

白殭菌液態發酵產生胜類抗生物素的研究

陳宴淑、張耀南、曾耀銘

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

摘要

白殭菌素已知為環狀胜 β -毒素之一，主要為白殭菌生成產物。本研究以農業藥物毒物試驗所提供之白殭菌 *Beauveria bassiana* A1 為培養菌株，嘗試利用不同培養基進行液態發酵培養白殭菌生成其代謝物-白殭菌素，初步結果顯示以 25 g/L sucrose、25 mL/L edible molasses、10 g/L malt extract、10 g/L NZ broth、2 g/L K₂HPO₄ 為培養基質時，幾乎無白殭菌素之產生，但若以玉米水萃取液為基礎培養基質，再添加上述培養基質濃度，基質起始調配 pH 值為 6.2，在 25℃ 下以 150 rpm 振盪培養 76 h 後，可得到本實驗室目前最佳白殭菌素產量為 699 μ g/L，由此可知玉米水萃取液為影響白殭菌生成白殭菌素的重要組成成分之一。

關鍵詞：白殭菌、白殭菌素、玉米水萃取液

目錄

| | | | |
|-----------------------------------|----|-----------------------------|----|
| 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 白殭菌屬分種 | 8 |
| 2.2 白殭菌屬分種 | 8 | 2.3 白殭菌殺蟲原理 | 10 |
| 2.4 白殭菌之寄生範圍及標的害蟲 | 12 | 2.5 微生物農藥 | 12 |
| 2.6 白殭菌素 | 15 | 2.7 農用抗生素 | 16 |
| 2.8 胜 β -類抗生素 | 17 | 2.9 HPLC 之儀器原理 | 19 |
| 2.9 HPLC 之儀器原理 | 19 | 第三章 材料與方法 | 21 |
| 3.1 實驗材料 | 21 | 3.2 儀器設備 | 21 |
| 3.2 儀器設備 | 21 | 3.3 實驗方法 | 22 |
| 3.3 實驗方法 | 22 | 3.3.1 培養方法 | 22 |
| 3.3.1 培養方法 | 22 | 3.3.2 白殭菌素以 HPLC 定量分析 | 23 |
| 3.3.2 白殭菌素以 HPLC 定量分析 | 23 | 第四章 結果與討論 | 28 |
| 4.1 培養基之最適化研究 | 28 | 4.2 最適玉米之測試 | 28 |
| 4.2 最適玉米之測試 | 28 | 4.3 最適玉米水萃取液之測試 | 29 |
| 4.3 最適玉米水萃取液之測試 | 29 | 第五章 結論與展望 | 33 |
| 5.1 結論 | 33 | 參考文獻 | 34 |
| 5.2 展望 | 34 | 附錄 | 43 |

參考文獻

- 李秦林 (1998) 以米為基質利用固態發酵生產黑殭菌素 Destruxins。台灣大學農業化學研究所碩士論文。洪文凱 (1995) 黑殭菌素之分離、生物檢定及安全性評估。大葉工學院碩士論文。施錫彬 (1998) 白殭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) 黑殭菌素之萃取研究。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 *Diatraea 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. Genthner, 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. Higgins, 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. Krska, R., R. Schuhmacher, M. Grasserbauer, S.R. Kanhere, and P.M. Scott. (1995) Spurenanalyse des *Fusarium* Mykotoxins beauvericin in Mais, *Braunschweig-Volkenrode*, 115-118. Krska, R., R. Schuhmacher, M. Grasserbauer, and P.M. Scott. (1996) Determination of the *Fusarium* mycotoxin beauvericin at $\mu\text{g/kg}$ levels in corn by high performance liquid chromatography with diode array detection, *Journal of Chromatography*, 746:233-238. Krska, 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. Krska, R., R. Schuhmacher, M. Grasserbauer, M. Lemmens, and P. Ruckebauer. (1996) *Fusarium* mycotoxin beauvericin: Optimisation of analytical methodology and natural occurrence in maize ears in Austria, *Mycotoxins and Phycotoxins*, 27-31. Krska, R., R. Schuhmacher, and M. Grasserbauer. (1996) Optimierung eines Analysenverfahrens zur Spurenbestimmung von beauvericin in natürlich und künstlich infiziertem Mais, *Mykotoxin-Workshop*, 18:36-41. Krska, 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. Tanticharoen, 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 *Metarhizium 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.