中國蛇蟲草菌液態培養條件對菌絲體生物質量及生物活性成分之影響

張雅雯、徐泰浩, 林芳儀

E-mail: 382076@mail.dyu.edu.tw

摘 要

中國蛇蟲草菌（Ophiocordyceps sinensis； syn. Cordyceps sinensis ； syn. Hirsutella sinensis）為源自於知名冬蟲夏草藥材之藥用真菌，其嗜低溫（21℃以下）特性與灰黑色菌落與冬蟲夏草屬（Cordyceps spp.）及其他以C. sinensis（培養於23-25℃）為學名發表之所謂「冬蟲夏草」具有顯著之區別性。雖然過去已有不少C. sinensis國際期刊研究論文，但有關具有嗜低溫特性之O. sinensis（中國被毛孢H. sinensis，冬蟲夏草之無性型，依據「國際藻類、真菌與植物命名法規（International Code of Nomenclature for algae, fungi, and plants）」，一真菌僅能有一學名）其液態發酵、生物活性成分與抗氧化相關性之研究非常稀少。本研究主要以嗜低溫O. sinensis H101品系，探討其液態發酵產程中培養基碳源與氮源之種類與濃度、培養基啟始pH值及培養條件（溫度、震盪轉速與培養期間）對其菌絲體生物質量、生物活性成分（胞外多醣體、胞內多醣體、腺?、蟲草素、麥角固醇及總多酚）之影響。此外，並進一步探討菌絲體與發酵培養液之抗氧化生物活性與胞外多醣體及胞內多醣體中β-葡聚醣之含量與特性。研究結果顯示中國蛇蟲草菌H101品系之菌絲體與發酵培養液具有極佳之抗氧化能力，發酵培養液中發現含有高含量之胞外多醣體與總多酚。本研究主要結論為嗜低溫中國蛇蟲草菌H101品系在液態培養適當條件下除可產生菌絲體外，其培養液中含多種生物活性成分，菌絲體萃取物及培養液含高抗氧化活性，主要抗氧化物為多醣體與總多酚。

關鍵詞 : 中國蛇蟲草菌、冬蟲夏草、中國被毛孢、液態培養、生物活性成分、抗氧化

目錄

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。 2.王?。2011。中國被毛孢固體和液體培養中生長特性的研究。蘭州交通大學。碩士論文。 3.王尊生、顧宇翔、周麗、袁勤生、俞永信。2005。冬蟲夏草(Cordyceps sinensis)菌絲體固體發酵粉化學成份分析。天然產物研究與開發(Natural Product Research And Development) 3:331-336 4.王淑芳、朱慧新、朱平。2003。中國被毛孢的初步培養與生活力的觀察。中國食用菌22:(6)。 5.田雅嵐。2001。培養基與培養基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。大葉大學食品工程學系研究所碩士論文。 6.行政院衛生署。2011。行政院公報。台北市。行政院衛生署中醫年報。10(2):477-499。 7.何育澤。2012。豆類與穀類基質應用於樟芝與銀耳共培養液態發酵與其生物活性探討。大葉大學生物產業科技學系碩士論文。 8.呂志豪。2012。培養基與培養條件對茯苓與嗜酸乳酸桿菌共培養生成生物活性成分之影響。大葉大學生物產業科技學系碩士論文。 9.李玉玲。2001。冬蟲夏草菌生長溫度的研究。青海畜牧獸醫雜誌 31(6)8-9。 10.李春如、彭凡、樊美珍、李曾智。2004。中國被毛孢RCEF0273培養工藝研究。安徽農業大學學報31(4):460~465。 11.李珮玲。1993。機能性食品冬蟲夏草之形態發生、發酵培養與分析檢定。大葉大學食品工程研究所碩士論文。 12.李鋒、高興玉、饒邦複、劉利、崔連群。2006。冬蟲夏草提取液對小鼠病毒性心肌炎心肌脂質過氧化作用的影響。南京中醫藥大學學報。22(l)21-23。 13.尚才人、白蘭芳、王金主、鄭軍科。2011。冬蟲夏草液態深層培養的優化工藝研究。山東化工。40(4):72-74。 14.林桂英。2000。不同冬蟲夏草菌株發酵產程中機能性指標成份之探討。大葉大學食品工程研究所碩士論文。 15.俞永信。2005。冬蟲夏草粉化學成分的組成特點。第七屆海峽兩岸學術研討會論文集 79-87。 16.胡梅。2008。

長根菇靜置培養液體菌種培養基的優化。安徽農業科學。36(5):1926-1928。 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.唐臻筑。2012。培養基組成與饋料控制對桑黃Phellinus igniarius胞外多醣體量產之影響。大葉大學生物產業科技學系碩士論文。 19.柴建萍、白興榮、謝道燕。2003。蛹蟲草主要有效成分及其藥理功效。雲南農業科技4: 22-23。 20.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。 21.張力元。2010。不同鮮菇萃取液其多醣與重金屬含量之檢測。南台科技大學生物科技研究所碩士論文。 22.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。 23.梁志弘。2009。桑黃之液態培養及其生理活性。國立中興大學食品暨應用生物科技學系研究所博士論文。 24.莊曉莉、李祥麟、黃檀溪。2003。蠶蛹草具有顯著之抗氧化性與自由基清除能力。師大學報 48(1,2):13-24。 25.許瑞祥。1984。人工培養冬蟲夏草之性質及其代謝物之探討。台灣大學農業化學所碩士論文。 26.許瑞祥。2011。冬蟲夏草(Cordyceps sinensis)無性世代菌種鑑定之研究。蟲草類保健食品國際研討會:1-43。2011年5月3日。財團法人食品工業發展研究所。台北。 27.郭惠菁。2005。以固態發酵製備蟲草米及其品質與抗氧化性質。國立中興大學食品科學系碩士論文。 28.郭錫勇、郭莉莉、陳芳。1995。代氏蟲草與冬蟲夏草化學成份的比較。中藥材18(8):403-405。 29.陳召南。1992。細腳擬青黴(固培物)與冬蟲夏草為主治療慢性腎功能衰竭28例初步觀察。上海中醫藥雜誌2:11。 30.陳佳玲。2006。培養環境對蛹蟲草菌絲球型態及生物活性成份生產之相關性影響。大葉大學生物產業科技系碩士論文。 31.陳宗融。1995。冬蟲夏草的抗癌療效研究。明通醫藥34-35。 32.陳怡倩。2001。利用批次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。 33.陳林垮、許瑞祥、郭嘉信。2011。高效液相色譜與液相色譜-質譜分析冬蟲夏草相關產品之麥角固醇與其衍生物。分析化學39(9):1380-1386。 34.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。大葉大學生物產業科技系博士論文 35.陸毅航。2012。蔓荊子之抗氧化活性研究。大葉大學生物產業科技系碩士論文。 36.陸震鳴。2009。樟芝深層液態發酵及其三?類化合物的研究。江南大學博士論文 37.麥焯棉。2008。不同生態因子對蛹蟲草菌絲生長影響的研究。佛山科學技術學院學報。自然科學版26: 56-59。 38.程秀芳、楊軍方、施安輝、米振清。2006。紫外光光度法測定冬蟲夏草D-甘露醇的含量。肅魯藥事25: 286-287。 39.馮天保。2009。中國被毛孢菌絲體水提物質量分析與控制方法研究。上海師範大學碩士論文。 40.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。 41.楊佳蓉。2012。應用玉米軸芯以靜置培養雲芝Trametes versicolor LH1生產木寡醣之探討。大葉大學生物產業科技學系碩士論文。 42.楊雅萍。2008。烏苓參最佳液態醱酵條件研究。大仁科技大學生物科技研究所碩士論文。 43.葉淑幸。2003。培養基中碳氮源與培養方式對蛹蟲草菌。Cordyceps militaris醱酵產程中生質、菌絲球及生物活性成分之影響。大葉大學生物產業科技學系研究所碩士論文。 44.劉永霞。2004。冬蟲夏草無性型中國被毛孢生物學特性及馴化、菌絲體發酵研究。貴州大學碩士論文 45.劉佳茹。2005。油脂與介面活性劑對舞菇生長與多醣生產之影響。大葉大學生物產業科技學系碩士論文。 46.劉彥威、蘇敬良、韓博、田向榮。2006。不同培養條件對冬蟲夏草菌絲體甘露醇的影響。食品科學27(1)90-92。 47.樊美珍、陳民、郭超、鄭有鵬。1990。蛹蟲草菌絲體和冬蟲夏草化學成份的比較。中國蟲生真菌研究與應用。中國農業科技出版社2:81-85。 48.蔡仲軍、尹定華、黃天福、陳仕江、李泉生。2003。不同產地冬蟲夏草甘露醇含量比較。中國藥房14(8):505-506。 49.鄭林。2011。中國被毛孢甾醇提取最優條件的正交法研究。上海師範大學碩士論文。 50.鞏伯梁、徐長豪、周建華、宋細忠、李剛。2011。利用原生質體紫外誘變技術選育可常溫發酵的中國被毛孢新菌株 39(1):1-6 51.韓洪坤，楊曉彤，楊慶堯，馮慧琴，楊明俊。2011。中?被毛孢(Hirsutella sinensis)菌?体提取物的抗氧化活性研究。中國菌物學會第三屆藥用真菌學術研討會論文集。 52.蘇慶華。1993。利用分子篩-高效液態層析儀(SE-HPLC)分析真菌中藥材之1-3-BETA多醣體。 53.Ahmad, N. and Mukhtar, H. 1999. Green tea polyphenols andcancer: biologic mechanisms and practical implications. Nutrition Review 57:78-83. 54.Alexopolus, C. J., Mims, C. M. and Blackwell, M. 1996. Introductory Mycolgy.Wily, New York. 55.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 171-176. 56.Bok, J. W., Lermer, L., Chilton, J., Klingeman, H. G. and Towers, G. H. 1999. Antitumor sterols from the mycelia of Cordyceps sinensis. Phytochemistry 51:891-8. 57.Brown, G. D., and Gordon, S. 2003. Fungal β-glucans and mammalian immunity. Immunity 19(3):311 – 315. 58.Cai, S. L., Zheng, Y. B., Wang, M. Z., Lin, Q. Q., Huang Y. and Huang, J. Z. 2013. Enhanced production of mycelia biomass and Cordycepin by submerged culture of Cordyceps militaris with response surface methodology. Academic Periodical of Farm Products Processing 3:13-21. 59.Catley, B. J. 1980. The extracellular polysaccharide, pullulan, produced by Aureobasidium Pullulans: a relationship between elaboration rate and morphology. Journal of Microbiology 120(1): 265-268. 60.Cha, S. H., Lim, J.S., Yoon, C.S., Koh, J. H., Chang, H .I. and Kim, S. W. 2007. Production of mycelia and exo-biopolymer from molasses by Cordyceps sinensis 16 in submerged culture. Bioresour Technol 98:165-8. 61.Chaplin, M. F. and Kennedy, J. F. 1994. Carbohydrate analysis: A practical approach. Oxford, UK: IRL Press Ltd. 62.Chen, J., Zhang, W., Lu, T., Li, J., Zheng, Y. and Kong, L. 2006. Morphological and genetic characterization of a cultivated Cordyceps sinensis fungus and its polysaccharide component possessing antioxidant property in H22 tumor-bearing mice. Life Sci 78:2742-8. 63.Chen, S. D., Lin, S.Y., Lai, Y. S. and Cheng, Y. H. 2008. Effect of Cordyceps sinensis adlay fermentative products on antioxidant activities and macrophage functions. Taiwan J Agric Chem Food Sci 46:223-33. 64.Chen, S. Y., Ho, K.J., Hsieh, Y. J., Wang, L. T. and Mau, J. L. 2012. Contents of lovastatin, γ-aminobutyric acid and ergothioneine in mushroom fruiting bodies and mycelia. LWT - Food Sci Technol 47:274-8. 65.Chen, S., Li, Z., Krochmal, R., Abrazado, M., Kim, W. and Cooper, C. B. 2010. Effect of Cs-4R (Cordyceps sinensis) on exercise performance in healthy older subjects: A double-blind, placebo-controlled trial. J Altern Complement Med 16:585-90. 66.Chen, W., Zhang, W., Shen, W. and Wang, K. 2010. Effects of the acid polysaccharide fraction isolated from a cultivated Cordyceps sinensis on macrophages in vitro. Cell Immunol 262:69-74. 67.Chen, Y., Chen, Y. C., Lin, Y. T., Huang, S. H. and Wang, S. M. 2010. Cordycepin induces apoptosis of CGTH W-2 thyroid carcinoma cells through the calcium-calpain-caspase 7-PARP pathway. J Agric Food Chem 58:11645-52. 68.Cheung, J. K., Li J., Cheung, A. W., Zhu, Y., Zheng, K. Y. and Bi, C. W. 2009. Cordysinocan, a polysaccharide isolated from cultured Cordyceps, activates immune responses in cultured T-lymphocytes and macrophages: Signaling cascade and induction of cytokines. J Ethnopharmacol 124:61-8. 69.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. 70.Cunningham, K. G., Hutchinson, S. A., Manson, W. and Spring, F. S. 1950. Cordycepin a metabolic product form cultures of Cordyceps militaris. Link Natural (London)166:949-951. 71.Dai, Y. C. and Li, Y. 2011. Notes on the nomenclature of six important medicinal fungi in China. Mycosystema 30(4): 515-518 72.Decker, E. and Welch, B. 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. J. Agric. Food Chem. 38: 674~677. 73.Dong, C. H. and Yao, Y. J. 2005. Nutritional requirements of mycelial growth of Cordyceps sinensis in submerged culture. J Appl Microbiol 99:483-92. 74.Dong, C. H. and Yao, Y. J. 2011. On the reliability of fungal materials used in studies on Ophiocordyceps sinensis. J Ind Microbiol Biotechnol 38:1027-35. 75.Dong, C. H. and Yaoa, Y. J. 2008. In vitro evaluation of antioxidant activities of aqueous extracts from natural and cultured mycelia of Cordyceps sinensis .Food Science and Technology 41(4): 669 – 677. 76.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. 77.Forage, R. G., Harrison, D. E. F. and Pitt, D. E. 1985. Effect of environment on microbial activity. Comprehensive Biotechnology 1:253-279. 78.Furuya, T., Hirotani, M. and Matsuzawa, M. 1983. N6-(2-hydroxyethyl) adenosine, a biologically active compound from cultured mycelium of cordyceps and isaria species. Phytochemistry 22: 2509-2512. 79.Ge, F., Li, C. R., Hu, F. L., Gui, L., Zhou Q., Chen A. H. and Fan, M. Z. 2007. Effects of different culture condition on nucleoside content in the mycelia of Hirsutella sinensis. Mycosystema 26(2):234-242. 80.Ghikas, D. V., Kouvelis, V. N., Typas, M. A. Crisan, E. V. and Sands, A. 1978. Nutritional value. In Chang, S. T.&Hayes, W. A.(Eds.),The Biology and Cultivation of Edible Mushrooms(pp.137-165).Academic Press,New York. 81.Glazebrook, M. A., Vining, L. C. and White, R. L. 1992. Growth morphology of Streptomyces akiyoshiensis in submerged culture: influence of pH, inoculum, and nutrients. Canadian journal of microbiology 38(2): 98-103. 82.Glazebrook, M. A., Vining, L. C. and White, R. L. 1992. Growth morphology of Streptomyces akiyoshiensis in submerged culture: influence of pH, inoculum, and nutrients. Canadian journal of microbiology 38(2): 98-103. 83.Gu, Z., Wu, X. and Zhang, H. 2007. Determination of cordycepin and cordycep Acid in Cordyceps Sinensis by high-performance Liquid Chromatography. Journal of Chinese Institute of Food Science and Technology 7(3):144-148. 84.Guihua, Y. and Guoshan, W. 1988. A preliminary report on growth habit of Cordyceps militaris. Edible fungi 4:110-111. 85.Halliwell, B. J. and Gutteridge, M. C. 1989. Free radicals in biology and medicine. Oxford, Clarendon press 123. 86.Hallwell, B., Murcia, M. A., Chirico, S. and Aruoma, O. I. 1995. Aruoma, free radicals and antioxidants in food and in vivo: What they do and how they work. Critical Reviews in Food Sci.and Nutrition 35:7-20. 87.He, S. Q., Wang, S. X., Luo, J. C., Li, F. Q., Jin, X. L., Wen, Z. H., Ma, F. Q., Zhou, Z. X. and Tang, D. Z. 2011. The re-study for morphology of Ophiocordyceps sinensis and Hirsutella sinensis. Microbiology China 38(11): 1730-1738 88.Heim, K. E., Tagliaferro, A. R. and Bobilya, D. J. 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. Journal of Nutritional Biochemistry 13:572-584. 89.Houng, J. Y., Chang, M. Y. and Tsai, G. J. 2006. Optimization of the medium composition for the submerged culture of Ganoderma lucidumby Taguchi array design and steepest ascent method. Enzyme Micro.Tech 38:407-414. 90.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. 91.Hsu, J. T. 1997. China Medicinal and Edible Fungi. Beijing: Beijing Medical University and China Xiehe Medical University. 345-510. 92.Hsu, T. H., Shiao, L. H., Hsieh, C. and Chang, D. M. 2002. Acomparison of the chemical Composition and bioactive ingredient of the Chinese medicinal mushroom Dong Chong Xia CAO, its counterfeit and mimic, and fermented mycelium of Cordyceps sinensis. Food Chemistry 78(4): 463-469. 93.Huang, Z. J., Ji, H., Li, P., Xie, L., Zhao, X. C. 2002. Hypoglycemic effect and mechanism of polysaccharides from cultured mycelium of Cordyceps sinensis. J China Pharm Univ 33:51-4. 94.Jia, J. M., Tao, H. H. and Feng, B. M. 2009.Cordyceamides A and B from the culture liquid of Cordyceps sinensis (Berk.) Sacc. Chem Pharm Bull (Tokyo) 57:99-101 95.Kiho, T., Hui, J., Yamane, A. and Ukai, S. 1993. Polysaccharides in Fungi. XXXII. Hypoglycemic activity and chemical properties of a polysaccharide from the cultural mycelium of Cordyceps sinensis. Biol Pharm Bull 16:1291-3. 96.Kiho, T., Yamane, A., Hui, J., Usui, S. and Ukai, S. 1996. Polysaccharides in fungi. XXXVI.1 Hypoglycemic activity of a polysaccharide (CS-F30) from the cultural mycelium of Cordyceps sinensis and its effect on glucose metabolism in mouse liver. Biol Pharm Bull 19:294-6. 97.Kim, S. D. 2010. Isolation, structure and cholesterol esterase inhibitory activity of a polysaccharide, PS-A, from Cordyceps sinensis. J Appl Biol Chem 53:784-9. 98.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. 99.Ko, Y. T. and Lin, Y. L. 2004. 1, 3- β -glucan quantification by a fluorescence microassay and analysis of its distrivution in foods. Journal of Agricultural and Food Chemistry 52:3313-3318. 100.Kubo, E., Sato, A., Yoshikawa, N., Kagota, S., Shinozuka, K. and Nakamura, K. 2012. Effect of Cordyceps sinensis on TIMP-1 secretion from mouse melanoma cell. Cent Eur J Biol 7:167-71. 101.Kuo, M. C., Chang, C. Y., Cheng, T. L. and Wu, M. J. 2007. Immunomodulatory effect of exo-polysaccharides from submerged cultured Cordyceps sinensis: Enhancement of cytokine synthesis, CD11b expression, and phagocytosis. Appl Microbiol Biotechnol 75:769-75. 102.Leu, S. F., Poon, S. L., Pao, H. Y. and Huang, B.M. 2011. The in vivo and in vitro stimulatory effects of cordycepin on mouse Leydig cell steroidogenesis. Biosci Biotechnol Biochem 75:723-31. 103.Leung, P. H., Zhao, S., Ho, K. P. and Wu, J. Y. 2009. Chemical properties and antioxidant activity of exopolysaccharides from mycelial culture of Cordyceps sinensis fungus Cs-HK1. Food Chem 114:1251-6. 104.Li, C., Li, Z., Fan, M., Cheng, W., Long, Y., Ding, T. and Ming, L. 2006. The composition of Hirsutella sinensis, anamorph of Cordyceps sinensis. Food Composition and Analysis 19:800-805. 105.Li, H. P., Hu, Z., Yuan, J. L., Fan, H. D., Chen, W. and Wang, S. J. 2007. A novel extracellular protease with fibrinolytic activity from the culture supernatant of Cordyceps sinensis: Purification and characterization. Phytother Res 21:1234-41. 106.Li, S. P., Su, Z .R. and Dong, T. X. 2002. The fruiting body and its caterpillar host of Cordyceps sinensis show close resemblance in main constituents and anti-oxidation

activity. Phytomedicine 9:319-324 107.Li, S. P., Yang, F. Q. and Tsim, K. W. 2006a. Quality control of Cordyceps sinensis, a valued traditional Chinese medicine. Journal of Pharmaceutical and Biomedical Analysis 41: 1571-1584. 108.Li, S. P., Zhang, G. H., Zeng, Q., Huang, Z. G., Wang, Y. T and Dong, T. T. 2006. Hypoglycemic activity of polysaccharide, with antioxidation, isolated from cultured Cordyceps mycelia. Phytomedicine 13:428-33. 109.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). pp: 93~145. Academic Press. New York. 110.Liu, Y. W., Su, J. L., Han, B. and Tian, X. R. 2006. Effects on determining mannitol content of cyceliun of Cordyceps Sinensis by different culture condition. Food Science. 27(1):90-92. 111.Lo, H. C., Hsieh, C., Lin, F. Y. and Hsu, T. H. 2013. A Systematic Review of the Mysterious Caterpillar Fungus Ophiocordyceps sinensis in Dong ChongXiaCao and Related Bioactive Ingredients. Journal of Traditional and Complementary Medicine 3: 16-32 112.Matsuda, H., Akaki J., Nakamura, S., Okazaki, Y., Kojima, H. and Tamesada, M. 2009. Apoptosis-inducing effects of sterols from the dried powder of cultured mycelium of Cordyceps sinensis. Chem Pharm Bull 57:411-4. 113.Mau, J. L. 2005. The umami taste of edible and medicinal mushrooms. International Journal of Medicinal Mushrooms 7:119-125. 114.Mau, J. L., Chang, C. N., Haung, S. H. and Chen, C. C. 2004. Antioxidant properties of methanolic extracts from Grifola frondosa Morchella esculenta and Termitomyces albuminosus mycelia. Food Chemistry. 87(1):111-118. 115.Muller, E. and Ja, V. A. 1973. Pyrenomycetes, Spahaeriales, in the Fungi V,IV,Chap.5 116.Nagy, M. and Grancai, D. 1996. Colorimetric determination of flavanones in propolis. Pharmazie 51,100-101. 117.Olanow, C. W. 1997. Attempts to obtain neuroprotection in Parkinson's disease. Neurology 49: 26-33. 118.Oyaizu, M. 1986. Studies on products of brwning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr 44: 307. 119.Pao, H. Y., Pan, B. S., Leu, S.F. and Huang, B. M. 2012.Cordycepin stimulated steroidogenesis in MA-10 mouse Leydig tumor cells through the protein kinase C pathway. J Agric Food Chem 60:4905-13. 120.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. 121.Robak, J. and Gryglewski, I. 1988. Flavonoids are scavengers of superoxide anions. Biochem. Pharma. 37: 837~841. 122.Sheng, L., Chen, J., Li, J. and Zhang, W. 2011. An exopolysaccharide from cultivated Cordyceps sinensis and its effects on cytokine expressions of immunocytes. Appl Microbiol Biotechnol 163:669-78. 123.Shih, I. L., Tsai, K. L. and Hsieh, C. 2007. Effects of culture conditions on the mycelial growth and bioactive metabolite production in submerged culture of Cordyceps militaris. Biochemical Engineering Journal 33 : 193-201. 124.Shimada, K., Fujikawa, K., Yahara, K. and Nakamura T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Food Chemistry 40(6):945-948. 125.Shimizu, D., 1994. Color Iconography of Vegetable Wasps and Plant Worms. Seibundo Shinkosha, Tokyo. 126.Sun, L. J., Wang, Y. P. and Li, Y. G. 2008. Study on fermented condition of Cordyceps Sinensis. Science and technology of food industry. 4:158-162 127.Sung, G. H., Nigel, L. H. J., Sung, J. M., Luangsa-ard, J. J., Shrestha, B. and Joseph, W. S. 2007. Phylogenetic classification of Cordyceps and the clavicipitaceous fungi. Studies in Mycology 57: 5-59. 128.Suzuki, I., Hashimoto, K., Oikawa, S., Sato, K., Osawa, M. and Yadomae, T. 1989. Antitumor and immunomodulating activities of a beta-glucan obtained from liquid-cultured Grifola frondosa. Chem Pharm Bull 37(2) 410-413. 129.Tai, H. H., Li, H. S. and Chien, Y. H. 2002. A companion of the chemical composition and bioactive ingredients of the Chinese medical mushroom dongchongxiacao, it sc ounterfeit and mimic, and fermented mycelium of Cordyceps sinensis. Food Chernistry 78:463-469. 130.Tang, Y. J. and Zhong, J. J. 2004. Modeling the kinetics of cell growth and ganoderic acid production in liquid static cultures of the medicinal mushroom Ganoderma lucidum. Biochemical Engineering Journal 21:259-264. 131.Wang, C., Xie F., Zhang, N. and Li, J. H. 2011. Infuence of temperature on liquid culture of Hiesutella sinensis. Hunan agricultural sciences 23:21-24. 132.Wang, J., Liu, Y. M., Cao, W., Yao, K. W., Liu, Z. Q. and Guo, J. Y. 2012. Anti-inflammation and antioxidant effect of cordymin, a peptide purified from the medicinal mushroom Cordyceps sinensis, in middle cerebral artery occlusion-induced focal cerebral ischemia in rats. Metab Brain Dis 27:159-65. 133.Wang, L., Wang, G., Zhang, J., Zhang, G., Jia, L. and Liu, X. 2011. Extraction optimization and antioxidant activity of intracellular selenium polysaccharide by Cordyceps sinensis SU-02. Carbohydr Polym 86:1745-50. 134.Wang, S. F., Zhu, H. X. and Zhu, P. 2004. The preliminary cultivation and observation on the vitality of Hirsutella sinensis.Edible fungi of china 22(6):4-6. 135.Wang, S. H., Yang, W. B., Liu, Y. C., Chiu, Y. H., Chen, C. T. and Kao, P. F. 2011. A potent sphingomyelinase inhibitor from Cordyceps mycelia contributes its cytoprotective effect against oxidative stress in macrophages. J Lipid Res 52:471-9. 136.Wang, X. L., Liu, G. Q., Zhu, C. Y., Zhou, G. Y. and Kuang, S. M. 2011. Enhanced production of mycelial biomass and extracellular polysaccharides in caterpillar-shaped medicinal mushroom Cordyceps sinensis CS001 by the addition of palmitic acid. J Med Plant Res 5:2873-8. 137.Wang, Y., Wang, M., Ling, Y., Fan, W. and Yin, H. 2009. Structural determination and antioxidant activity of a polysaccharide from the fruiting bodies of cultured Cordyceps sinensis. Am J Chin Med 37:977-89. 138.Wang, Y., Yin, H., Lv, X., Gao, H. and Wang, M. 2010. Protection of chronic renal failure by a polysaccharide from Cordyceps sinensis. Fitoterapia 81:397-402. 139.Wang, Z. M., Peng, X., Lee, K. L., Tang, J. C., Cheung, P. C. and Wu, J. Y. 2011. Structural characterisation and immunomodulatory property of an acidic polysaccharide from mycelial culture of Cordyceps sinensis fungus Cs- HK1. Food Chem 125:637-43. 140.Wang, Z. S., Gu, Y. X., Zhou, L., Yuan, Q. S. and Yu, Y. X. 2005. Chemical constituents of Cordyceps sinensis mycelial fermentation preparations in solid media.Natural product research and development 17(3):331-336. 141.Wu, Y., Sun, H., Qin, F., Pan Y. and Sun, C. 2006. Effect of various extracts and a polysaccharide from the edible mycelia of Cordyceps sinensis on cellular and humoral immune response against ovalbumin in mice. Phytother Res 20:646-52. 142.Xiao, Z. H., Zhou, J. H. and Wu, H. S. 2011. Effect of myriocin on the expression of cyclinD1 in high glucose-induced hypertrophy mesangial cells. Zhongguo Dang Dai Er Ke Za Zhi 13:677-9. 143.Yan, J. K., Li, L., Wang, Z. M., Leung, P. H., Wang, W. Q. and Wu, J. Y. 2009. Acidic degradation and enhanced antioxidant activities of exopolysaccharides from Cordyceps sinensis mycelial culture. Food Chem 117:641-6. 144.Yan, J. K., Wang, W. Q., Li, L. and Wu, J. Y. 2011. Physiochemical properties and antitumor activities of two

α -glucans isolated from hot water and alkaline extracts of Cordyceps (Cs-HK1) fungal mycelia. Carbohydr Polym 85:753-8. 145.Yang M. J., Feng, H. Q., Yang, Q. Y. and Yang, X. T. 2011. HPLC determination of nucleosides of Hirsutella sinensis mycelium. Food research and development 32(11)96-98. 146.Yang, Q. Y. and Jong, S. C. 1989. Medicinal mushroom in China. Mushroom Science 12:631-641. 147.Yoon, T. J., Yu, K. W., Shin, K. S. and Suh, H. J. 2008. Innate immune stimulation of exo-polymers prepared from Cordyceps sinensis by submerged culture. Appl Microbiol Biotechnol 80:1087-93. 148.Yoshikawa, N., Nakamura, K., Yamaguchi, Y., Kagota, S., Shinozuka, K. and Kunitomo, M. 2007. Cordycepin and Cordyceps sinensis reduce the growth of human promyelocytic leukaemia cells through the Wnt signalling pathway. Clin Exp Pharmacol Physiol 34:S61-3. 149.Yu, L., Zhao, J., Zhu, Q. and Li, S. P. 2007. Macrophage biospecific extraction and high performance liquid chromatography for hypothesis of immunological active components in Cordyceps sinensis. J Pharm Biomed Anal 44:439-43. 150.Yu, Q. F. 2010. Determination of Adenosine in Cordyceps sinensis(Berk)Sacc.and Tablets by HPLC. Journal of Guangxi Normal University:Natural Science Edition 28(2):65-68 151.Yu, W. S. 1998. Review of polyunsaturated fat acids. Guowai Yixue,W eishengxwe Fence 11(4):61 -63. 152.Zhang, W., Li, J., Qiu, S., Chen, J. and Zheng, Y. 2008. Effects of the exopolysaccharide fraction (EPSF) from a cultivated Cordyceps sinensis on immunocytes of H22 tumor bearing mice. Fitoterapia 79:168-73. 153.Zhang, W., Yang, J., Chen, J., Hou, Y. and Han, X. 2005. Immunomodulatory and antitumour effects of an exopolysaccharide fraction from cultivated Cordyceps sinensis (Chinese caterpillar fungus) on tumour-bearing mice. Biotechnol Appl Biochem 42:9-15. 154.Zhao, Y. Y., Shen, X., Chao, X., Ho, C. C., Cheng, X. L. and Zhang Y. 2011. Ergosta-4,6,8(14),22-tetraen-3-one induces G2/M cell cycle arrest and apoptosis in human hepatocellular carcinoma HepG2 cells. Biochim Biophys Acta 1810:384-90. 155.Zhou, X., Luo, L., Dressel, W., Shadier, G., Krumbiegel, D. and Schmidtke, P. 2008. Cordycepin is an immunoregulatory active ingredient of Cordyceps sinensis. Am J Chin Med 3:967-80. 156.Zhou, Y., Du, J. and Tsao, G. T. 2000. Mycelial pellet formation by Rhizopus oryzae ATCC 20344. Applied Biochemistry and Biotechnology 84-86:779-789. 157.Zhu, S. R., Lu, Wen, Wu, M. Y. and Jiang, C. G. 2007. Research cultivation and use of the bacterium growth liquid of Cordyceps militaris. Journal of Anhui Agri 35(36):11908-11909.