

# Studies on Production of Peptide Antibiotics-Beauvericin by Beauveria bassiana in Liquid Culture

陳宴淑、張耀南、曾耀銘

E-mail: 9300174@mail.dyu.edu.tw

## ABSTRACT

Beauvericin, one of cyclodepsipeptide toxins, is one kind of metabolic products of Beauveria species. The preliminary culture media were studied for beauvericin production of Beauveria bassiana A1, kindly donated by Professor Suey-Sheng kao at the Biopesticide Department, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, in liquid culture. There was much little beauvericin production with the culture medium composed of sucrose (25g/L), edible molasses (25mL/L), malt extract (10g/L), NZ broth (10g/L), k<sub>2</sub>HPO<sub>4</sub> (2g/L). The aqueous (water) extract liquid of corn was as a basic medium. When the medium positions described above were dissolved in the water extract liquid, which was as the culture medium, the beauvericin production was 699 μg/L for 76h shaken culture (150rpm) at 25° and the initial pH6.2 of the medium. The corn water extract liquid becomes a suitable source and represents a potent role for B. bassiana A1 in beauvericin production.

Keywords : 白僵菌、白僵菌素、玉米水萃取液

## Table of Contents

第一章 緒論 .....	1	第二章 文獻回顧 .....	4	2.1蟲生真菌的分類
.....4 2.2白僵菌屬分種 .....	4	2.3白僵菌殺蟲原理	8	
.....10 2.4白僵菌之寄生範圍及標的害蟲 .....	10	2.5微生物農藥	12	
.....12 2.6白僵菌素 .....	12	2.7農用抗生素	15	
.....16 2.8勝?類抗生素 .....	16	2.9 HPLC之儀器原理	17	
.....19 第三章 材料與方法 .....	19	3.1實驗材料	21	
.....21 3.2儀器設備 .....	21	3.3實驗方法	21	
.....22 3.3.1培養方法 .....	22	3.3.2白僵菌素以HPLC定量分析	22	
.....23 第四章 結果與討論 .....	23	4.1培養基之最適化研究	28	
.....28 4.2最適玉米之測試 .....	28	4.3最適玉米水萃取液之測試	28	
.....29 第五章 結論與展望 .....	29	參考文獻	33	
.....34 附錄 .....	34		43	

## REFERENCES

- 李秦林 (1998) 以米為基質利用固態發酵生產黑僵菌素Destruixins。台灣大學農業化學研究所碩士論文。洪文凱 (1995) 黑僵菌素之分離、生物檢定及安全性評估。大葉工學院碩士論文。施錫彬 (1998) 白僵菌產生的幾種重要毒素。桃園區農業改良場。高清文、蔡勇勝 (1989) 利用蟲生真菌防治甜菜夜蛾。重要蔬菜害蟲綜合防治研討會，214-225。中華昆蟲特刊第四號。高穗生，蔡勇勝 (1995) 蟲生病源真菌在蟲害防治上之利用（上）。藥試所專題報導，38:122。高穗生，蔡勇勝 (1995) 蟲生病源真菌在蟲害防治上之利用（下）。藥試所專題報導，39:16。陳堅 (1999) 環境生物技術。457-495，中國輕工業出版社，北京。黃明章 (1997) 黑僵菌素之萃取研究。大葉工學院碩士論文。Arcas, J.A., B.M. Diaz, and R.E. Lecuona. (1999) Bioinsecticidal activity of conidia and dry mycelium preparations of two isolates of Beauveria bassiana against the sugarcane borer Diaatraea saccharalis, Journal of Biotechnology, 67:151-158. Bernardini, M., A. Carilli, G. Pacioni, and B. Santurbano. (1975) Isolation of beauvericin from Paecilomyces fumoso-roseus, Phytochemistry, 14:1865. Bidochka, M.J., and G.G. Khachatourians. (1990) Identification of Beauveria bassiana extracellular protease as a virulence factor in pathogenicity toward the migratory grasshopper, melanoplus sanguinipes, Journal of invertebra pathology, 56:362-370. Bidochka, M.J., and G.G. Khachatourians. (1992) Growth of the entomopathogenic fungus Beauveria Bassiana on cuticular components from the migratory grasshopper, Melanoplus sanguinipes, Journal of invertebra pathology, 59:165-173. Bidochka, M.J., and G.G. Khachatourians. (1993) Oxalic acid hyperproduction in Beauveria bassiana mutants is related to a utilizable carbon source but not to virulence, Journal of invertebra pathology, 62:53-57. Boucias, D.G., S.Y. Hung, I. Mazet, and J. Azbell. (1994) Effect of the fungal pathogen, Beauveria bassiana, on the lysozyme activity in spodoptera exigua larvae, J. Insect Physiol, 40(5):385-391. Boman, H.G. (1995) Peptide antibiotics and their role innate immunity, Annu. Rev. Immunol., 13:61-92. Buchanan, G.O., and P.B. Reese. (2001) Biotransformation of diterpenes and diterpene derivatives by Beauveria bassiana ATCC7159, Phytochemistry, 56:141-151.

Champlin, F.R., and E.A. Grula. (1979) Noninvolvement of beauvericin in the entomopathogenicity of *Beauveria bassiana*, App. Environ. Microbiol, 37:1122-1126. Deol, B. S., D.D. Ridley, and P. Singh. (1978) Isolation of cyclodepsipeptides from plant pathogenic fungi, Aust. J. Chem., 31: 1397-1399. Gupta, S., S.B. Krasnoff, N.L. Underwood, J.A.A. Renwick, and D.W. Roberts. (1991) Isolation of beauvericin as an insect toxin from *Fusarium semitectum* and *Fusarium moniliforme* var. subglutinans., Mycopathologia, 115: 185-189. Griffiths, D.A., D.E. Brown, and S.G. Jezequel. (1993) Metabolism of xenobiotics by *Beauveria bassiana*, Xenobiotica,23(10)1085-1100. Gentner, F.J., G.M. Cripe, and D.J. Crosby. (1994) Effect of *Beauveria bassiana* and its toxins on *Mysidopsis bahia*(Mysidacea), Arch. Environ. Contam. Toxicol., 26:90-94. Gupta, S., C. Montllor, and Y.S. Hwang. (1995) Isolation of novel beauvericin analogues from the fungus *Beauveria bassiana*, Journal of Natural Products, 58(5): 733-738. Hamill, R.L., C.E. Higgens, H.E. Boaz, and M. Gorman. (1969) The structure of beauvericin, a new depsipeptide antibiotic toxic to *Artemia salina*, Tetrahedron Lett., (49): 4255-4258. Havukkala, I., C. Mitamura, S. Hara, K. Hirayae, Y. Nishizawa, and T. Hibi. (1993) Induction and purification of *Beauveria bassiana* chitinolytic enzymes, Journal of invertebrate pathology, 61: 97-102. Holland, H.L., T.A. Morris, P.J. Nava, and M. Zabic. (1999) A new paradigm for biohydroxylation by *Beauveria bassiana* ATCC7159, Tetrahedron, 55:7441-7460. Ignoffo, C.M. (1975) Entomopathogens as insecticides, Environmental Letters, 8(1):23-40. Ignoffo, C.M. (1988) CRC handbook of natural pesticides volume v microbial insecticides part A entomogenous protozoa and fungi. 151-237. Kraska, R., R. Schuhmacher, M. Grasserbauer, S.R. Kanhere, and P.M. Scott. (1995) Spurenanalyse des Fusarium Mykotoxins beauvericin in Mais, Braunschweig-Volkenrode, 115-118. Kraska, R., R. Schuhmacher, M. Grasserbauer, and P.M. Scott. (1996) Determination of the Fusarium mycotoxin beauvericin at  $\mu\text{g}/\text{kg}$  levels in corn by high performance liquid chromatography with diode array detection, Journal of Chromatography, 746:233-238. Kraska, R., M. Lemmens, R. Schuhmacher, M. Grasserbauer, M. Pronczuk, H. Wisniewska, and J. Chelkowski. (1996) Accumulation of the mycotoxin beauvericin in kernels of corn hybrids inoculated with *Fusarium subglutinans*, J. Agric. Food Chem., 44(11):3665-3667. Kraska, R., R. Schuhmacher, M. Grasserbauer, M. Lemmens, and P. Ruckenbauer. (1996) Fusarium mycotoxin beauvericin: Optimisation of analytical methodology and natural occurrence in maize ears in Austria, Mycotoxins and Phycotoxins, 27-31. Kraska, R., R. Schuhmacher, and M. Grasserbauer. (1996) Optimierung eines Analysenverfahrens zur Spurenbestimmung von beauvericin in natürliche und künstlich infiziertem Mais, Mykotoxin-Workshop, 18:36-41. Kraska, R., R. Schuhmacher, M. Grasserbauer, M. Lemmens, R. Lemmens-Gruber, A. Adler, and H. Lew. (1997) Toxicity of beauvericin to mammalian cells and its production by Austrian isolates of *Fusarium proliferatum* and *Fusarium subglutinans*, Mycotoxin Research. Logrieco, A., A. Moretti, C. Altomare, A. Bottalico, and E.C. Torres. (1993) Occurrence and toxicity of *Fusarium subglutinans* from Peruvian maize, Mycopathologia, 122: 185-190. Mazet I., S.Y. Hung, and D.G. Boucias. (1994) Detection of toxic metabolites in the hemolymph of *Beauveria bassiana* infected *Spodoptera exigua* larvae, Birkhauser Verlag Basel, 50:142-147. Ngoka, L.C.M., M.L. Gross, and P.L. Toogood. (1999) Sodium-directed selective cleavage of lactones:a method for structure determination of cyclodepsipeptides, International Journal of Mass Spectrometry, 182(183):289-298. Nilanonta, C., M. Isaka, P. Kittakoop, S. Trakulnaleamsai, M. Tantcharoen, and Y. Thebtaranonth. (2002) Precursor-directed biosynthesis of beauvericin analogs by the insect pathogenic fungus *Paecilomyces tenuipes* BCC1614, Tetrahedron, 58:3355-3360. Ouchinnikov, T.A., V.T. Ivanov, and I.I. Mikhaleva. (1971) The synthesis and some properties of Beauvericin, Tetrahedron Letters, 2:159-162. Roberts, D.W. (1981) Toxins from the entomogenic fungi in microbial control of pests and plant disease, Academic Press, 441-464. Raadt A.D., B. Fetz, H. Griengl, M.F. Klingler, B. Krenn, K. Mereiter, D.F. Munzer, P. Plachota, H. Weber, and R. Saf. (2001) Chiral auxiliaries as docking/protecting groups:biohydroxylation of selected ketones with *Beauveria bassiana* ATCC7159, Tetrahedron, 57:8151-8157. Shimizu S., Y.Tsuchitani, and T. Matsumoto. (1993) Production of an extracellular protease by *Beauveria bassiana* in the haemolymph of the silkworm, *Bombyx mori*, Letters in Applied Microbiology, 16:291-294. Samsinakova, A. (1966) Growth and Sporulation of Submersed Cultures of the Fungus *Beauveria bassiana* in Various Media, Journal of invertebrate pathology, 8:395-400. Suzuki, A., M. Kanaoka, A. Isogai, S. Murakoshi, M. Ichinoe, and S. Tamura. (1977) Bassianolide, a new insecticidal cyclodepsipeptide from *Beauveria bassiana* and *Verticillium lecanii*, Tetrahedron Lett., (25): 2167-2170. Suresh P.V., and M. Chandrasekaran. (1999) Impact of process parameters on chitinase production by an alkalophilic marine *Beauveria bassiana* in solid state fermentation, Process Biochemistry, 34:257-267. Urtz B.E., and W.C. Rice. (2000) Purification and characterization of a novel extracellular protease from *Beauveria bassiana*, Mycol. Res., 104(2): 180-186. Vilcinskas A., and M. Wedde. (1997) Inhibition of *Beauveria bassiana* proteases and fungal development of *Galleria mellonella* larvae, Biocontrol Science and Technology, 7:591-601. Zimmermann, G. (1993) The entomopathogenic fungus *Metarrhizium anisopliae* and its potential as a biocontrol agent, Pestic. Sci. 37:375-379. Zizca, J., and J. Weiser. (1993) Effect of beauvericin a toxic metabolite of *Beauveria bassiana*, on the ultrastructure of *Culex pipiens autogenicus* larvae, Cytobios, 75:13-19.