同碳源濃度對G. lucidum 生成菌絲體與多醣體產量之影響.....
76 4.7 不同氮源濃度對G. lucidum 生成菌絲體與多醣體產量之影響.....	78 4.8 G. lucidum 於培養7天後添加入不同基質對菌絲體與多醣體產量變化之探討.....	78 4.8 G. lucidum 於培養7天後添加入不同基質對菌絲體與多醣體產量變化之探討.....
80 4.9 批次發酵培養G. lucidum 生成菌絲體與多醣體之探討.....	84 4.10 餵料批次發酵培養G. lucidum 生成菌絲體與多醣體之探討.....	84 4.10 餵料批次發酵培養G. lucidum 生成菌絲體與多醣體之探討.....
88 第五章 結論.....	92 參考文獻.....	92 參考文獻.....
94 附錄一 酵母粉與玉米浸粉之成分表.....	104 附錄二 多醣體濃度檢量線.....	104 附錄二 多醣體濃度檢量線.....
105 附錄三 多醣分子量分佈檢量線.....	106 表目錄 表2.1 靈芝的一般化學成分.....	106 表目錄 表2.1 靈芝的一般化學成分.....
.....6 表2.2 菇類子實體與菌絲體之比較.....	14 表4.1 以GPC分析不同起始pH值之發酵液中水溶性多醣之濃度(M.W.>10,000)與分子量.....	14 表4.1 以GPC分析不同起始pH值之發酵液中水溶性多醣之濃度(M.W.>10,000)與分子量.....
之菌絲熱萃液之水溶性多醣濃度(M.W.>10,000)與分子量.....	52 表4.2 以GPC分析不同起始pH值之菌絲熱萃液之水溶性多醣濃度(M.W.>10,000)與分子量.....	52 表4.2 以GPC分析不同起始pH值之菌絲熱萃液之水溶性多醣濃度(M.W.>10,000)與分子量.....
水溶性多醣濃度(M.W.>10,000)與分子量.....	53 表4.3 以GPC分析不同氮源之發酵液中水溶性多醣濃度(M.W.>10,000)與分子量.....	53 表4.3 以GPC分析不同氮源之發酵液中水溶性多醣濃度(M.W.>10,000)與分子量.....
61 表4.4 以GPC分析不同氮源之菌絲熱萃液之水溶性多醣濃度(M.W.>10,000)與分子量.....	63 表4.5 以GPC分析不同碳源之發酵液中水溶性多醣濃度(M.W.>10,000)與分子量.....	63 表4.5 以GPC分析不同碳源之發酵液中水溶性多醣濃度(M.W.>10,000)與分子量.....
70 表4.6 以GPC分析不同碳源之菌絲熱萃液之水溶性多醣濃度(M.W.>10,000)與分子量.....	71 表4.7 比較培養基質中鹽類的添加對G. lucidum 生長之影響.....	71 表4.7 比較培養基質中鹽類的添加對G. lucidum 生長之影響.....
75 表4.8 以不同碳源濃度對G. lucidum 於搖瓶發酵培養生長之影響.....	77 表4.9 以不同氮源濃度對G. lucidum 於搖瓶發酵培養生長之影響.....	77 表4.9 以不同氮源濃度對G. lucidum 於搖瓶發酵培養生長之影響.....
79 表4.10 G. lucidum 搖瓶發酵培養7天後餵入10mL不同基質對pH之影響.....	81 表4.11 G. lucidum 搖瓶發酵培養7天後餵入10mL不同基質對菌體重之影響.....	81 表4.11 G. lucidum 搖瓶發酵培養7天後餵入10mL不同基質對菌體重之影響.....
82 表4.12 G. lucidum 搖瓶發酵培養7天後餵入10mL不同基質對胞外多醣之影響.....	83 表4.13 以不同濃度磷源對G. lucidum 於搖瓶發酵培養生長之影響.....	83 表4.13 以不同濃度磷源對G. lucidum 於搖瓶發酵培養生長之影響.....
91 圖目錄 圖2.1 靈芝的生長週期.....	11 圖2.3 微生物生長和產物形式的動力形式.....	11 圖2.3 微生物生長和產物形式的動力形式.....
4 圖2.2 靈芝多醣之結構.....	34 圖4.1 G. lucidum 以不同起始pH經搖瓶發酵培養7天後pH...48 圖4.2 G. lucidum 以不同起始pH經搖瓶發酵培養7天後之乾菌重.....	34 圖4.1 G. lucidum 以不同起始pH經搖瓶發酵培養7天後pH...48 圖4.2 G. lucidum 以不同起始pH經搖瓶發酵培養7天後之乾菌重.....
49 圖4.3 G. lucidum 以不同起始pH經搖瓶發酵培養7天後之胞外多醣.....	50 圖4.4 G. lucidum 以不同起始pH經搖瓶發酵培養7天後之胞內多醣.....	49 圖4.3 G. lucidum 以不同起始pH經搖瓶發酵培養7天後之胞外多醣.....
51 圖4.5 G. lucidum 於不同起始pH值發酵培養7天其發酵培養液中胞外多醣分子量分佈.....	52 圖4.6 G. lucidum 於不同起始pH值發酵培養7天其發酵培養液中胞內多醣分子量分佈.....	51 圖4.5 G. lucidum 於不同起始pH值發酵培養7天其發酵培養液中胞外多醣分子量分佈.....
53 圖4.7 G. lucidum 以不同氮源經搖瓶發酵培養7天後之乾菌...57 圖4.9 G. lucidum 以不同氮源經搖瓶發酵培養7天後之胞外多醣.....	58 圖4.10 G. lucidum 以不同氮源經搖瓶發酵培養7天後之胞內多醣.....	53 圖4.7 G. lucidum 以不同氮源經搖瓶發酵培養7天後之乾菌...57 圖4.9 G. lucidum 以不同氮源經搖瓶發酵培養7天後之胞外多醣.....
59 圖4.11 G. lucidum 於不同無機氮源發酵培養7天其發酵培養液中胞外多醣分子量分佈.....	60 圖4.12 G. lucidum 於不同有機氮源發酵培養7天其發酵培養液中胞外多醣分子量分佈.....	59 圖4.11 G. lucidum 於不同無機氮源發酵培養7天其發酵培養液中胞外多醣分子量分佈.....
60 圖4.13 G. lucidum 於不同無機氮源發酵培養7天其菌絲熱萃		60 圖4.13 G. lucidum 於不同無機氮源發酵培養7天其菌絲熱萃

液中胞內多醣分子量分佈.....	62	圖4.14 G. licidum於不同有機氮源發酵培養7天其菌絲熱萃液中胞內多醣分子量分佈.....	62	圖4.15 G. licidum以不同碳源經搖瓶發酵培養7天後之pH....	66	圖4.16 G. licidum以不同碳源經搖瓶發酵培養7天後之乾菌重.....	67
以不同碳源經搖瓶發酵培養7天後之胞外多醣.....	68	圖4.17 G. licidum以不同碳源經搖瓶發酵培養7天後之胞內多醣.....	69	圖4.18 G. licidum以不同碳源發酵培養7天其發酵培養液中胞外多醣分子量分佈.....	70	圖4.20 G. licidum於不同碳源發酵培養7天其菌絲熱萃液中胞內多醣分子量分佈.....	71
之pH與乾菌種及胞內、胞外多醣之化.....	73	圖4.21 G. licidum於搖瓶發酵培養13天.....	73	圖4.22以2%葡萄糖為碳源發酵槽批次發酵培養13天.....	73	圖4.23以4%葡萄糖為碳源發酵槽批次發酵培養.....	86
85	圖4.24以4%葡萄糖為碳源發酵槽批次發酵培養轉速提高到350rpm.....	87	圖4.25餌料批次發酵培養，在培養第三天餌入1%CSP1L(pH4.68)，在第七天餌入4%葡萄糖1L，發酵培養13天.....	89	圖4.26餌料批次發酵培養，在培養第三天餌入1%CSP2L(pH7)，發酵培養13天.....	90	

## REFERENCES

- 1.丁懷謙(2000)食藥用菇多醣體之免疫生理活性，食品工業，32(5):28-42。
- 2.尤新(2001)機能性醣酵製品，藝軒圖書出版社。
- 3.王伯徹、黃麗娜(2001)保健用菇類發酵工業之開發，農業世界雜誌，281:100-106。
- 4.王伯徹(2000a)具開發潛力食藥用菇介紹，食品工業，32(5):1-17。
- 5.王伯徹(2000b)食藥用菇保健食品之開發，食品工業，32(5):18-27。
- 6.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。
- 7.王伯徹(1990)藥用真菌系列報導(一)靈芝，食品工業，22(1):23-33。
- 8.王進琦、李聰明、賴敏男(1998)猴頭菇以液體浸漬培養產製水溶性多醣類之探討，食品科學，26(5):714-726。
- 9.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。
- 10.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。
- 11.白壽雄、羅道蘊(1994)生物性多醣體及應用，生物產業，5(34):167-173。
- 12.江國瑛、段國仁、許塗棋(1998)利用深層發酵法生產靈芝多醣之研究，大同學報，28:353-358。
- 13.江國瑛、張欣暘、許塗棋、段國仁(1997)利用深層醣酵法生產靈芝多醣的研究，第二屆生化工程研討會論文集，139-142。
- 14.任一平、黃百芬、陳俊青(1996)應用高效液相色譜法測定香菇多醣，食品與發酵工業，5:31-35。
- 15.李平作、徐柔、章克昌(1999)靈芝發酵過程中胞外多醣快速測定模型的建立，無錫輕工大學學報，18(3):62-65。
- 16.李秉征(2001)以液態培養生成香菇菌絲體之研究，東海大學化學工程研究所碩士論文。
- 17.李明彥(1990)松杉靈芝浸漬發酵的培養條件對產物的影響，台灣大學農業化學研究所碩士論文。
- 18.李玲玲、王正怡、蘇慶華(1995)利用流動細胞分析儀測植生蟲草抗腫瘤多醣體(PN-2)對小白鼠巨噬細胞吞噬能立即輔助淋巴細胞活性之影響，北醫學報，23(1):11-19。
- 19.李泰興(1998)氣氣傳送介質及消泡劑對模擬醣酵液中氣氣傳送之影響，技術學刊，13(1):103-108。
- 20.李俊賢、高寶璧、詹美華、蘇慶華(1992)真菌性中藥材水溶性多醣之分析，北醫學報，21(1):25-32。
- 21.吳景陽(2001)-聚葡萄糖之生理功能與理化特性，食品工業，33(6):1-9。
- 22.林俊清(1990)生藥的解說 灵芝的介紹，藥學介紹，6(3):104-111。
- 23.武梅、周應揆、趙永昌、李亞輝(1999)靈芝菌絲體液體發酵 培養產靈芝多醣的動態研究，雲南大學學報，21(2):165-166。
- 24.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。
- 25.唐瑞菁、程梅萍(1992)靈芝培養基的探討 - 酵母抽出物的取代，國立雲林技術學院學報，1:145-156。
- 26.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。
- 27.高益槐(2000)世紀奇草話靈芝。元氣齋出版社，台北市。
- 28.郭倩、周昌艷、高軍輝(1998)無苦靈芝子實體多醣的研究，食用菌學報，5(3):21-25。
- 29.許瑞祥(1995)靈芝的研究現況與展望，生物產業，6(4):289-296。
- 30.陸文樸、林忠平、林志彬(1992)靈芝的科學應用，渡假出版社。
- 31.梁志欽(1991)松杉靈芝浸漬發酵培養生產的1,3-glucanase對菌體外多醣的影響，國立台灣大學農業化學研究所碩士論文。
- 32.陳大為、黃壤基、李旭生(1991)靈芝對體外培養之口腔癌細胞的毒殺效應，中華醫誌，48:54-57。
- 33.陳健祺(2000)，食用菇類在醫學上的應用，食品工業，32(5):54-69。
- 34.張為憲、李敏雄、呂政義、張永和、陳昭雄、孫璐西、陳怡宏、張基郁、顏國欽、林志城、林慶文(1996)，食品化學，華香園出版社。
- 35.黃仕政、陳勁初(2000)發酵生物技術在菇類食品的應用，中華食品工業，40:62-66。
- 36.黃家樑(1997)液態培養靈芝菌絲體與靈芝多醣體之研究，東海大學化學工程研究所碩士論文。
- 37.黃雪芳、劉柯俊、管育慧、董光世、蘇慶華、董大成(1989)口服靈芝之菌絲培養液之抗癌人工轉移作用，中華癌醫會誌，5(1):10-15。
- 38.黃賜源(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。
- 39.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。
- 40.黃麗娜(1998)菇類菌絲體深層培養在食品工業上之應用，食藥用菇類的培養與應用，144-150，食品工業研究所出版。
- 41.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。
- 42.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。
- 43.趙純一(1997)，微差黏度計與光散射儀在膠體滲透層析儀的原理與應用，科儀新知，19(3):26-40。
- 44.楊革(1997)靈芝菌絲體深層培養及多糖提取工藝研究，食用菌學報19(2):8-9。
- 45.游英欽(1996)以搖瓶振盪及小型發酵槽培養探討培養基組成及物理化學因子對靈芝多醣生長形態變化的影響，國立交通大學生物科技研究所碩士論文。
- 46.劉峻?(1996)靈芝液態培養及多醣生成之研究，東海大學化學工程研究所碩士論文。
- 47.劉柯俊、黃雪芳、蘇慶華、董大成(1989)口服靈芝多醣體之吸收ICR老鼠口服標識碳14靈芝培養液之研究，中華癌醫會誌，5(2):22-30。
- 48.劉國柱(1990)現代科學看靈芝，雙利實業有限公司，台北。
- 49.韓紹英、趙傳孝、姜彥祥、龔國華(1989)食用菌高產栽培及科學加工。中國食品出版社，北京。
- 50.蘇慶華(1991)靈芝之分類學及生理活性物質，北醫學報20:1-16。
- 51.Alexopolus, C. J. and C. W. Mims (1979) Introductory Mycology. John Wiley and Sons, Inc. N. Y.
- 52.Arcidiacono, S. and Kaplan, D. L. (1992) Molecular weight distribution of chitosan isolated from *Mucor rouxii* under different culture and processing conditions. Biotech. Bioeng. 39:281-286.
- 53.Breene, W. M. (1990) Nutritional and medicinal value of specialty mushrooms. J. of Food

Protection. 53(10)883-894. 54.Chen, W. C., Hau, D. M., Wang, C. C., Lin, I. H., and Lee, S. S. (1995) Effect of Ganoderma lucidum and Krestin on subset T-cell in spleen of g-irradiated mice. J. of Chinese Med. 23(1):71-80. 55.Choi, J. H., Oh, D. K., Kim, J. H. and Lebeault, J. M. (1991) Characteristics of novel high viscosity polysaccharide, methylan, produced by *Methylbacterium organophilum*. Biotech. Lett. 13(6):417-420. 56.De la Vega, M. G., Cejudo, F. J. and Panque (1991) Production of exocellular polysaccharide by *Azotobacter chroococcum*. Appl. Biochem. Biotech. 30:273-284. 57.Dubois, M., Gilles, K. A., Hamilton, J. K., Reber, P. A. and Smith F. (1956) Colorimetric method for determination of sugars and related substances. Anal. chem. 28(3):350-356. 58.Eyal, J. (1991) Mushroom mycelium growth in submerged culture potential food applications. In Biotechnology and Food Ingredients. ed. Goldberg, I. and Williams, R., Van Nostrand Reinhold. New York. p.31-64. 59.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 Biochem. 37:769-774. 60.Gutierrez, A., Prieto, A. and Martinez, A. T. (1996) Structural characterization of extracellular polysaccharide produced by fungi from the genus *Pleurotus*. Carb. Res. 281:143-154. 61.Hikino, H., Konno, C., Mirin, Y., and Hayashi T. (1985) Isolation and hypoglycemic activity of ganoderans A and B, glycans of *Ganoderma lucidum* fruit bodies. Planta Medica. 4:339-404. 62.Hosono, A., Lee, J., Ametin, A., Natsume, M., Hirayama, M., Adachi, T. and Kaminogawa, S., (1997) Characterization of a water-soluble polysaccharide fraction with immunopotentiating activity form *Bifidobacterium adolescentis* M 101-4. Biosci. Biotech. Biochem. 64(2):312-316. 63.Israilides, C., Bocking, M., Smith, A. and Scanlon, B. (1994) A novel rapid coupled enzyme assay for the estimation of pullulan. Biotech. Appl. Biochem. 19:285-291. 64.Kawagishi, H. (1995) Mushroom lectins. Food Rev. Int. 11(1):62-68. 65.Kiho, T., Hui, J., Yamane, A. and Ukai, S. (1993) Polysaccharide in fungi XXXII. Hypoglycemic activity and chemical properties of polysaccharide from the cultural mycelium of *Cordyceps sinensis*. Biol. Pharm. Bull. 16(12):1291-1293. 66.Kim, K. C. and Kim, I. G. (1999) *Ganoderma lucidum* extract protects DNA from strand breakage caused by hydroxyl radical and UV irradiation. Int. J. Mol. Med. 4:273-277. 67.Kim, D. H., Shim, S. B., Kim, N. J. and Jang, I. S. (1999) Beta-glucuronidase inhibitory activity and hepatoprotective effect of *Ganoderma lucidum*. Biol. Pharm. Bull. 22:162-164. 68.Lacroix C., Ledy A., Noel G. and Choplin L. (1985) Effect of pH on the batch fermentation of pullulan from sucrose medium. Biotech. Bioeng. 27:202-207. 69.Lee, S. S., Wei, Y. H. Chen, C. F., Wang, S. Y., and Chen, K. Y. (1995) Antitumor effects of *Ganoderma lucidum*. J. of Chinese Med. 6(1):1-12. 70.Lei L. S. and Z. B. Lin (1992) Effect of *Ganoderma* polysaccharides on T cell subpopulations and production of interleukin 2 in mixed lymphocyte response. Acta Pharmaceutica Sinica. 27(5):331-335. 71.Lieu, C. W., Lee, S. S. and Wang S. Y. (1992) The effect of *Ganoderma lucidum* on induction of differentiation in leukemic U937 cells. Anticancer Res. 12:1211-1216. 72.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. 73.Litchfield, J. H. (1967) Submerged culture of mushroom mycelium. In : Microbial Technology(Peppler, H. J., ed), Reinhold, New York, 107-144. 74.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 polysaccharide. J. Chromato. A. 823:339-347. 75.Luo, J., Huang, R., Chen, L., Li, H. and Z, Z. (1998) Fermentation of *Ganoderma lucidum* and studies on polysaccharides. 98" Nanjing International Symposium on Science & Cultivation of Mushrooms. Nanjing, China. 76.Machova, E., Kvapilova, K., Kogan, G. and Sandual, J. (1999) Effect of ultrasonic treatment on the molecular weight of carboxymethylated chitin-glucan complex from *Aspergillus niger*. Ultra. Sonochem. 5:169-172. 77.Maruyama, H., Yamazaki, K., Murofushi, S., Konda, C., and Ikekawa, T. (1989) Antitumor activity of *Sarcodon aspratus* (Berk.) S. Ito and *Ganoderma lucidum* (Fr.) Karst. J. of Pharmacobio-Dynamics. 12:118-123. 78.Miyazaki, T. and Nishijima M. (1981) Studies on fungal polysaccharides XXVII. Structural examination of a water-soluble, anti-tumor polysaccharides of *Ganoderma lucidum*. Chem. Parm. Bull. 29(12):3611-3616. 79.Mizuno, T., Nishitoba, T., Saito, H. and Kawagishi, H. (1995a) Antitumor-active substances from mushrooms. Food Rev. Int. 11(1)23-61. 80.Mizuno, T., Wang, G., Zhang, J., Kawagishi, H., Nishitoba, T. and Li J. (1995b) Reishi, *Ganoderma lucidum* and *Ganderma tsugae* : Bioactive substance and medicinal effects. Food Rev. Int. 11(1)151-166. 81.Mizuno, T., T. Sakai and G. Chihara (1995c) Health foods and medicinal usages of mushroom. Food Rev. Int. 11(1)69-81. 82.Morigiwa, A., Kitabatake, K., Fujimoto, Y., and Ikekawa, N. (1986) Angiotensin converting enzyme-inhibitory triterpenes from *Ganoderma lucidum*. Chemical and Pharmaceutical Bulletin. 34:3025-3028. 83.Pan, J., Cao, X., Li, F., Zuo, Z., Sun, Q. and Wang, L. (1996) Studies on the nutritional requirement of *Ganoderma lucidum* in submerged culture. Acta Edulis Fungi. 3(4):31-34. 84.Park, E. J., Ko, G., Kim, J., and Sohn, D. H. (1997) Antifibrotic effects of a polysaccharide extracted from *Ganoderma lucidum*, glycyrrhizin, and pentoxifylline in rats with cirrhosis induced by biliary obstruction. Biol. Pharm. Bull. 20:417-420. 85.Peter, H., Herbst, H., Hessselink, P. M., Lunsdorf, H., Schumpe, A. and Deckwer, W. (1989) The influence of agitation rate on xanthan production by *Xanthomonas campestris*. Biotechnol. Bioeng. 34:1393-1397. 86.Seviour, R. J. and Kristiansen, B. (1983) Effect of ammonium ion concentration on polysaccharide production by *Aureobasidium pullulans* in batch culture. Appl. and MicroBiol Biotechnol. 17:178-181. 87.Smith, I. H. and Pace, G. W. (1982) Recovery of microbial polysaccharides. J. of Chem. Technol. and Biotechnol. 32:119-129. 88.Sone, Y., Okuda, R., Wada, N., Kishida, E., and Misaki, A. (1985)Structure and antitumor activities of the polysaccharide isolated from fruiting body and growing culture of mycelium of *Ganoderma lucidum*. Agric. and Biol. Chem. 49(9):2641-2653. 89.Song, C. H., and Nair, N. G. (1987) A synthetic medium for the production of submerged cultures of *Lentinus Edodes*. Mycologia. 79(6):866-876. 90.Staats, N., Stal, L. J. and Mur, L. R.(2000) Exopolysaccharide production by the epipellic diatom *Cylindrotheca closterium*: effect of nutrient conditions. J. of Exp. Marine Biol. and Ecolo. 249:13-27. 91.Stasinopoulos, S. I. and Seviour, R. J. (1992) Exopolysaccharide production by *Acremonium persicinum* in stirred-tank and air-lift fermentors. Appl. and Microbiol Biotechnol. 36:465-468. 92.Stasinopoulos, S. J., Seviour, R. J. (1990) Stimulation of exopolysaccharide production in the fungus *Acremonium persicinum* with fatty acids. Biotech. and Bioeng. 36:778-782. 93.Stasinopoulos, S. J., Seviour, R. J. and Auer, D. F. (1989) Inhibition of fungal exopolysaccharide production

by chemical antifoams. Lett. in Appl. Microbiol. 8:91-93. 94. Stejskal, J. and Potucek, F. (1985) Oxygen transfer in liquids. Biotech. and Bioeng. 27:503-508. 95. Tseng, T. C., Shiao, M. S., Shieh, Y. S. and Hao, Y. Y. (1984) Study on Ganoderma lucidum 1. Liquid culture and chemical composition of mycelium. Bot. Bull. Academia Sinica. 25:149-157. 96. Wang, S. Y., Hsu, M. L., Hsu, H. C., Tzeng, C. H., Lee, S. S., Shiao, M. S., and Ho, C. K. (1997) The anti-tumor effect of Ganoderma lucidum is mediated by cytokines released from activated macrophages and T lymphocytes. Int. J. of Cancer. 70:699-705. 97. William C. and Wernau, G. C. (1981) Fermentation Process for Production of Xanthan. U.S. Patent. 4(80):195-203. 98. Williams, D. L., Pretus, H. A. and Browder, I. W. (1992) Application of aqueous gel permeation chromatography with in-line multi-angle laser light scattering and differential viscometry detectors for the characterization of natural product carbohydrate pharmaceuticals. J. Liquid Chromatogr. 15:2297-2309. 99. Wood, P. J., Weisz, J. and Blackwell, B. A. (1991) Molecular characterization of cereal  $\beta$ -D-glucans. Structural analysis of oat I  $\beta$ -D-glucans from different source by high-performance liquid chromatography of oligosaccharides released by lichenase. Cereal. Chem. 68(1):31-39. 100. Yamane, T. and S. Shimizu (1984) Fed-batch Techniques in Microbial Processes, Adv. Biochem. Eng. Biotechnol., 30, 147-194. 101. Yang, F. C. and Liau, C. B. (1998a) Effects of cultivating conditions on the mycelial growth of Ganoderma lucidum in submerged flask cultures. Bioprocess Eng. 19:233-236. 102. Yang, F. C. and Liau, C. B. (1998b) The influence of environmental conditions on polysaccharide formation by Ganoderma lucidum in submerged cultures. Process Biochem. 33(5):547-553. 103. Yang, F. C. and Hwang S. Y. (1998c) Nutritional studies on submerged culture of Ganoderma lucidum. Tunghai J. 39:1-10. 104. 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 Technol. 27(3-5):295-301. 105. 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. Biotechnol. Lett. 9(9):621-624. 106. Youssef, F., Roukas, T. and Biliaderis, C. G. (1999) Pullulan production by a non-pigmented strain of Aureobasidium Pullulans using batch and Fed-batch culture. Process Biochem. 34:355-366. 107. Xu, H., Lee, S. H., S., Lee, S. F., White, R. L. and Blay J. (1999) Isolation and characterization of an anti-HSV polysaccharide from Prunella vulgaris. Antiviral Res. 44:43-54.