

# Effects of Cultural Environment on the Relationships of Pellets Morphology and Bioactive Ingredients by *Cordyceps milita*

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## ABSTRACT

The effects of inoculum age, temperature, initial pH of the medium, inoculum size, carbon and nitrogen source and C/N ratio on mycelial morphology, mycelial biomass and bioactive ingredients by *Cordyceps militaris* was investigated. The mycelial morphology was quantified and characterized by means of image analysis, which included average diameter, roughness, circularity and core area ratio of the pellets. It indicated spores from grown on YMA medium for 2 weeks, 20 °C, initial pH 6.0,  $5 \times 10^4$  spores/mL and glucose as carbon source were best suited for mycelial biomass and bioactive ingredients biosynthesis. The majority of the mycelial morphology were fluffy and large pellets in initial pH 5.5–6.5 of medium. It showed that increasing inoculum size caused the pellets to increase in number per unit volume and roughness but to decrease in size and circularity. When glucose was used, the maximum number and roughness of pellets was obtained. The majority of the mycelial morphology was pellets in malt extract or yeast extract powder medium. The majority of the mycelial morphology was free mycelia in peptone,  $\text{NH}_4\text{NO}_3$ , or  $(\text{NH}_4)_2\text{SO}_4$  medium. When yeast extract powder was used, bioactive ingredients content were the highest among those tests. The maximum mycelial biomass, production of cordycepin in broth and content of IPS, adenosine and cordycepin in mycelia in C/N ratio as 2 of medium was 12.94 g/L, 172.60 mg/L, 58.3, 0.75 and 1.48 mg/g, respectively. While the fermentation proceeded, the pellets became fluffy and compact. In shake flask cultures, the cordycepin production in broth reached a maximum level of 225.89 mg/L on day 9 ; the maximum content of IPS, adenosine and cordycepin in mycelia was obtained on day 7. It was found that the fluffy and compact pellet was favorable for bioactive ingredients biosynthesis of *C. militaris*.

Keywords : *Cordyceps militaris*, polysaccharides, cordycepin, pellet, morphology, image analysis

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## REFERENCES

1. 沈齊英及陳順志。2001。北蟲草對正己烷所致大鼠損傷的保護作用。中藥材24(2):112-116。
2. 沈均、陶榮芬及胡錫澄。1999。蛹蟲草治療癌症療效初探。中成藥17(5):22-23。
3. 洪臻、江傳及馬德恩。2000。蟲草素對小鼠S180瘤抑制作用研究。時珍國醫藥11(10):873-874。
4. 宋振玉。1995。中草藥現代研究(第一卷)。第100、101頁。北京醫科大學中國協和醫科大學聯合出版社。中國，北京。
5. 郁利平、李華娟、李修義、閻秀欣及孫竹萍。1994。蛹蟲草對癌誘變劑-MMS所致BALB/c小鼠脾細胞DNA損傷的拮抗作用。實用腫瘤學雜誌2:7-8。
6. 莊曉莉、李祥麟及黃檀溪。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:數理與科技類48(1,2):13-24。
7. 貢成良、吳衛東、徐承智、楊昆及陳國剛。2002。家蠶蛹蟲草的化學成分分析。蠶業科學28(2):168-172。
8. 梁宗琦。1999。真菌次代謝產物多樣性及其潛在應用價值。生物多樣性7:145-150。
9. 孫月、卜寧及劉建華。1999。蛹蟲草蟲草酸蟲草素含量測定與分析。中國食用菌18(6):19。
10. 陳長安。1998。常用藥物治療手冊。第534、592、706頁。全國藥品年鑑雜誌社。台灣，臺北。
11. 湯新強、楊彤、李傳勛、周琴、齊艷、張厚利及安家彥。2004。人工蛹蟲草胞外多糖對卡鉑抗癌和骨髓抑制作用的影響。中醫藥學刊22(3):403-406。
12. 彭國平、李紅陽及袁永泰。1996。冬蟲夏草與人工蛹蟲草的成分比較。南京中醫藥大學學報12(5):26-27。
13. 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。大葉大學食品工程研究所碩士論文。台灣，彰化。
14. 楊雅瑤。2001。蟲草屬菌種之深層培養及其區分物之抗氧化性評估。台灣大學食品科技研究所碩士論文。台灣，台北。
15. 楊企震及郭用莊。1995。蛹蟲草治療癌症療效初探。中成藥17(5):22-23。
16. 劉潔、楊旭、陳正、梁曼義及李景洛。1994。蠶蛹蟲草鎮靜及性激素樣作用的研究。白恩求醫科大學學報20(1):14-16。
17. 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. *J. Agric. Food. Chem.* 48:2744-2748.
18. Agutter, P. S. and McCaldin, B. 1979. Inhibition of ribonucleic acid efflux from isolated SV40-3T3 cell nuclei by 3'-deoxyadenosine (cordycepin). *Biochem. J.* 180(2):371-378.
19. Araujo, R. and Rodrigues, A. G. 2004. Variability of germinative potential among pathogenic species of *Aspergillus*. *J. Clin. Microbiol.* 42(9):4335-4337.
20. Bae, J. T., Park, J. P., Song, C. H., Yu, C. B., Park, M. K. and Yun, J. W. 2001. Effect of carbon source on the mycelial growth and exo-biopolymer production by submerged culture of *Paecilomyces japonica*. *J. Biosci. Bioeng.* 91(5):522-524.
21. Bai, D. M., Jia, M. Z., Zhao, X. M., Ban, R., Shen, F., Li, X. G. and Xu, S. M. 2003. L(+)-Lactic acid production by pellet-form *Rhizopus oryzae* R1021 in a stirred tank fermentor. *Chem. Eng. Sci.* 58:785-791.
22. Braun, S. and Vecht-Lifshitz, S. E. 1991. Mycelial morphology and metabolite production. *Trends Biotechnol.* 9:63-68.
23. Carlsen, M., Spohr, A. B., Nielsen, J. and Villadsen, J. 1996. Morphology and physiology of an  $\alpha$ -amylase producing strain of *Aspergillus oryzae* during batch cultivations. *Biotechnol. Bioeng.* 49:266-276.
24. Casas Lopez, J. L., Sanchez Perez, J. A., Fernandez Sevilla, J. M., Rodriguez Porcel, E. M. and Chisti, Y. 2005. Pellet morphology, culture theology and lovastatin production in cultures of *Aspergillus terreus*. *J. Biotechnol.* 116:61-77.
25. Chang, H. L., Chao, G. R., Chen, C. C. and Mau, J. L. 2001. Non-volatile taste components of *Agaricus blazei*, *Antrodia camphorate* and *Cordyceps militaris* mycelia. *Food Chem.* 74:203-207.
26. Chassy, B. M. and Suhadolnik, R. J. 1969. Nucleoside antibiotics IV. Metabolic fate of adenosine and cordycepin by *Cordyceps militaris* during cordycepin biosynthesis. *Biochim. Biophys. Acta.* 182:307-315.
27. Chen, W. C. and Liu, C. H. 1996. Production of beta-fructofuranosidase by *Aspergillus japonicus*. *Enzyme Microb. Technol.* 15:153-160.
28. Cho, Y. J., Park, J. P., Hwang, H. J., Kim, S. W., Choi, J. W. and Yun, J. W. 2002. Production of red pigment by submerged culture of *Paecilomyces sinclairii*. *Lett. Appl. Microbiol.* 35:195-202.
29. Choi, M. A., Lee, W. K. and Kim, M. S. 2001. Identification and antibacterial activity of volatile flavor components of *Cordyceps militaris*. *J. Food. Sci. Nutr.* 4(1):18-22.
30. 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. *Biosci. Biotechnol. Biochem.* 68:2257-2264.
31. Cox, P. W., Paul, G. C. and Thomas, C. R. 1998. Image analysis of the morphology of filamentous micro-organisms. *Microbiology.* 144:817-827.
32. Cox, P. W. and Thomas, C. R. 1992. Classification and measurement of fungal pellets by automated image analysis. *Biotechnol. Bioeng.* 39:945-952.
33. Cui, Y. Q. and Luyben, K. C. A. M. 1997. Effect of agitation intensities on fungal morphology of submerged fermentation. *Biotechnol. Bioeng.* 55(5):715-726.
34. Cui, Y. Q., van der Lans, R. G. J. M., and Luyben, K. C. A. M. 1998. Effect of dissolved oxygen tension and mechanical forces on fungal morphology in submerged fermentation. *Biotechnol. Bioeng.* 57:409-419.
35. Cunningham, K. G., Manson, W., Spring, F. S. and Hutchinson, S. S. 1950. Cordycepin, a metabolic product isolated from cultures of *Cordyceps militaris* Link. *Nature.* 166:949.
36. Domingues, F. C., Queiroz, J. A., Cabral, J. M. S. and Fonseca, L. P. 2000. The influence of culture conditions on mycelial structure and cellulase production by *Trichoderma reesei* Rut C-30. *Enzyme Microb. Technol.* 26:394-401.
37. Du, L. X., Jia, S. J. and Lu, F. P. 2003. Morphological changes of *Rhizopus chinesis* 12 in submerged culture and its relationship with antibiotic production. *Process Biochem.* 1643-1646.
38. Edelstein, L. 1983. A model for pellet size distributions in submerged mycelial cultures. *J. Theor. Biol.* 105:427-452.
39. el-Enshasy, H., Hellmuth, K. and Rinas, U. 1999. Fungal morphology in submerged cultures and its relation to glucose oxidase excretion by recombinant *Aspergillus niger*. *Appl. Biochem. Biotechnol.* 81(1):1-11.
40. Fang, Q. H., Tang, Y. J. and Zhong, J. J. 2002. Significance of inoculation density control in production of polysaccharide and ganoderic acid by submerged culture of *Ganoderma lucidum*. *Process Biochem.* 37:1375-1379.
41. Fang, Q. H. and Zhong, J. J. 2002. Submerged fermentation of higher fungus *Ganoderma lucidum* for production of valuable bioactive metabolites - ganoderic acid and polysaccharide. *Biochem. Eng. J.* 10:61-65.
42. Furuya, T., Hirofani, M. and Matsuzawa. 1983. N<sup>6</sup>-(2-hydroxyethyl) adenosine, a biologically active compound from cultured mycelium of *Cordyceps* and *Isaria* species. *Phytochemistry.*

22:2509-2512. 43. Gerlach, S. R., Siedenberg, D., Gerlach, D., Schugerl, K., Giuseppin, M. L. F. and Hunik, J. 1998. Influence of reactor systems on the morphology of *Aspergillus awamori*. Application of neural network and cluster analysis for characterization of fungal morphology. *Process Biochem.* 33 ( 6 ) :601-615. 44. Hammerschmidt, D. E. 1980. *Szechwan purpur*. *N. Engl. J. Med.* 302:1191-1193. 45. Haq, I. U., Ali, S., Qadeer, M. A. and Iqbal, J. 2002. Effect of copper ions on mould morphology and citric acid productivity by *Aspergillus niger* using molasses media. *Process Biochem.* 37:1085-1090. 46. Higashiyama, K., Fujikawa, S., Park, E. and Okabe, M. 1999. Image analysis of morphological change during arachidonic acid production by *Mortierella alpina* 1S-4. *J. Bioscience Bioeng.* 87 ( 4 ) :489-494. 47. Jin, B., Leeuwen, J. V. and Patel, B. 1999. Mycelial morphology and fungal protein production from starch processing wastewater in submerged cultures of *Aspergillus oryzae*. *Process Biochem.* 34:335-340. 48. Johansen, C. L., Coolen, L. and Hunik, J. H. 1998. Influence of morphology on product formation in *Aspergillus awamori* during submerged fermentations. *Biotechnol. Prog.* 14:233-240. 49. Kim, H. O. and Yun, J. W. 2005. A comparative study on the production of exopolysaccharides between two entomopathogenic fungi *Cordyceps militaris* and *Cordyceps sinensis* in submerged mycelial culture. *J. Appl. Microbiol.* 99:728-738. 50. Kim, J. R., Yeon, S. H., Kim, H. S. and Ahn, Y. J. 2002. Larvicidal activity against *Plutella xylostella* of (cordycepin) from the fruiting body of *Cordyceps militaris*. *Pest Manag. Sci.* 58:713-717. 51. Kim, S. W., Hwang, H. J., Xu, C. P., Sung, J. M., Choi, J. W. and Yun, J. W. 2003a. Optimization of submerged culture process for the production of mycelial biomass and exo-polysaccharides by *Cordyceps militaris* C738. *Appl Microbiol.* 94:120-126. 52. Kim, S. W., Xu, C. P., Hwang, H. J. and Choi, J. W. 2003b. Production and characterization of exopolysaccharides from an entomopathogenic fungus *Cordyceps militaris* NG3. *Biotechnol. Prog.* 19:428-435. 53. Koc, Y., Urbano, A. G., Sweeney, E. B. and McCaffrey, R. 1996. Induction of apoptosis by cordycepin in ADA-inhibited TDT-positive leukemia cells. *Leukemia.* 10 ( 6 ) :1019-1024. 54. Kodama, E. N., Mccaffrey, R. P., Yusa, K. and Mitsuya, H. 2000. Antileukemic activity and mechanism of action of cordycepin against terminal deoxynucleotidyl transferase positive (TdT+) Leukemic cell. *Biochem. Pharmacol.* 59:273-281. 55. Kredich, N. M. 1980. Inhibition of nucleic acid methylation by cordycepin. *J. Biol. Chem.* 255 ( 15 ) :7380-7385. 56. Lai, L. T., Pan, C. C. and Tzeng, B. K. 2003. The influence of medium design on lovastatin production and pellet formation with a high-producing mutant of *Aspergillus terreus* in submerged cultures. *Process Biochem.* 38:1317-1326. 57. Lallas, G. C., Courtis, N. and Havredaki, M. 2004. K562 cell sensitization to 5-fluorouracil- or interferon-alpha-induced apoptosis via cordycepin ( 3 ' -deoxyadenosine ) :fine control of cell apoptosis via poly ( A ) polymerase upregulation. *Int. J. Biol. Markers.* 19 ( 1 ) :58-66. 58. Lee, B. C., Bae, J. T., Pyo, H. B., Choe, T. B., Kim, S. W., Hwang, H. J. and Yun, J. W. 2004. Submerged culture conditions for the production of mycelial biomass and exopolysaccharides by the edible Basidiomycete *Grifola frondosa*. *Enzyme Microb. Technol.* 35:369-376. 59. Lee, K. M., Lee, S. Y. and Lee, H. Y. 1999. Bistage control of pH for improving exopolysaccharide production from mycelia of *Ganoderma lucidum* in an air-lift fermentor. *J. Biosci. Bioeng.* 88 ( 6 ) :646-650. 60. Liu, J. M., Zhong, Y. R., Yang, Z., Cui, S. L. and Wang, F. H. 1989. Chemical constituents of *Cordyceps militaris* ( L. ) Link. *Zhongguo Zhong Yao Za Zhi.* 14:608-609, 639. 61. Liu, J. Y. S., Yang, X., Chen, Z. and Li, J. 1997. Anticarcinogenic effect and hormonal effect of *Cordyceps militaris* Link. *Zhongguo Zhongyao Zanzhi.* 22 (2):111-113. 62. Mao, X. B., Eksriwong, T., Chauvatcharin, S. and Zhong, J. J. 2005. Optimization of carbon source and carbon / nitrogen ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. *Process Biochem.* 40:1667-1672. 63. Mao, X. B. and Zhong, J. J. 2006. Significant effect of NH<sub>4</sub><sup>+</sup> on cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. *Enzyme Microbial. Technol.* 38:343-350. 64. 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. *Biotechnol. Prog.* 20 ( 5 ) :1408-1413. 65. Marks, D. B. and Keller, B. J., 1971. Growth of unicellular forms of the fungus *Cordyceps militaris* and analysis of the chemical composition of their walls. *J. Gen. Microbiol.* 69: 235-239. 66. Metz, B. and Kossen, N. W. F. 1977. The growth of molds in the form of pellets-a literature review. *Biotechnol. Bioeng.* 19:781-799. 67. Montreau, N., Vaur, S., Dautry, F. and Andeol, Y. 2003. Injection of exogenous RNA in amphibian oocytes leads to RNA level fluctuations which are sensitive to cordycepin, an RNA chain elongation terminator. *C. R. Biol.* 326 ( 12 ) :1135-1147. 68. Nakamura, K., Konoha, K., Yoshikawa, N., Yamaguchi, Y., Kagota, S., Shinozuka, K. and Kuntiomio, M. 2005. Effect of cordycepin ( 3 -deoxyadenosine ) on hematogenic lung metastatic model mice. *In Vivo.* 19 ( 1 ) :137-141. 69. 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 Rat. *Arch Pharm Res.* 24 ( 4 ) :327-332. 70. Nielsen, J., Johansen, C. L., Jacobsen, M., Krabben, P. and Villadsen, J. 1995. Pellet formation and fragmentation in submerged cultures of *Penicillium chrysogenum* and its relation to penicillin production. *Biotechnol. Prog.* 11:93-98. 71. Papagianni, M. 2004. Fungal morphology and metabolite production in submerged mycelial processes. *Biotechnol. Adv.* 22:189-259. 72. Papagianni, M., Matthey, M. and Kristiansen, B. 1999. The influence of glucose concentration on citric acid production and morphology of *Aspergillus niger* in batch and culture. *Enzyme Microb. Technol.* 25:710-717. 73. Papagianni, M. and Moo-Young, M. 2002. Protease secretion in glucoamylase *Aspergillus niger* cultures: fungal morphology and inoculum effects. *Process Biochem.* 37:1271-1278. 74. Pastrana, L., Blane, P. J., Santerre, A. L., Loret, M. O. and Goma, G. 1995. Production of red pigments by *Monascus ruber* in sythetic media with a strictly controlled nitrogen source. *Process Biochem.* 30:333-341. 75. 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 cells by aqueous extract of *Cordyceps militaris* trough induction of apoptosis. *Oncol. Rep.* 13:1211-1216. 76. Park, E. Y., Koike, K. Higashiyama, Fujikawa, S. and Sonoda, Y. 1999. Effect of Nitrogen source on mycelia morphology and arachidonic acid production in cultures of *Mortierella alpina*. *J. Brosci. Bioeng.* 88:61-67. 77. 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*. *Lett. Appl. Microbiol.* 33:76-81. 78. Park, J. P., Kim, S. W.,

Hwang, H. J., Cho, Y. J. and Yun, J.W. 2002a. Stimulatory effect of plant oils and fatty acid on the exo-biopolymer production in *Cordyceps militaris*. *Enzym. Microb. Technol.* 31:250-255. 79. 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*. *Lett. Appl. Microbiol.* 34 (6):433-438. 80. Park, J. P., Kim, Y. M., Kim, S. W., Hwang, H. J., Cho, Y. J., Lee, Y. J., Song, C. H. and Yun, J. W. 2002c. Effect of aeration rate on the mycelial morphology and exo-biopolymer production in *Cordyceps militaris*. *Process. Biochem.* 37:1257-1262. 81. Paul, G. C., Priede, M. A. and Thomas, C. R. 1999. Relationship between morphology and citric acid production in submerged *Aspergillus niger* fermentations. *Biochem. Eng. J.* 3:121-129. 82. Paul, G. C. and Thomas, C. R. 1998. Characterisation of mycelial morphology using image analysis. *Adv. Biochem. Eng. Biotechnol.* 60:1-59. 83. Rinas, U., el-Enshasy, H., Emmeler, M., Hille, A., Hempel, D. C. and Horn, H. 2005. Model-based prediction of substrate conversion and protein synthesis and excretion in recombinant *Aspergillus niger* biopellets. *Chem. Eng. Sci.* 60:2729-2739. 84. Rodriguez Porcel, E. M., Casas Lopez, J. L., Sanchez Perez, J. A., Fernandez Sevilla, J. M. and Chisti, Y. 2005. Effect of pellet morphology on broth rheology in fermentations of *Aspergillus terreus*. *Biochem. Eng. J.* 26:139-144. 85. Sautour, M., Dantigny, P., Guilhem, M. C., and Bensoussan, M. 2003. Influence of inoculum preparation on the growth of *Penicillium chrysogenum*. *J. Appl. Microbiol.* 95 ( 5 ) :1034-1038. 86. Schugerl, K., Gerlach, S. R. and Siedenberg, D. 1998. Influence of the process parameters on the morphology and enzyme production of *Aspergilli*. *Adv. Biochem. Eng. Biotechnol.* 60:195-266. 87. Siedenberg, D., Gerlach, S. R., Schugerl, K., Giuseppin, M. L. F. and Hunik, J. 1998. Production of xylanase by *Aspergillus awamori* on synthetic medium in shake flask cultures. *Process Biochem.* 33 ( 4 ) :429-433. 88. Singh, O. V., Sharma, A. and Singh, R. P. 2001. Optimisation of fermentation conditions for gluconic acid production by a mutant of *Aspergillus niger*. *Indian J. Exp. Biol.* 39 ( 11 ) :1136-1143. 89. Sinha, J., Bae, J. T., Park, J. P., Song, C. H. and Yun, J. W. 2001. Effect of substrate concentration on broth rheology and fungal morphology during exo-biopolymer production by *Paecilomyces japonica* in a batch bioreactor. *Enzyme Microb. Technol.* 29:392-399. 90. Sugar, A. M. and McCaffrey, R. P. 1998. Antifungal activity of 3' -deoxyadenosine ( cordycepin ) . *Antimicrob. Agents Chemother.* 42 ( 6 ) :1424-1427. 91. Takahashi, J. and Yamada, K. 1959. Studies on the effect of some physical conditions on the submerged mold culture:Part II. On the two types of pellet formation in the shaking culture. *J. Agric. Chem. Soc.* 33:707-709. 92. Thomas, C. R. 1992. Image analysis: putting filamentous microorganisms in the picture. *Trends Biotechnol.* 10:343-348. 93. Thomas, C. R. and Paul, G. C. 1996. Applications of image analysis in cell technology. *Curr. Opin. Biotechnol.* 7:35-45. 94. Tucker, K. G., Kelly, T., Delgrazia, P. and Thomas, C. R. 1992. Fully-automatic measurement of mycelial morphology by image analysis. *Biotechnol. Prog.* 8:353-359. 95. Wen, L., Tang, Y. L., Yin, Q. F., Xia, M. and Yang, Y. L. 2005. Assays on nutrient and effective ingredients in different parts of *Cordyceps militaris*. *Zhongguo Zhong Yao Za Zhi.* 30:659-661. 96. Won, S. Y. and Park, E. H. 2005. Anti-inflammatory and related pharmacological activities of cultured mycelia and fruiting bodies of *Cordyceps militaris*. *J. Ethnopharmacol.* 96:555-561. 97. 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 Biochem & Function.* 18 (2): 93-97. 98. Xu, C. P., Kim, S. W., Hwang, H. J., Choi, J. W. and Yun, J. W. 2003. Optimization of submerged culture conditions for mycelial growth and exo-biopolymer production by *Paecilomyces tenuipes* C240. *Process Biochemistry.* 38:1025-1030. 99. Xu, C. P. and Yun, J. W. 2004. Influence of aeration on the production and the quality of the exopolysaccharides from *Paecilomyces tenuipes* C240 in a stirred-tank fermenter. *Enzyme Microb. Technol.* 35:33-39. 100. Yoo, H. S., Shin, J. W., Cho, J. H., Son, C. G., Lee, Y. W., Park, S. Y. and Cho, C. K. 2004. Effects of *Cordyceps militaris* extract on angiogenesis and tumor growth. *Acta. Pharmacol. Sin.* 25:657-665. 101. Yoshikawa, N., Nakamura, K., Yamaguchi, Y., Kagota, S., Shinozuka, K. and Kunitomo, M. 2004. Antitumour activity of cordycepin in mice. *Clin. Exp. Pharmacol. Physiol.* 31:51-53. 102. Zhou, Y., Du, J. and Tsao, G. T. 2000. Mycelial pellet formation by *Rhizopus oryzae* ATCC 20344. *Appl. Biochem. Biotechnol.* 84-86:779-789. 103. Znidarsic, P., Komel, R. and Pavoko, A. 2000. Influence of some environmental factors on *Rhizopus nigricans* submerged growth in the form of pellets. *World J. Microbiol. Biotechnol.* 16:589-593. 104. Znidarsic, P., Komel, R. and Pavko, A. 1998. Studies of a pelleted growth form of *Rhizopus nigricans* as a biocatalyst for progesterone 11 -hydroxylation. *J. Biotechnol.* 60:207-216.