

Effect of medium and cultural condition in submerged fermentation on mycelium biomass and extracellular polysaccharide pr

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ABSTRACT

Wolfiporia cocos is a medicinal fungi, its sclerotium has been long been used as traditional Chinese herb with diuretic, sedative and tonic. Pharmacology studies have proven that its avtive ingredients possess anti-inflammatory, anti-aging, immunity, anti-tumor, sedative, diuretic and anti-emetic properties. Triterpenes and pachymaran have been reported as major biochemical activities. Mycelium is cultured through fermentation and used in health food products to develop product into commercial quantities. Fermentation culture using the chemically defined media culture followed. The process not only prevented interference during the effective ingredient extraction, but also further controlled the Wolfiporia cocos growth within the condition required for the effective ingredients. Then chemically defined media was added to Wolfiporia cocos and cultured in 25 °C controlled temperature oscillator to evaluate the effects of the initial pH, different carbon-nitrogen ratio, glucose concentration, nitrogen concentration and inoculation dosage of the culture medium on the mycelium biomass , reducing sugar and the mycelium extracellular polysaccharide composition, and analyzed the crude protein, crude fat, total sugar ,ash and water content of mycelia and sclerotium. Findings showed that initial pH was 3.0 ; carbon-nitrogen ratio was 30:1 ; glucose concentration was 5.0% ; and the mycelium biomass content of the nitrogen concentration was 1% fermented culture mycelium biomass was higer. And extracellular polysaccharid and showed that initial pH was 3.0; carbon-nitrogen ratio was 30:1; glucose concentration was 5.0%; and the mycelium biomass content of the nitrogen concentration was 1% fermented culture was higer. A 5L fermentation tank was used to stuffy the physicochemical factor including the different temperature, different stirring speed, changes of the mycelium biomass, reducing sugar, pH in supernatant and extracellular polysaccharide under 168-hours fermentation process. Finding showed that maximum mycelium biomass was achieved under 30 °C and 200 rpm stirring speed. Extracellular polysaccharide was achieved under a 30 °C , 200 rpm stirring speed.

Keywords : medicinal fungi ; Wolfiporia cocos ; submerged culture ; mycelium ; extracellular polysaccharide

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REFERENCES

- 1.尤新編著。2001。機能性醣酵製品。p119-1670。
- 2.王伯徹。1990。藥用真菌系列報導(四)。茯苓。食品工業。22 (5) :32~38。
- 3.王伯徹。1990。藥用真菌系列報導(一)靈芝。食品工業。22 (5) :23-30。
- 4.王利業、方惠杰、陳逢喜、張紅敏及喻宗源。1993。茯苓中三?系化學成分之研究。現代應用藥學 10:8-11。
- 5.王秋穎及徐錦堂。1993。蜜環菌發酵液在豬苓菌發酵過程中的應用。中國藥學雜誌 28(8): 466-468。
- 6.王進琦、李聰明及賴敏男。1998。猴頭菇以液態浸漬培養製水溶性多醣之探討。食品科學25(6):714-726。
- 7.卯曉嵐。1988。中國野生真菌種類及生態習性。真菌學報7: 36-43。
- 8.田雅嵐。2001。培養基與培養條件對冬蟲夏草菌菌絲體生物質量、化學組成及水溶性胞內多醣體生成之影響。大葉大學食品工程研究所碩士論文。
- 9.宋愛榮、郭立忠、段方猛及劉作亭。1999。pH對灰樹花液體深層發酵的影響。中國食用菌。18 (3) :29-31。
- 10.李兆蘭。1987。裂褶菌深層培養及多糖測定。真菌學報。6 (3) :170-177。
- 11.李俊賢、高寶璧、詹美華及蘇慶華。1992。真菌性中藥材水溶性多糖成分之分析。北醫學報21 (1) : 25-33。
- 12.李曉明、戴如琴及朱勤。1989。冬蟲夏草發酵濾液多糖的組成分分析。中國中藥雜誌14 (2) : 31-33。
- 13.杜自疆。1980。食用菇栽培技術。豐年社 p219。
- 14.周立、張瑋、蘭惠顏及許津。1994。茯苓素誘生腫瘤壞死因子 (TNF) 的作用。中國抗生素雜誌。19 (5) :376-380。
- 15.柯麗霞及楊曉彤。1998。金針菇菌絲體糖蛋白(FMGP)的提取及其理化特性。中國食用菌 17(4): 40-42。
- 16.柳勇。1994。茯苓純菌種速生高產栽培技術。生物學通報 29:41-42。
- 17.胡琦桂。1994。真菌球狀菌絲體生長之探討。食品工業。26(9):37-45。
- 18.徐錦堂。1997。中國藥用真菌學。聯合出版社。p547-573。
- 19.張土善、張丹參、朱桐君及陳醒言。1992。冬蟲夏草胺基酸成分的藥理分析。藥學學報26 (5) : 326-330。
- 20.張建軍。1995。藥用真菌多糖體成分的分離方法之研究。交通大學生物科技研究所碩士論文。
- 21.許益民、李學農及陳建偉。1992。中成藥。14 (4) :37。
- 22.陳春霞。1985。茯苓多糖體的藥理藥化研究及臨床應用初探。中草藥。16(4): 40-44。
- 23.陳偉盈、馮觀泉、許堯興、弗迪波、許少春及袁亞。1992。冬蟲夏草工藝深層發酵研究。中草藥23 (8) : 409-411, 416。
- 24.陳萍、陳桂良及陳並恆。1995。比色法測定甲基茯苓多糖之含量。中國中藥雜誌 20:543-544。
- 25.陶雪娟、徐崇敬、宋鳳菊、張建敏及陳建華。1999。蕈菌液體生物醣酵技術的研究現狀與進展。上海農學院學報 17: 141-147。
- 26.閔三弟。1996。真菌的藥用價值。食用菌學報3(4): 55-64。
- 27.黃仁彰。2000。菇類多醣體製劑的研發與應用。食品工業 32(10): 45-58
- 28.黃賜源。1996。靈芝液體培養及氣舉式生化反應槽之研究。東海大學化工所碩士論文。
- 29.黃麗娜。1996。食用菇菌絲體深層培養在食品工業上的應用。食品工業。28(9): 20-26。
- 30.楊新美。1988。中國食用菌栽培學。農業出版社2584頁。
- 31.雷敬敷、張玲及李與義。1993。香菇發酵工藝及香菇多糖的提取。中國食用菌 12(3): 31-35。
- 32.趙吉福、麼雅娟、陳英杰、徐綏緒及姚新生。1996。磺醯化新茯苓多糖的製備及抗腫瘤作用。瀋陽藥科大學學報13 (2) : 125-128。
- 33.劉明哲。1988。茯苓。藥用植物栽培 9:347-354。
- 34.劉松青及武成榮。1999。灰樹花栽培技術研究。中國食用菌 18(1):16。
- 35.劉波。1984。中國藥用真菌。山西人民出版社。p53-60。
- 36.劉勝宇。2001。探討培養溫度對巴西蘑菇液態醣酵之影響。國立中央大學化學工程研究所碩士論文。新竹，台灣。
- 37.潘琦及何蘭茜。1998。茯苓多糖的結構改造及分析測定。雲南中醫學院學報21 (4) : 20-21。
- 38.蔣先明及石清東。1996。茯苓多糖與羧甲基茯苓多糖的結構表現。廣西師範大學學報11 (3) : 40-46。
- 39.蔡淑娟。1997。茯苓與豬苓核醣體基因18 s-28s間隔區域核甘酸序列之探討。東吳大學微生物學系碩士論文。。
- 40.鄧剛民及許津。1992。茯苓素:一種潛在的醛固酮拮抗劑17 (1) : 34-37。
- 41.蕭麗華。1997。冬蟲夏草藥材真偽品與發酵培養製備物之結構特徵及成份分析比較。大葉大學食工所碩士論文。
- 42.賴慶亮譯。水野卓及川合正允編。。1997。菇類的化學、生化學。國立編譯館。
- 43.戴郁軌及朱凱俊。1982。真菌名詞辭典。名山出版社2467頁。
- 44.顏新泰。1992。茯苓有性繁殖研究報告。中藥材 15:6-7。
- 45.魏玎玲、吳榮燦、張由美、葉小帆。1990。藥用真菌茯苓對癌細胞之分化誘導作用研究。行政院國家科學委員會專題研究計畫成果報告。
- 46.Ainsworth, G. C., Sparrow, F. K., and Sussman, A. S. 1973a. The fungi. . A taxonomic review with keys:Ascomycetes and fungi imperfecti, Academic press, Inc. New York.
- 47.Catley. 1980. The extracellular polysaccharide, pullulan, produced by Aureobasidium pullulans-A relationship between elaboration rate and morphology. J. Gen. M. 120:265-268.

- 48.Cheung, P. C-K. 1997. Dietary fiber content and composition of some edible fungi determined by two methods of analysis. *J. Sci. Food. Agric.* 73:255-260. 49.Chihara, G., Hamuro, J., Makda, Y., Arat, Y. and Fukuoka, F. 1970. Antitumour polysaccharide derived chemically from natural glucan (pachyman) . *Nat.* 225:943-944. 50.Cochrane , V. W. 1958. Physiology of fungi. John. Willey, New York. p524. 51.Cuellar, M. J., Giner, R. M., Recio, M. C., Just, M. J., Manez, S. and Rios, J. L. 1996. Effect of the Basidiomycete *Poria cocos* on experimental dermatitis and other inflammatory conditions. *Chem. Pharm. Bull.* 45(3): 492-494. 52.Cuellar, M.J., Giner, R.M., Recio, M. C. Just, M.J., Manez, S., Rios, J. L. 1997. Two fungal lanostane derivatives as phospholipase A2 inhibitors. *J. Natl. Products.* 59: 977-979. 53.Desmond-T, Lalor-FJ, Osullivan-B Ferguson-G. Synthesis. 1990. Structure and properties of stereochemically non-rigid molybdenum pyrazolylborato complexes containing a Dihapto-Thiocarboxyamido ligand. *J. Org. Metallic Chem.* 381:33-37. 54.Ding, Q., Jiang, S., Zhang, L. and Wu, C. 1998. Laser light-scattering studies of pachyman. *Carbohydrate Res.* 308: 339-343. 55.Dobois, M., Gilles, K. A., Hamilton, J. K., Rebers, P.A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28(3): 350-356. 56.Eyal, J. 1991. Mushroom mycelium grown in submerged culture-potential food applications. (Goldberg, I. And Williams, R., eds) p31-64. Van Nostrand Reinhold, New York.
- 57.Franz, G. 1989. Polysaccharides in pharmacy: Current applications and future concepts. *Plant Med.* 55:493-497. 58.Giner-Larza, E. M., Manez, S., Giner-Pons, R. M., Recio, M. C. and Rios, J. L. 2000. On the anti-inflammatory and anti-phospholipase A2 activity of extracts from lanostane-rich species. *J. Ethnopharmacology.* 73: 61-69. 59.Hamuro, J., Yamashita, Y., Ohsaka, Y., Maeda, Y. Y.and Chihara, G. 1971. Carboxymethylpachymaran, a new water soluble polysaccharide with marked antitumour activity. *Nat.* 233: 486-488. 60.Hattori, T., Hayashi, K., Nagao, T., Furuta, K., Ito, M. and Suzuki, Y. 1992. Studies of antinephritic effects of plant components (3) . Effects of pacyman, a main component of *Poria cocos* Wolf on original —type anti-GBM nephritis in rats and its mechanisms. *Jpn. J. Pharmacol.* 59: 89-96. 61.Hatvani, N. 2001. Antibacterial effect of the culture fluid of *Lentinus edodes* mycelium International J. Antimicrobial Agents. 17:71-74. 62.Hayes, W. A. 1978. Biological nature. p191-237. In: The biology and cultivation of edible mushroom. Eds., S. T. Chang and W. A. Hayes. Academic Press. New York.
- 63.Hikino, H. 1985.Recent research on oriental medicinal plants. In: Wanger, H., Hikino, H., Farnsworth, N.R. (Eds), Economic and medicinal plant research. 1. Acaemic Press, London. p64-65. 64.Hsu, H.Y., Chen, Y. P., Shen, S. J., Hsu, C. S., Chen, C C. and Chang, H. C.1986. Oriental material medica, A concise guide. Oriental healing arts institute, Long beach. p 305-306. 65.Humfeld, H. 1948. The production of mushroom mycelium *Agaricus campestris* in submergec culture. *Sci.* 107:133. 66.Jong , S. C., Birmingham. J. M., and Pai, S. H. 1991. Immunodulatory substances of fungal origin *J. Immunol. Immunopharmacol.* 11:115-122. 67.Jong, S. C. and Birmingham, J. M. 1993. Medicinal and therapeutic value of the Shitake mushroom. *Adv. Appl. Microbiol.* 39:153-184. 68.Jong, S. C. and Donovick, R. 1989. Antiumor and antiviral substances from fungi. *Adv. Appl. Microbiol.* 34: 183-262. 69.Jong, S. C., Birmingham, J. M. and Pai, S. H. 1991. Immumomodulatory substances of fungal origin. *EOS Rev. Immunol. Immunopharmacol.* 11: 115-122. 70.Kanayama, H., Adachi, N. and Togami, M. 1983. A new antitumor polysaccharide from the mycelia of *Poria cocos* Wolf. *Chem. Pharm. Bull.* 31(3):1115-1118. 71.Kosaric, N., LeDuy, A. and Zajic, J. E. 1973. Submerged culture growth of edible mushroom on waste sulphite liquors. *Can. J. Chem. Eng.* 51:186-190. 72.Kurtzmann, R. H. and Y. Chang-Ho. 1982. Physiological consideration for cultivation of Volvariella mushrooms. p 139-161. In: Tropical mushrooms: biological nature and cultivation methods. Eds., S. T. Chang and T. H. Quimio. The Chinese Univ. Press. Hong Kong. 73.Litchfield, J. H. 1967. Submerged culture of mushroom mycelium. In: Microbial Technology. (Peppler, H. J. ed) p107-144. Reinhold, New York. 74.Litchfield, J. H. 1979. Production of single cell protein for use in food and feed. In: Microbial Technology. 2nd ed (Peppler, H. J. and Perlmann, D., eds). p93-145. Academic press. New York.
- 75.Litchfield, J. H. and Overbeak, R. C. 1965. Submerged culture growth of *Morchella* species in food processing waste substances. Proceedings of the 1st Inter. Congress on Food Sci and Technol. London vol.2. p 511-520. 76.Margaritis, A. and Pace, G. W. 1985. Microbial polysaccharides. In: Comprehensive biotechnology. Vol.3 eds. M-Y, Murrsy, H. W. Blanch., S. Drew. And D. I. C. Wang. Pergamon Press, Oxford. p1004-1005.
- 77.Mcneil, B. and Kristiansen, B. 1989. Influence of impellar speed upon the pullulan fermentation. *Biotechnol. Lett.* 9:101. 78.Milagres, A. M.F. and Sales, R. M. 2001. Evaluating the basidiomycetes *Poria medulla-panis* and *Wolfiporia cocos* for xylanase production. *Enzyme and Microbial Technology.* 28: 522-526. 79.Mizuno, T. 1999. The extraction and development of antitumor-active polysaccharides from medicinal mushrooms in Japan (Review) . *Inter. J. Medicinal mushroom.* 1:9-29. 80.Mizuno, T., Kinoshita, T., Zhuang, C., Ito, H. and Mayuzumi, Y. 1995b. Antitumor-active substances from mushrooms. *Food Rev. Int.* 11: 23-61. 81.Narui, T., Takahashi, K., Kobayashi, M. and Shibata, S. 1980. A polysaccharide produced by laboratory cultivation of *Poria cocos* Wolf. *Carbohydrate Res.* 87:161-163. 82.Ono, K., Yasuda, N. and Ueda, S.1997. Effect of pH on puppulan elaboration by *Aureobasidium pullulans* S-1. *Aga. Biol. Chem.* 41(11): 2113. 83.Park, J. P. Kim, S. W. Hwang, H. J. and Yun, J.W. 2001. Optimization of submerged culture conditions for the mycelial growth and exo-biopolymer production by *Cordyceps militaris*. *Letters Appl. Microbiol.* 33: 76-81. 84.Resser, F. J., Spencer, F. T. and Sallans, H. R. 1958b. Protein and fat content of some mushroom growth in submerged cultured . *Appl. Microbiol.* 6: 5-8. 85.Saito, H., Misaki, A. and Harada, T. 1968. A comparison of the structure of curdlan and pachyman. *Agr. Biol. Chem.* 32 (10) : 1261-1269. 86.Schugerl, K., Wittler, R. and Lorenz, T. 1983. The use of molds in pellent from Trends . *Biotechnol.* 1: 120-122. 87.Seviour, R. J., Stasinopoulos, S. J., Auer, D. P. F. and Gibbs, P. A. 1992. Production of pullulan and other exopolysaccharides by filamentous fungi. *Crit. Rev. Biotechnol.* 13:279-298. 88.Sone, Y., Okuda, R., Wada, N., Kishida, E. and Misaki, A. 1985. Structures and antitumor activities of the polysaccharides isolated from fruiting body and the growing culture of mycelium of *Ganoderma lucidum*. *Agric. Biol. Chem.* 49 (9) :2641-2653. 89.Song, C. H. and Cho, K. Y. 1987. A synthetic medium of the production of submerged culture of *Lentinus edodes*. *Mycologia.* 79: 866-876. 90.Tai, T., Akahori, A. and Shingu, T. 1993. Triterpenes of *Poria cocos* . *Phytochemistry.* 32:1239-1244. 91.Tai, T., Akita, Y., Kinoshita, K., Koyama, K., Takashi, K. and Watanabe, K. 1995. Anti-emetic principles of *Poria cocos*. *Plant*

Med. 61(6): 527-530. 92.Tseng, J. and Chang, J. G. 1992. Suppression of tumor necrosis factor- α , interleukin-1 β , interleukin-6 and granulocytmonocyte by an extract of *Poria cocos*. Chinese J. Microbiol. Immunol. 25: 1-11. 93.Wu, B., Liang, M., Tong, L., Huang, T., Liang, N. and Li, J. 1994. Zhongguo Yaolixue Tongbao. 10: 300-304. 94.Yamada, H., Kiyohara, H., Takemoto, N., Zhao, J. F., Kawamura, H., Komatsu, Y., Cyong, J. C., Aburada, M. and Hosoya, E. 1992. Plant Med. 58: 166-170. 95.Yang, F. C. and Liau, C. B. 1998. Effect of cultivating conditions on the mycelial growth of *Ganoderma lucidum* in submerged flask cultures. Bioprocess Engineering.19: 233-236. 96.Yang, F. C. and Liau, C. B. 1998. The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged cultures. Process Biochem. 33(5): 547-553. 97.Yang, F. C., Ke, Y. F. and Kuo, S. S. 2000. Effect of fatty acids on the mycelial growth and polysaccharides formation by *Ganoderma lucidum* in shake flask cultures. Enzyme and Microbial Technol. 27:295-301. 98.Yasukawa, K., Kaminaga, T., Kitanaka, S., Tai, T., Nunoura, Y., Natori, S. and Takido, M. 1998. 3- β -Hyftocynrnzoyldehydrotumulosic acid from *Poria cocos*, and its anti-inflammatory effect. Phytochemistry. 48: 1357-1360. 99.Yu, S. J. and Tseng, J. 1996. Fu-Ling,a Chinese herbal drug, modulates cytokine secretion by human peripheral blood monocytes. Int. J. Immuno. Pharmacol. 18: 37-44. 100. Zadrazi, F. 1978. Cultivation of *Pleurotus*. p521-557. In: The biology and cultivation of edible mushroom. Eds., S. T. Chang and W. A. Hayes. Academic Press. New York.