

二階段醱酵時不同生長條件對綠殭菌產孢之影響

林明申、謝建元

E-mail: 9300346@mail.dyu.edu.tw

摘要

蟲生真菌綠殭菌能感染30多種鱗翅目害蟲，特別對夜蛾科幼蟲致病力強，其分生孢子對害蟲具感染性，是極具開發潛力的殺蟲微生物。本研究以*Nomuraea rileyi* CCRC 35515為試驗菌株，探討二階段醱酵時不同生長條件對綠殭菌產孢之影響。於醱酵中途加入營養源試驗中，液態醱酵第五天時加入20 mL氮源(80% V8 juice及0.6%玉米澱粉)，液態醱酵至第六天接種至固態基質其產孢量可達 6.97×10^9 conidia/g-dry material。此外在添加界面活性劑於液態培養基試驗中，以添加全透力於液態培養基，菌體生長至第五天時菌體濃度最高可達0.0453 g/mL。在產量方面，亦是以添加全透力產孢量最高達 4.89×10^9 conidia/g-dry material。研究結果發現，在搖瓶試驗中，使用棉花塞(瓶塞32 mm、棉花塞重量約4.8 g)為封瓶者，菌體濃度較使用橡皮塞(瓶塞中心之通氣孔洞，直徑約4 mm，孔隙中填充0.25 g棉花)佳，可達0.03 g/mL，產量亦是以棉花塞者最佳達 1.11×10^{10} conidia/g-dry material。此外在添加幾丁聚醣試驗中，以添加1%幾丁聚醣於固態基質中最佳，產孢量達 5.69×10^9 conidia/g-dry material。生物檢定中對三齡期之甜菜夜蛾死亡率並無助益，死亡率為 $45\% \pm 3.1\%$ 。

關鍵詞：二階段醱酵、綠殭菌、分生孢子、幾丁聚醣

目錄

第一章、緒論.....1	第二章、文獻回顧.....3
2.1 綠殭菌之簡介.....3	2.2 流行疾病之誘發.....4
2.3 不同來源分離株致病力比較.....5	2.4 接種型態及接種濃度.....6
2.5 作用機制.....7	2.6 化學農藥對綠殭菌抑制作用.....8
2.7 綠殭菌之安全性.....9	2.8 營養需求對綠殭菌產孢之影響.....10
2.9 環境對綠殭菌之影響.....10	2.10 固態與液態醱酵.....12
2.11 真菌製劑.....14	第三章、材料與方法.....15
3.1 實驗設備.....15	3.2 實驗材料.....15
3.2.1 實驗培養基.....15	3.2.2 試驗菌種.....16
3.2.3 試驗昆蟲.....17	3.3 實驗方法.....17
3.3.1 孢子懸浮液之製備.....17	3.3.2 幾丁聚醣之製備.....17
3.3.3 二階段醱酵.....17	3.3.4 添加幾丁聚醣之影響.....18
3.3.5 物理因子對二階段產孢之探討.....19	3.3.6 化學因子對二階段產孢之探討.....20
3.3.7 分析方法.....24	第四章、結果與討論.....27
4.1 幾丁聚醣對綠殭菌之影響.....27	4.2 物理因子對二階段產孢之探討.....30
4.2.1 醱酵液接種量對二階段產孢之影響.....30	4.2.2 固態基質對二階段產孢之影響.....35
4.2.3 不同光源對二階段產孢之影響.....38	4.2.4 氧氣濃度對二階段產孢之影響.....43
4.3 化學因子對二階段產孢之探討.....47	4.3.1 中途加入碳源及氮源對產孢之影響.....47
4.3.2 添加營養源於固態基質對產孢之影響.....51	4.3.3 添加食用油對二階段產孢之影響.....58
4.3.4 界面活性劑對二階段產孢之影響.....58	第五章 結論.....67
參考文獻.....69	附錄一、培養基之碳、氮、氫元素分析表.....78
附錄二、酵母粉及玉米澱粉之成分表.....79	附錄三、營養源A成分表.....80
附錄四、營養源B成分表.....81	附錄五、甜菜夜蛾人工飼料配方.....82
論文口試問題.....83	

參考文獻

1. 林秀芬(2002)利用蟲生真菌對溫室內蚜蟲之防治探討。國立屏東科技大學植物保護系碩士論文，屏東。
2. 高穗生、蔡勇勝(1995)蟲生病原真菌在蟲害防治上之利用。藥試所專題報導38、39:16-18。
3. 唐立正(1997)本地產綠殭菌感染玉米穗夜蛾之研究。國立中興大學昆蟲學系博士論文，台中。
4. 唐心潔(1998)綠殭菌感染甜菜夜蛾之研究。國立中興大學昆蟲學系碩士論文，台中。
5. 蔡淑珍(1988)蟲生真菌之調查及綠殭菌感染斜紋夜盜之生理學與病理學。國立台灣大學植物病蟲害學研究所碩士論文，臺北。
6. 謝建元、洪文凱、高穗生、王順成、曾耀銘(1998)本土黑殭菌以固態和液態醱酵生產黑殭菌素之探討。中國農業化學會誌36(4):371-379。
7. 薛一祥(2001)綠殭菌醱酵最適化條件之探討。大葉大學食品工程學系碩士論文，彰化。
8. Balardin, R. S., and Loch, L. C. (1989) Semisynthetic culture media for

Nomuraea rileyi mass production, Pesqui. Agropecu. Bras., 24: 375-381. 9. Bateman, R. P., Carey, D. M., and Prior, C. (1993) The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. *Ann. Appl. Biol.*, 122: 145-152. 10. Bell, J. V. (1975) Production and pathogenicity of the fungus *Spicaria rileyi* from solid and liquid media, *J. Invertebr. Pathol.*, 26: 129-130. 11. Boucia, D. G. and Pendland, J. C. (1984) Nutritional requirements for conidial germination of several host range pathotypes of the entomopathogenic fungus, *Nomuraea rileyi*, *J. Invertebr. Pathol.*, 43: 288-292. 12. Boucia, D. G., Schoborg, E. A. and Allen, G. E. (1982) The relative susceptibility of six noctuid species to infection by *Nomuraea rileyi* isolated from *Anticarsia gemmatialis*, *J. Invertebr. Pathol.*, 39: 238-240. 13. Cherry, A. J., Jenkins, N. E., Heviefio, G., Batemen, R. and Lomer, C. J. (1999) Operational and economic analysis of a West African pilotscale production plant for aerial conidia of *Metarhizium* spp. For use as a mycoinsecticide against locust and grasshoppers, *Biocontrol Science and Tecnol.*, 9: 35-51. 14. Cliguest, S. and Scheffer, R. J. (1997) Influence of culture conditions on growth and survival of conidia of *Trichoderma* spp. coated on seeds, *Biocontrol Science and Tecnol.*, 7: 171-181. 15. Deacon, J. W. (1983) *Microbial Control of Plant Pests and Disease*, American Microbiological Society Press, 88pp. 16. Gardner, W. A., Sutton, R. M. and Noblet, R. (1997) Persistence of *Beauveria bassiana*, *Nomuraea rileyi* and *Nosema necatrix* on soybean foliage, *Environ. Entomol.*, 6: 616-618. 17. Garcia, C. and Ignoffo, C. M. (1997) Dislodgment of *Nomuraea rileyi* from cadavers of cabbage looper, *Trichoplusia ni*, *J. Invertebr. Pathol.*, 30: 114-116. 18. Getizin, L. W. (1961) *Spicaria rileyi* (Farlow) Charles, an entomopathogenous fungus of *Trichoplusia ni* (Huebner), *J. Invertebr. Pathol.*, 50: 67-69. 19. Glare, T. R. (1987) Effect of host species and light conditions on production of conidia by an isolate of *Nomuraea rileyi*, *J. Invertebr. Pathol.*, 50: 71-74. 20. Horton, D. L., Carner, G. R. and Turnipseed, S. G. (1980) Pesticide inhibition of the entomogenous fungus *Nomuraea rileyi* in soybean, *Environ. Entomol.*, 9: 304-308. 21. Hajek, A. E. and St. Leger, R. J. (1994) Interaction between fungal pathogens and insect host, *Annu. Rev. Entomol.*, 39: 293-322. 22. Ignoffo, C. M. (1981) The fungus *Nomuraea rileyi* as a microbial insecticide. In "Microbial Control of Pest and Plant Diseases 1970-1980." (H. D. Burges, Ed.) Academic Press, New York, pp. 513-537. 23. Ignoffo, C. M. and Garcia, C. (1985) Host spectrum and relative virulence of an Ecuadoran and Mississippian biotype of *Nomuraea rileyi*, *J. Invertebr. Pathol.*, 45: 346-352. 24. Ignoffo, C. M. and Garcia, C. (1978) *In vitro* inactivation conidia of the entomopathogenic fungus *Nomuraea rileyi* by human gastric juice, *Environ. Entomol.*, 7: 217-218. 25. Ignoffo, C. M., Garcia, C. and Hostetter, D. L. (1976) Effects of temperature on growth and sporulation for the entomopathogenic fungus *Nomuraea rileyi*, *Environ. Entomol.*, 5: 935-936. 26. Ignoffo, C. M., Garcia, C. and Hostetter, D. L. and Pienll, R. E. (1977) Vertical movement of conidia of *Nomuraea rileyi* through sand and loam soil, *J. Econ. Entomol.*, 70: 163-164. 27. Ignoffo, C. M., Garcia, C. and Kroha, M. J. (1982) Susceptibility of larvae of *Trichoplusia ni* and *Anticarsia gemmatialis* to intrahemocoelic injections of conidia and blastospores of *Nomuraea rileyi*, *J. Invertebr. Pathol.*, 39: 198-202. 28. Ignoffo, C. M., Garcia, C., Kapp, R. W. and Coate, W. B. (1979) An evaluation of the risk to mammals of the use of an entomopathogenic fungus, *Nomuraea rileyi*, as a microbial insecticide, *Environ. Entomol.*, 3: 354-358. 29. Ignoffo, C. M., Garcia, C., Hostetter, D. L. and Pienll, R. E. (1975) Sensitivity of the entomopathogenic fungus *Nomuraea rileyi* to chemical pesticides used on soybeans, *Environ. Entomol.*, 4: 765-768. 30. Ignoffo, C. M., Garcia, C. and Samson, R. A. (1989) Relative virulence of *Nomuraea* spp. (*N. rileyi*, *N. atypicola*, *N. anemonoides*) originally isolated from an insect, a spider, and soil, *J. Invertebr. Pathol.*, 54: 373-378. 31. Im, D. J., Aguda, R. M., and Rombach, M. C. (1988) Effect of nutrients and pH on the growth and sporulation of four entomogenous hyphomycetes fungi, *Korean J. Appl. Entomol.*, 27:41-46. 32. John N. W. and Michael E. B. (1999) Kinetics and manipulation of hyphal breakage and its effect on antibiotic production, *Enzyme Microbial. Technol.*, 25: 404-410. 33. Kish, L. P., Samon, R. A. and Allen, G. E. (1974) The genus *Nomuraea* Maublanc, *J. Invertebr. Pathol.*, 24: 154-158. 34. Kawakami, K. (1960) On the changes of characteristics of the silkworm muscardines through successive cultures, *Bull. Sericult. Exp. Stn.*, 16: 83-99. 35. Kiuch, M., Yasui, H., Hayasaka, S. and Kamimura, M. (2003) Entomogenous fungus *Nomuraea rileyi* inhibits host insect molting by C22-oxidizing inactivation of hemolymph ecdysteroids, *Archives of Insect Biochem. Physiol.*, 52: 35-44. 36. Liu, B. L. and Tzeng, Y. M. (1999) Water content and water activity for the production of cyclodepsipeptides in solid-state fermentation by *Metarhizium anisopliae*, *Biotechnol. Lett.*, 21: 657-661. 37. Lonsane, B. K., Saucedo, C. G., Rainbault, M., Roussos, S., Viniegera, G. G., Ghildyal, N. P. and Ramakrishna, M. M. (1992) Scale-up strategies for solid state fermentation system (review), *Process Biochem.*, 27: 259-273. 38. Preez, J. C. Du, Jong, F. De, Botes, P. J., and Lategon, T. M. (1985) Fermentation alcohol from grain sorghum starch, *Biomass*, 8: 101-117. 39. Prior, C., Jollands, P. and Le-Patourel, G.. (1988) Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina: hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae), *J. Invertebr. Pathol.*, 52: 66-72. 40. Ramoska, W. A. (1984) The influence of relative humidity on *Beauveria bassiana* infectivity and replication in the chinch bug, *Blissus leucoperus*, *J. Invertebr. Pathol.*, 43: 389-394. 41. Riba, G. and Glandard, A. (1980) Establishment of a nutritive medium for the deep culture of the entomopathogenic fungus *Nomuraea rileyi*, *Entomophaga*, 25: 317-322. 42. Ramesh, M. V. and Lonsane, B. K. (1990) Critical importance of moisture content of the medium in alpha-amylase production by *Bacillus licheniformis* M27 in a solid-state system, *Appl. Microbiol. Biotechnol.*, 33: 501-501. 43. Stuart, D. M., Mitchell, D. A., John, M. R., and Lister, J. D. (1999) Solid-State Fermentation in Roatating Durm Bioreactors: operating variables affect performance through their effects on transport phenomena, *Biotechnol. Bioeng.*, 63: 383-391. 44. Sun, T. L., Beihui, L. P., Liu, D. and Li, Z. (1998) New solid-state fermentation process for repeated batch production of fibrinolytic enzyme by *Fusarium oxysporum*, *Process Biochem.*, 33: 419-422. 45. Tanada, Y. and Kaya, H. K. (1993) Fungal infections. pp. 318-387, In "Insect Pathology", Academic Press, California. 46. Tang, L. C. and Hou, R. F. (1998) Potential application of the entomopathogenic fungus, *Nomuraea rileyi*, for control of the earworm, *Helicoverpa argimera*, *Entomologia Exp. Appl.*, 88: 25-30. 47. Tang, L. C. and Hou, R. F. (2001) Effects of environmental factors on virulence of the entomopathogenic fungus, *Nomuraea rileyi*, against the cyrn earworm, *Helicoverpa argimera*, *J. Appl. Ent.*, 125: 243-248. 48. Vimala Devi, P. S. (1994) Conidia production of the entomopathogenic fungus *Nomuraea rileyi* and its evaluation for

control of *Spodoptera litura* (Fab) on *Ricinus communis*, J. Invertebr. Pathol., 63: 145-150. 49. Vimala Devi, P. S. (1995) Soil treatment with *Nomuraea rileyi*: a promising technique for the control of *Spodoptera litura* on groundnut, Biocontrol, 5: 361-364 50. Vimala Devi, P. S. and Prasad, Y. G. (1996) Compatibility of oils and antifeedants plant origin with the entomopathogenic fungus *Nomuraea rileyi*, J. Invertebr. Pathol., 68: 91-93 51. Yang, Z., Wieger, K., Jan, P. S. and Jan, B. (1996) Medium Optimization for nuclease P1 production by *Penicillium citrinum* in solid-state fermentation using polyurethane foam as inert carries, Enzyme Microbial. Technol., 18: 108-112. 52. Xu, F. and Chen, H. (2002) Effect of periodically dynamic changes of air on cellulase production in solid-state fermentation, Enzyme Microbial. Technol., 30: 45-48.