

Phylogenetic analysis, contents of bioactive ingredients and antioxidant activity of cordyceps milit

徐胤桓、徐泰浩

E-mail: 345418@mail.dyu.edu.tw

ABSTRACT

Cordyceps militaris belongs to the same genus as C. sinensis which is a well known traditional Chinese medicine with pharmacological activities. Both C. sinensis and C. militaris are rare and expensive. However, the mycelial of C. militaris strains have been isolated and successfully cultivated to manufacture in large quantities by fermentation technology. To investigate the phylogenetic and chemical characterizations of 6 mycelia strains and one fruiting body of C. militaris from different regions, sequence analysis of nuclear and mitochondria ribosomal genes including internal transcribed spacers (ITS) and bioactive ingredients comparison were applied. Although phylogenetic trees of nuclear and mitochondria ribosomal genes showed slightly differences by geographical origins, all sequences formed single cluster, which suggested that intraspecies variations were small. In contrast, statistical differences were found by analysis of proximate composition, intracellular polysaccharide (IPS), exopolysaccharide (EPS) and cordycepin of mycelium in submerged cultured of 6 C. militaris strains. These results suggest that cultured mycelia of different C. militaris strains would be different in content of bioactive ingredients. Furthermore, investigation of antibacterial activities from C. militaris DYU1 showed that fermentation fluid of DYU1 has a good suppression for pathogen Staphylococcus aureus.

Nevertheless, analysis of polysaccharide structure by FTIR from C. militaris DYU1 revealed that the crude polysaccharide contain -1,3 glucan functional group

Keywords : Cordyceps militaris、phylogenesis、bioactive ingredients、antioxidant activity、antibacterial test、FTIR

Table of Contents

封面內頁 簽名頁 中文摘要iii 英文摘要iv 誌謝 v 目錄vi 表目錄x 圖目錄 xi 1.前言 1 2.文獻回顧 3 2.1蛹蟲草生物學特性 3 2.2蛹蟲草成分 3 2.2.1一般化學成分 3 2.2.2微量元素成分及胺基酸組成 4 2.2.3其他成分 4 2.3生物活性成分 6 2.3.1蟲草多醣 6 2.3.2蟲草素 7 2.3.3腺苷 8 2.3.4甘露醇 8 2.3.5麥角固醇 10 2.3.6超氧化物歧化酶(SOD) 10 2.4藥理作用 12 2.4.1抗氧化 12 2.4.2抗腫瘤作用 13 2.4.3固腎及保肝作用 13 2.4.4調節免疫功能 13 2.4.5降低血糖作用 14 2.4.6降血脂作用 14 2.4.7抗菌作用 14 2.4.8保護心臟功能 15 2.4.9鎮靜作用 15 2.4.10促進睪固酮內分泌功能 16 2.5液態釀酵 16 2.6液態釀酵影響因子 16 2.6.1碳源 17 2.6.2氮源 17 2.6.3無機鹽類 18 2.6.4溫度 18 2.6.5通氣量 18 2.6.6 pH值 19 2.6.7攪拌速度 19 2.6.8其他添加物 19 2.7食用安全性 20 2.8親源分析 20 3.材料與方法 22 3.1實驗流程 22 3.2試驗菌株 24 3.3菌種保存 24 3.4菌種培養 25 3.5搖瓶培養試驗 26 3.5.1蛹蟲草菌株搖瓶試驗培養基 26 3.6分析方法 26 3.6.1分子生物鑑定 26 3.6.2菌體之生物質量 31 3.6.3基本成分分析 32 3.6.4胞內外多醣測定 34 3.6.5還原糖測定 35 3.6.6生物活性成分分析 36 3.6.7抗氧化活性分析 37 3.6.9傅立葉紅外線光譜儀(FTIR)圖譜分析 39 3.7統計分析 39 4.結果與討論 40 4.1不同品系蛹蟲草菌種外觀觀察與比較 40 4.2不同品系蛹蟲草菌種基因序列分析結果之探討 44 4.3相同培養條件對於不同品系蛹蟲草菌絲體基本成份分析之比較 53 4.4不同品系蛹蟲草菌種在相同培養條件下其胞內外多醣之比較 53 4.5不同品系蛹蟲草培養在相同條件下釀酵液中蟲草素之比較 56 4.6不同品系蛹蟲草菌種在相同液態基培養7天後以顯微鏡觀察比較 56 4.7相同培養條件對於不同品系蛹蟲草菌釀酵液抗oxidative ability之比較 59 4.7.1還原力試驗 59 4.7.2亞鐵離子螯合能力試驗 59 4.7.3 DPPH自由基清除能力試驗 62 4.8蛹蟲草菌(DYU1)液態培養期間菌絲乾重、殘糖、PH值及胞外多醣含量之變化 67 4.9蛹蟲草菌DYU1液態培養期間釀酵液中腺苷及蟲草素之含量變化 67 4.10蛹蟲草菌(DYU1)釀酵液抑制細菌生長試驗 71 4.11 FTIR圖譜分析蛹蟲草菌(DYU1)之粗多醣 75 5.結論 77 參考文獻 79 附錄 90 表目錄 表3.1蛹蟲草編號、來源地與形態 24 表3.2種菌培養基組成 25 表3.3複合培養基組成 26 表3.4本次試驗所使用之DNA序列 27 表4.1蛹蟲草菌絲體外觀之比較 41 表4.3蛹蟲草菌絲體萃取液之還原力 61 表4.4蛹蟲草菌絲體萃取液之亞鐵離子螯合能力 64 表4.5蛹蟲草菌絲體萃取液之DPPH自由基清除能力 66 表4.6蛹蟲草菌(DYU1)液態培養期間菌絲乾重、殘糖、pH值及胞外多醣含量之變化 69 表4.7蛹蟲草菌(DYU1)培養第七天以不同濃度釀酵液之抑菌效果 74 圖目錄 圖2.1野生蛹蟲草子實體 5 圖2.2人工培養蛹蟲草子實體 5 圖2.3蟲草素結構式 9 圖2.4腺苷結構式 9 圖2.5甘露醇結構式 11 圖2.6麥角固醇結構式 11 圖3.1實驗流程圖(一) 22 圖3.2實驗流程圖(二) 23 圖4.1蛹蟲草菌絲第7天形態 42 圖4.2蛹蟲草菌絲第21天形態 43 圖4.3不同品系蛹蟲草以SSU rDNA呈現之結果圖 46 圖4.4不同品系蛹蟲草以LSU rDNA呈現之結果圖 47 圖4.5不同品系蛹蟲草粒腺體SSU rDNA呈現之結果圖 48 圖4.6細胞核rDNA序列以Neighbor-joining法重建的親緣樹 49 圖4.7粒腺體SSU rDNA以Neighbor-joining法重建的親緣樹 50 圖4.8 ITS序列以Neighbor-joining法重建的親緣樹 51 圖4.9蛹蟲草及冬蟲夏草ITS rDNA序列比對圖 52 圖4.10不同品系蛹蟲草在相同培養條件下EPS及IPS含量之比較 55 圖4.11不同品系蛹蟲草於相同液態培

養條件下蟲草素含量之比較 57 圖4.12 MIC-D顯微鏡下蛹蟲草菌絲體形態圖(22X) 58 圖4.13蛹蟲草菌絲體萃取液之還原力 60 圖4.14蛹蟲草菌絲體萃取液之亞鐵離子螯合能力 63 圖4.16蛹蟲草菌(DYU1)液態培養期間菌絲乾重、殘糖、pH值及胞外多醣含量之變化 68 圖4.16蛹蟲草菌(DYU1)液態培養期間腺?及蟲草素含量之變化 70 圖4.18蛹蟲草菌(DYU1)以不同濃度釀酵液之抑菌效果第六小時 72 圖4.19蛹蟲草菌(DYU1)以不同濃度釀酵液之抑菌效果第十小時 73 圖4.20 -1,3-Glucan與蛹蟲草菌(DYU1)粗多糖之FTIR圖譜分析 76

REFERENCES

- 王建芳、楊春清。2005。蛹蟲草有效成分及藥理作用研究進展。中藥研究發展22:30-32。
- 王琦。2003。蛹蟲草SOD活力的測定與分析。遼寧師專學報5: 99-100。
- 王瑞華、楊昕、楊仁、銳李高。2008。人工蛹蟲草子實體中游離麥角甾醇的提取及含量測定。中國藥師11:412-414。
- 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。
- 林桂英。2000。不同冬蟲夏草菌株釀酵過程中機能性指標成分之探討。大葉大學生物產業科技學系研究所碩士論文。
- 冉翠香、王莉、許智宏。2001。人工培植蛹蟲草子實體原基的誘發形成。食用菌23(4): 9-10。
- 朱雅?、桂仲爭。2009。蛹蟲草液體菌種通氣釀酵培養及其營養成分分析。食品與生物技術學報28: 699-704。
- 何佳樺。2006。冬蟲夏草Cordyceps sinensis加工食品分子檢測之研究。國立台灣大學園藝學研究所碩士論文。
- 李柏儀。2009。培養基組成與釀酵條件對蛹蟲草生物活性指標成分之影響。大葉大學生物產業科技學系研究所碩士論文。
- 汪多仁。2002。甘露醇的開發與應用。廣西蔗糖3: 34-35。
- 肖亦農、韓梅、趙春燕。2009。不同培養基對蛹蟲草子實體甘露醇、多醣和礦質元素含量的影響。瀋陽農業大學學報40: 227-229。
- 孟兆麗、朱凱、馮雲、孟慶繁、侯阿澧。2008。蛹蟲草多糖抑菌及抗氧化作用研究。科學研究29: 31-33。
- 武忠傳、王遠兵、趙現方、竇艷萍。2008。冬蟲夏草和蛹蟲草釀酵液抗菌活性研究。微生物學雜誌28: 47-50。
- 姜泓、劉珂、孟舒。2000。人工蛹蟲草子實體化學成分。藥學學報35: 663-668。
- 胡亞叮。2004。功能性食藥用菌蛹蟲草的開發技術研究。湖南農業大學。
- 桂仲爭、朱雅紅。2008。蛹蟲草的人工培育、有效成分及藥理作用研究進展。蠶業科學34: 178-184。
- 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。
- 秦建春、李曉明、張鞍靈、董艷?、高錦明。2006。蛹蟲草釀酵液抗菌活性初步研究。西北植物學報26: 402-406。
- 貢成良、吳衛東、徐承智、楊琨、陳國剛。2002。家蠶蛹蟲草的化學成份分析。蠶業科學28: 168-172。
- 貢成良、徐承智、楊昆、陳國剛、潘中華、吳衛東。2003。家蠶蛹蟲草的毒性研究。中國食用菌22: 54-56。
- 張東柱。1983。台灣數種靈芝生物學上之研究。國立台灣大學植病所碩士論文。
- 張淑芬。2001。食藥用菇類搖瓶液體培養條件之探討。食品工業33: 37-46。
- 習興梅、曾光明、郁紅艷、李建兵、黃國和。2006。真菌生物量指示劑麥角固醇的分離及測定方法。微生物學通報33: 128-132。
- 莊曉莉、李祥麟、陳香蘭、黃檀溪。2003。蠶蛹蟲草之特點與安全性。真菌學18: 151-161。
- 莊曉莉、李祥麟、黃檀溪。2003。蠶蛹蟲草具有顯著之抗氧化性與自由基清除能力。師大學報:數理科技類48: 13-24。
- 陳桂寶、羅梅初、劉寶晶。1997。蛹蟲草的藥理作用研究。中草藥28: 415。
- 陳敬民、李友娣、序驅、洪庚辛。1997。蛹蟲草的鎮靜催眠作用。中藥藥理與臨床13: 44-45。
- 陳美杏、呂均陞、石信德。2010。新興菇類的栽培與發展。科學發展446: 8-15。
- 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。
- 惠豐立、褚學英、魏明卉。2003。無機鹽對酵母菌產麥角固醇的影響。南陽師範學院學報。自然科學版2: 61-63。
- 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。
- 溫魯、翁梁、朱明傳、劉森琴。2008。不同林區蛹蟲草活性成分含量的比較。林業科學44: 149-151。
- 郭惠菁。2005。以固態釀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。台中、台灣。
- 解軍、郭欣、李培毅。1994。冬蟲夏草及人工菌絲體中蟲草菌素的定性定量研究。山西中醫10(4):36-38。
- 葉淑幸。2003。培養基中碳氮源與培養方式對蛹蟲草菌 Cordyceps militaris釀酵產程中生質、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。
- 廖春麗、方改霞、王蓮哲、王國貞、萬亞濤。2008。蛹蟲草主要有效成分分析。安徽農業科學36: 5050-5052。
- 趙林伊、王寶貴、張桂英。2004。柞蠶蛹蟲草對小鼠免疫功能的影響。中國公共衛生20: 230。
- 趙鵬、楊俊峰、李彬、劉榮珍、何為濤、李鳳文、王彥武、何勵。2004。蛹蟲草菌絲體降血脂作用的動物試驗研究。中國食品衛生雜誌16: 434-436。
- 劉洁、楊世杰、楊旭。1997。蠶蛹蟲草的抗腫瘤及激素樣作用。中國中藥雜誌22: 111-113。
- 潘子明。2005。真菌保健食品 - 紅麴製品介紹及國內研究現況。農業生技產業季刊3:28-36。
- 蔡仲軍、尹定華、?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: 505-506。
- 鄭劍玲、張中林、王美惠、秦?梅、楊秀珍。2006。食用真菌釀酵液SOD檢測。中國微生物學雜誌18: 464-465。
- 鄭慶委、高淑嫻、徐志本、閔宏林。2008。蛹蟲草菌絲生長特性的初步研究。蚌埠醫學院學報33。
- 謝雅惠、何佳靜、黃耿祥、楊智惠。2010。昆蟲的蛹裡竟然長出植物。科學發展454: 42-47。
- 呂均陞。2009。蛹蟲草之特性與栽培現況。農業試驗所技術服務78: 10-12。
- Ahn, Y. J., Park, S. J., Lee, S. G., Shin, S. C. and Choi, D. H., 2000. Cordycepin: selective growth inhibitor derived from liquid culture of Cordyceps militaris against Clostridium spp. Journal of Agricultural and Food Chemistry. 48: 2744-2748.
- 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.
- Belfrage, M., Sollevi, A., Segerdahl, M., Sjolund, K. and Hansson, P. 1995. Systemic adenosine infusion alleviates spontaneous and stimulus evoked pain in patients with peripheral neuropathic pain. Anesthesia and Analgesia. 81:713-717.
- Chaplin, M. F. and Kennedy, J. F. 1994. Carbohydrate analysis: A practical approach. Oxford, UK: IRL Press Ltd.
- Chang, H. L., Chao, G. R., Chen, C. C. and Mau, J. L. 2001. Non-volatile taste components of Agaricus blazei, Antrodia camphorata and Cordyceps militaris mycelia. Food Chemistry. 74: 203-207.
- Chang, W., Lim, S., Song, H., Song, B. W., Kim, H. J., Cha, M. J. Sung, J. M., Kim, T. W. and Hwang, K. C. 2008a. Cordycepin inhibits vascular smooth muscle cell proliferation. European Journal of Pharmacology. 597:64-69.
- Chang, Y., Jeng, K. C., Huang, K. F., Lee, Y. C. and Hou, C.W. 2008b. Effect of Cordyceps militaris Supplementation on Sperm Production, Sperm Motility and Hormones in

Sprague-Dawley Rats. *The American Journal of Chinese Medicine*. 36: 849- 859. 53. Chen, Y. Q., Wang, N., Qua, L. H., Lib, T. H. and Zhang, W. M. 2001. Determination of the anamorph of *Cordyceps sinensis* inferred from the analysis of the ribosomal DNA internal transcribed spacers and 5.8s rDNA. *Biochemical Systematics and Ecology*. 29:597 – 607. 54. Chen, Y. Q., Hu, B., Xu, F., Zhang, W., Zhou, H., and Qu, L. H., 2004. Genetic variation of *Cordyceps sinensis*, a fruit-body-producing entomopathogenic species from different geographical regions in China. *FEMS Microbiology Letters*. 230: 153-158. 55. Choi, S. B., Park, C. H., Choi, M. K., Jun., D.W. and Park, S. 2004. Improvement of insulin resistance and insulin secretion by water extracts of *Cordyceps militaris*, *phellinus linteus*, and *paecilomyces tenuipes* in 90% pancreatectomized rats. *Bioscience, Biotechnology and Biochemistry*. 68: 2257-2264. 56. Czub, J. and Baginski, M. 2006. Comparative Molecular Dynamics Study of Lipid Membranes Containing Cholesterol and Ergosterol. *Biophysical Journal* .90:2368-2382. 57. Das, S. K., Masuda, M., Hatashita, M., Sakurai, A. and Sakakibara, M. 2010a. Optimization of culture medium for cordycepin production using *Cordyceps militaris* mutant obtained by ion beam irradiation. *Process Biochemistry*. 45: 129-132. 58. Das, S. K., Masuda, M., Sakurai, A. and Sakakibara, M. 2010b. Medicinal uses of the mushroom *Cordyceps militaris*: Current state and prospects. *Fitoterapia*. 81: 961-968. 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 Biochemistry*. 37:769-774. 60. Feng, K., Wang, S., Hu, D. J., Yang, F. Q., Wang, H. X. and Li, S. P. 2009. Random amplified polymorphic DNA (RAPD) analysis and the nucleosides assessment of fungal strains isolated from natural *Cordyceps sinensis*. *Journal of Pharmaceutical and Biomedical Analysis*. 50: 522-526. 61. 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. 62. Ghikas, D. V., Kouvelis, V. N. and Typas, M. A. 2010. Phylogenetic and biogeographic implications inferred by mitochondrial intergenic region analyses and ITS1-5.8s-ITS2 of the entomopathogenic fungi *Beauveria bassiana* and *B.brongniartii*. *BMC Microbiology*. 10: 174. 63. Gong, Y. X., Li, S. P., Li, P., Liu, J. J., and Wang, Y. T., 2004. Simultaneous determination of six main nucleosides and bases in natural and cultured *Cordyceps* by capillary electrophoresis. *Journal of Chromatography A*. 1055: 215-221. 64. Gordon, M. H. 1990. The mechanism of antioxidant action in vitro. Ch 1, in *Food Antioxidants*, B. J. F. Hudson (Ed.),pp 1-18. Elsevier Applied Science, London and New York. 65. Halliwell, B. and Gutteridge, J. M. C. 1989. Free radicals in biology and medicine. Oxford, Clarendon press, 123. 66. Hsu, T. H., Shiao, L. H., Hsieh, C. Y. and Chang, D. M. 2002. A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom DongChongXiaCao, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis*. *Food Chemistry*. 78: 463-469. 67. Jiang, Y., Wong, J. H., Fu, M., Ng, T. B., Liu, Z. K., Wang, C. R., Li, N., Qiao, W. T., Wen, T. Y. and Liu, F. 2011. Isolation of adenosine, iso-sinensetin and dimethylguanosine with antioxidant and HIV-1 protease inhibiting activities from fruiting bodies of *Cordyceps militaris*. *Phytomedicine*. 18:189-193. 68. Jing, K. Y., Lin, L., Zhao, M. W., Po, H. L., Wen, Q. W. and Jian, Y. W. 2009. Acidic degradation and enhanced antioxidant activities of exopolysaccharides from *Cordyceps sinensis* mycelial culture. *Food Chemistry*. 117: 641-646. 69. Kinjo, N. and Zang, M. 2001. Morphological and phylogenetic studies on *Cordyceps sinensis* distributed in southwestern China. *Mycoscience*. 42: 567-574 70. 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. 71. Kim, H. G., Shrestha, B., Lim, S. Y., Yoon, D. H., Chang, W. C., Shin, D. J., Han, S. K., Park, S. M., Park, J. H. and Park, H., I. 2006. Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF-kappaB through Akt and p38 inhibition in RAW 264.7 macrophage cells. *European Journal of Pharmacology*. 545: 192-199 72. Lee, H., Kim, Y. J., Kim, H. W., Lee, D. H., Sung, M. K., and Park, T. 2006. Induction of apoptosis by *Cordyceps militaris* through activation of caspase-3 in leukemia HL-60 cells. *Biological and Pharmaceutical Bulletin*. 29: 670-674. 73. Lee, J. S., Kwon, J. S., Yun, J. S., Pahk, J. W., Shin, W. C., Lee, S.Y. and Hong, E. K. 2010. Structural characterization of immunostimulating polysaccharide from cultured mycelia of *Cordyceps militaris*. *Carbohydrate Polymers*. 80: 1011-1017 74. 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. 75. Li, S. P., Zhang, G. H., Zeng, Q., Huang, Z. G., Wang, Y. T., Dong, T. T., and Tsim, K. W., 2006b. Hypoglycemic activity of polysaccharide, with antioxidation, isolated from cultured *Cordyceps* mycelia. *Phytomedicine*. 13: 428-433 76. Lin, Y. and Chiang, B. 2008. Anti-tumor activity of the fermentation broth of *Cordyceps militaris* cultured in the medium of *Radix astragali*. *Process Biochemistry*. 43, 244-250. 77. Ling, J. Y., Zhang, G. Y., Lin, J. Q., Cui, Z. J. and Zhang, C. K. 2009. Supercritical fluid extraction of cordycepin and adenosine from *Cordyceps kyushuensis* and purification by high-speed counter-current chromatography. *Separation and Purification Technology*. 66: 625-629. 78. Liu, Z. 2002. Molecular evidence for teleomorph-anamorph connections in *Cordyceps* based on ITS-5.8s rDNA sequences. *Mycological Research*. 106: 1100-1108. 79. Liu, H., Zhang, S., Yu, A., Qu, L., Zhao, Y., Huang, H. and Li, J. 2004. Studies on intestinal permeability of cirrhotic patients by analysis lactulose and mannitol in urine with HPLC/RID/MS. *Bioorganic and Medicinal Chemistry Letters*. 14: 2339-2344. 80. Mao, X. B. and Zhong, J. J. 2004. Hyperproduction of cordycepin by two-stage dissolved oxygen control in submerged cultivation of medicinal mushroom *Cordyceps militaris* in bioreactor. *Biotechnology Progress*. 20: 1408-1413. 81. Metz, B. and Kossen, N. W. F. 1997. The growth of molds in the form of peltes-a literature review. *Biotechnol. Bioeng*. 19:781-799. 82. Miller, G. L. 1959. Use of dintrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry*. 31:426-428. 83. Nan, J. X., Park, E. J., Yang, B. K., Song, C. H., Ko, G. and Sohn, D. H. 2001. Antifibrotic effect of extracellular biopolymer from submerged mycelial cultures of *Cordyceps militaris* on liver fibrosis induced by bile duct ligation and scission in rats. *Archives of Pharmacal Research*. 24: 327-332. 84. Nakamura, K., Yoshikawa, N., Yamaguchi, Y., Kagota, S., Shinozuka, K. and Kunitomo, M. 2006. Antitumor effect of cordycepin (3'-deoxyadenosine) on mouse melanoma and lung carcinoma cells involves adenosine A3 receptor stimulation. *Aanticancer Research*. 26: 43-47. 85. Nikoh, N. and Fukatsu, T. 2000. Interkingdom host jumping underground:

phylogenetic analysis of entomoparasitic fungi of the genus cordyceps. Molecular Biology and Evolution. 17: 629 – 638. 86. Ohta, Y., Lee, J. B., Hayashi, K., Fujita, A., Park, D. K. and Hayashi, T. 2007. In vivo anti-influenza virus activity of an immunomodulatory acidic polysaccharide isolated from *Cordyceps militaris* grown on germinated soybeans. Journal of Agricultural and Food Chemistry. 55: 10194-10199. 87. Olanow, C. W. 1997. Attempts to obtain neuroprotection in Parkinson's disease. Neurology. 49: 26-33. 88. Paterson, R. R. 2008. Cordyceps: a traditional Chinese medicine and another fungal therapeutic biofactory. Phytochemistry. 69: 1469-1495. 89. Park, J. P., Kim, S. W., Hwang, H. J., Cho, Y. J., Lee, Y. S., Song, C. H. and Yun, J. W. 2001. Optimization of submerged culture conditions for the mycelial growth and exo-biopolymer production by *Cordyceps militaris*. Applied Microbiology. 33: 76-81. 90. Park, J. P., Kim, S. W., Hwang, H. J., Cho, Y. J. and Yun, J. W. 2002a. Stimulatory effect of plant oils and fatty acids on the exo-biopolymer production in *Cordyceps militaris*. Enzyme and Microbial Technology. 31:250-255. 91. 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. 2002b. Effect of agitation intensity on the exo-biopolymer production and mycelial morphology in *Cordyceps militaris*. Letters in Applied Microbiology. 34: 433-438. 92. Park, C., Hong, S. H., Lee, J. Y., Kim, G. Y., Choi, B. T., Lee, Y. T., Park, D. I., Park, Y. M., Jeong, Y. K. and Choi, Y. H. 2005. Growth inhibition of U937 leukemia cell by aqueous extract of *Cordyceps militaris* trough induction of apoptosis. Oncology reports. 13: 1211-1216. 93. Park, B. T., Na, K. H., Jung, E. C., Park, J. W. and Kim, H. H. 2009. Antifungal and Anticancer Activities of a Protein from the Mushroom *Cordyceps militaris*. Korean Journal of Physiology and Pharmacology. 13: 49-54. 94. Sandula, J., G. Kogan, M. Kacurakova, E. and Machova. 1999. Microbial (1 → 3)- β -D-glucans, their preparation, physico-chemical characterization and immunomodulatory activity. Carbohydrate Polymers. 38(3): 247-253. 95. Stensrud, O., Hywel-Jones, N. L. and Schumacher, T., 2005. Towards a phylogenetic classification of *Cordyceps*: ITS nrDNA sequence data confirm divergent lineages and paraphyly. Mycological Research. 109: 41-56. 96. Sung, G. H., Hywel-Jones, N. L., Sung, J. M., Luangsa-Ard, J. J., Shrestha, B. and Spatafora, J. W., 2007. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Studies in Mycology. 57: 5-59. 97. Tang, Y. J. and Zhong, J. J. 2003. Role of oxygen supply in submerged fermentation of *Ganoderma lucidum* for production of Ganoderma polysaccharide and ganoderic acid. Enzyme and Microbial Technology. 32:478-484. 98. Wang, L., Zhang, W. M., Hu, B., Chen, Y. Q. and Qu, L. H. 2008. Genetic variation of *Cordyceps militaris* and its allies based on phylogenetic analysis of rDNA ITS sequence data. Fungal Diversity. 31: 147-155. 99. Wu, Z. L., Wang, X. X. and Cheng, W. Y. 2000. Inhibitory effect of *Cordyceps sinensis* and *Cordyceps militaris* on human glomerular mesangial cell proliferation induced by native LDL. Cell Biochemistry and Function. 18:93-97. 100. Yoshikawa, N., Nakamura, K., Yamaguchi, Y., Kagota, S., Shinozuka, K., and Kunitomo, M., 2004. Antitumour activity of cordycepin in mice. Clinical and Experimental Pharmacology and Physiology. 31(2): 51-53. 101. Yu, R. M., Yang, W., Song, L. Y., Yan, C. Y. and Zhang, Z. Y. Z . 2007. Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured *Cordyceps militaris*. Carbohydrate Polymers. 70: 430-436. 102. Zhang, G., Huang, Y., Bian, Y., Wong, J. H., Ng, T. B. and Wang, H. 2006. Hypoglycemic activity of the fungi *Cordyceps militaris*, *Cordyceps sinensis*, *Tricholoma mongolicum*, and *Omphalia lapidescens* in streptozotocin-induced diabetic rats. Applied Microbiology and Biotechnology. 72:1152-1156. 103. 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.