

Effects of growth condition to conidial production of *Nomuraea rileyi* by two-stage fermentation

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ABSTRACT

Nomuraea rileyi has been reported that it is able to infect more than 30 species of lepidopterous pests and it makes the larva of several noctuid pests cause high mortality. Thus, it has great potential to be a microbial control agent. The purpose of this research was studying the effects of growth condition on conidia production of *Nomuraea rileyi* by using two-stage fermentation. 20 mL nitrogen source (80% V8 juice and 0.6% C.S.P.) was added to liquid culture at day 5, and then liquid culture was transferred to solid substrate at day 6. For another ten days' fermentation, the production of conidia was 6.97×10^9 conidia/g-dry material. Effect of addition of surfactant, the total wet addition was found to have the best production on both mycelia cell concentration and conidia concentration with 0.0453 g/mL and 4.89×10^9 conidia/g-dry material, respectively. In this study, we found that flask with cotton plug (using 4.8g cotton as the plug in flask bottle neck with I.D. 32mm) had better fungus growth and conidia production than flask with rubber plug (with a hole of I.D. 4mm with 0.25g cotton), and the best production on both mycelia cell concentration and conidia concentration with 0.03 g/mL and 1.11×10^{10} conidia/g-dry material, respectively. Furthermore, addition of 1% chitosan to product of conidia (5.69×10^9 conidia/g-dry material) was better than others, but the is high production was not favorite the mortality ($45\% \pm 3.1\%$) of *S. exigua*.

Keywords : two-stage fermentation, *Nomuraea rileyi*, conidia, chitosan

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REFERENCES

- 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。
- 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。
- 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發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)綠殭菌發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 gemmatalis*, *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 Technol.*, 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 Technol.*, 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 entomopathogenic 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 Ecuadorian 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 gemmatalis* 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.