

Submerged cultural fermentation for chlamyospore of *Trichoderma virens* using winery waste

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ABSTRACT

The main purpose of this study was to investigate the influence of different additives on the production of mycelium and chlamyospore in submerged culture of *Trichoderma virens* incubated with thin stillage. On the cell growth, the pH4 was found to be better than other pH condition and the 80 % of thin stillage as the main media was also found the higher cell dry weight (7.2 g/L) after three days cultivation. The effect of addition of carbon source was shown that the 3 % glucose have the best growth of cell in the thin stillage. On the other hand, the effect of addition of nitrogen source was not shown significant difference of cell growth between the organic nitrogen and inorganic nitrogen . In the aspect of chlamyospore the 60 % thin stillage at pH4 was found higher spore production 1.69×10^7 chlamyospore/mL after 6 days cell cultivation. The medium contains V8 juice and other additives such as Tween 80 and glycerol can increase the production of chlamyospore. In same manner of addition of carbon source or nitrogen source in thin stillage 1 % glucose addition was found to produce higher chlamyospore than other additive sugars. However, the addition of organic or inorganic nitrogen source was not significant increase the spore production. After two or three days' cultivation, the concentration of glucose in the broth was increased to 8 % by addition concentrated sugar solution. The mycelia was found become thinner and without any increase of spore production.

Keywords : *Trichoderma virens* ; Thin stillage ; chlamyospore ; Oxygen uptake rate

Table of Contents

目錄 頁次 封面內頁 簽名頁 大葉大學碩士論文全文授權書iii 中文摘要iv 英文摘要v 誌謝vi 目錄viii 圖目錄x 表目錄xii 第一章緒論1 第二章文獻回顧3 2.1 前言3 2.2 普遍存於植物殘骸及土壤中的腐生性真菌 - 木黴菌4 2.3 木黴菌的形態5 2.4 木黴菌的防治機制7 2.4.1 抗生素作用 (antibiosis) 8 2.4.2 微寄生作用 (mycoparasitism) 8 2.4.3 競爭作用 (competition) 8 2.5 木黴菌屬作為生物防治製劑應具備的條件9 2.5.1 優良及有效的菌株10 2.5.2 快速生長及較長存活期10 2.5.3 良好的傳送體系10 2.6 木黴菌的營養需求12 2.7 農產品廢棄物之應用13 第三章實驗之材料與方法16 3.1 實驗之材料16 3.1.1 菌株(Strain)16 3.1.2 試驗培養基16 3.2 實驗儀器與設備17 3.3 實驗方法19 3.3.1 菌體培養19 3.3.2 分析方法19 3.3.3 木黴菌液態發酵最適化之探討21 3.3.4 木黴菌液態培養厚膜孢子最適化之探討23 第四章結果與討論26 4.1 木黴菌生長情形26 4.2 木黴菌液態發酵最適化之探討26 4.2.1 不同濃度酒糟水對菌體生長之影響26 4.2.2 不同起始酸鹼值對菌體生長之影響30 4.2.3 不同碳源對菌體生長之影響32 4.2.4 不同碳源濃度對菌體生長之影響32 4.2.5 不同氮源對木黴菌菌體生長之影響35 4.3 木黴菌液態培養厚膜孢子最適化探討35 4.3.1 不同濃度酒水及起始酸鹼值對木黴菌厚膜孢子生長之影響35 4.3.2 界面活性劑對木黴菌厚膜孢子生長之影響44 4.3.3 不同濃度酒糟水及不同碳源木黴菌厚膜孢子生長之影響44 4.3.4 不同氮源對木黴菌厚膜孢子生長之影響49 4.3.5 滲透壓木黴菌厚膜孢子生長之影響49 第五章結論與未來展望53 參考文獻54

REFERENCES

- 1.吳幸娟,「以固定化酵母釀造米酒之試驗」,文化大學家政研究所碩士論文,(1990)。
- 2.林意欣,「以米酒糟亦產製酸蛋白質分解酵素之試驗」,東海大學化學工程研究所碩士論文,(1997)。
- 3.林俊杰、彭于瑞,「液態發酵高粱酒之研製(三)酒糟粕之再利用」,台灣省菸酒公賣局酒廠研究報告,239,85~96,(1993)。
- 4.官振儀,「利用酒糟水於靈芝液體培養之研究」,東海大學化學工程研究所碩士論文,(1998)。
- 5.黃賜源,「靈芝液體培養及氣舉式生物反應器應用之實驗」,東海大學化學工程研究所碩士論文,(1997)。
- 6.黃濬志、謝式垚鈺,「木黴菌(*Trichoderma*)及黏帚黴菌(*Gliocladium*)防治土黴病害的實例與展望」,農業世界雜誌,186,16-21,(1999)。
- 7.陳茂源、楊煥章、陳振輝,「米酒之酒糟水再利用」,台灣省菸酒公賣局酒廠研究報告,239,131~139,(1994)。
- 8.謝建元、洪文凱、高穗生、王順成、曾耀銘,「以本土黑殭菌以固態和液態發酵生產黑殭菌素之探討」,中國農化會誌,36:371-379,(1998)。
- 9.羅朝村,「生物防治在作物病害管理上的應用」,台灣省農業試驗所特刊,57,141-150,(1996)。
- 10.羅朝村「作物病害生物防治的應用與展望」,農業世界雜誌,35,11-22,(1999)。
- 11.羅朝村,「木黴菌防治土媒病害的實例與展望」,農業世界雜誌,186期:22-25,(1999)。
- 12.陳瑞祥、童伯開、蔡竹固,「拮抗性木黴菌菌株之分離篩選及其生理特性之研究」,嘉義技術學院學報,59:49-59,(1998)。
- 13.劉顯達,「紅豆根瘤病拮抗菌之開發與應用」,台灣農業,28:87-93(1992)。
- 14.Cho, C-F., Lee, W-C. 1999. Formulation of a Biocontrol Agent by Entrapping Biomass of *Trichoderma viride* in Gluten Matrix. *Journal of Bioscience and*

Bioengineering. 87 : 822-824. 15. Dahlberg, K. R., Etten, J. L. 1982. Physiology and biochemistry of fungal sporulation. *Ann Rev. Phytopathology*. 20 : 281-301. 16. DeLucca, A. J. II., Connick, W. J. Jr., and Fravel, D. R. 1990. The use of bacterial alginates to prepare biocontrol formulation. *J. Indust. Microbiol.* 6 : 129-134. 17. Eyal, J., Baker, C. P., Reeder, J. D., Devane, W. E., Lumsden, R. D. 1997. Large-scale production of chlamydo spores of *Gliocladium virens* strain GL-21 in submerged culture. *J. Indust. Microbiol.* 19 : 163 -168. 18. Fravel, D. R., Connick, W. J. J., Lewis, J. A. 1998. Formulation of microorganisms to control plant diseases in H. D. Burges edit *Formulation of Microbial Biopesticides* : 187-202. 19. Gary, E. H. 1991. Seed treatment for biological control of plant disease. *Crop Prot.* 10 : 166-171. 20. Huang, R. Z., and Hsieh, S. P. Y. 2000. Biocontrol of rice sheath blight and vegetable seedling damping off caused by *Rhizoctonia solani* by chlamydo spore formulation of *Gliocladium viride* isolate G-8. Third International Symposium of *Rhizoctonia*. National Chung Hsing Univ. Taiwan. 21. Latorre, B. A., Agosin, E., Martin, G. S., Vasquez, R. S. 1997. Effectiveness of conidia of *Trichoderma harzianum* produced by liquid fermentation against *Botrytis* bunch rot of table grape in Chile. *Crop Prot.* 16 : 209-214. 22. Lejeune, R., Nielsen, J., Baron, G. V. 1995. Influence of pH on the morphology of *Trichoderma reesei* QM 9414 in submerged culture. *Biotechnol. Lett.* 17: 341-344. 23. Lejeune, R., Baron, G. V. 1995. Effect of agitation on growth and production of *Trichoderma reesei* in batch fermentation. *Appl. Microbiol.* 43 : 249-258. 24. Lo, C. T. 1997. Biological Control of Turf Diseases Using *Trichoderma harzianum*. *Plant Protection Bulletin* 39 : 207-225. 25. Marcel, G-C, Leticia, P., Patricia, M., Robert, P. T. 1999. Mixed culture solid substrate fermentation of *Trichoderma reesei* with *Aspergillus niger* on sugar cane bagasse. *Tetrahedron Lett.* 41: 61-64. 26. Oh, S-U, Lee, S-J, Kim, J-H, Yoo, L-D. 2000. Structural elucidation of new antibiotic peptides, atroviridins A, B and C from *Trichoderma atroviride*. *Tetrahedron Lett.*, 41: 61-64. 27. Sachindra, N. M., Koji, K., Mitsuro, H. 1996. Chlamydo spore formulation in *Fusarium solani* F.sp. *phaseoli* in root extracts of crop plants and their virulence to kidney beans. *Soil Biol. Biochem.* 28:539-543. 28. Stack, J. P., Kenerley, C. M., Pettit, R. E. 1987. Influence of carbon and nitrogen sources, relative carbon, and nitrogen concentrations and soil moisture on the growth in nonsterile soil of soil-borne fungal antagonists. *Can. J. Microbiol.* 33 : 626-631. 29. Sun, L., Li, L. 1999. Effects of air pressure amplitude on cellulase productivity by *Trichoderma viride* SL-1 in periodic pressure solid state fermenter. *Process Bioch.* 34 : 25-29. 30. Zuoxing, Z., Kalidas, S. 1999. Effect of apple pomace-based *Trichoderma* inoculants on seeding vigour in pea germinated in potting. *Process Biochemistry*. 34 : 731-735.