枯草桿菌固態發酵生產 Iturin A 之最適化研究

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摘 要

許多枯草桿菌屬菌株對植物病原真菌和細菌具有拮抗作用，可製成粉劑或純化出其抗生物質， 進行種子覆被或土壤處理。Iturin A是由枯草桿菌所產生的二次代謝物，在培養中期會開始分 泌胞外分子，可對不同的病原菌產生抑制作用，為一具生物農藥發展潛力的菌種。本研究主要 是以固態發酵進行枯草桿菌（Bacillus subtilis）生產iturin A，探討枯草桿菌在不同條件下 對iturin A產生之影響，再以回應曲面法(RSM)進行枯草桿菌之固態發酵，分別探討其最適培養 基與最佳產量。在研究中發現使用高筋麵粉10 g與稻殼50 g時，能獲得較高iturin A產量。 含水量50%、20% (v/w)接種量、葡萄糖濃度為1%、通氣面積為4.15 cm2時，發酵培養5天之後， iturin A產量可達 9.26 mg/g-substrate。在添加界面活性劑方面，添加Span 80為1%時，iturin A 產量最高可達9.99 mg/g-substrate。而添加油脂方面以花生油1%對iturin A的生成達到最大的產量， 其iturin A產量 10.23 mg/g-substrate。在回應曲面法方面，固態發酵於太空瓶中進行培養，經回應 曲面試驗後，可得到最適培養基為使用高筋麵粉10 g與稻殼50 g時， 添加葡萄糖1.15%、KH2PO4 1.27 mM、MgSO4 5.08 mM、花生油1.01%、接種量19.49%、 含水量44.97%，iturin A產量為 11.435 mg/g-substrate。

關鍵詞 : 枯草桿菌 ; Iturin A ; 回應曲面法 ; 固態發酵

目錄

參考文獻

中文部份 1. 向明。1994。生物性農藥的研究現況。生物技術醫藥產業報導。3(4):2-14 2. 向明。1998。台灣生物農藥研發及其產業-枯草桿菌。生物產業與製藥產業(上)。75 3. 向明。1999。新穎無毒生物殺菌劑-台灣寶。農業世界雜誌。192: 70 4. 向明。2002。微生物發酵工程。生物技術方法 卷五。生物化學工程。P100-102 5. 曾德賜、黃文的、柯欣志。2003。枯草桿菌作為益生性生物製劑之應用。農業世界244:34-46。 6. 羅朝村。2003。Development and application of Trichoderma spp. in Taiwan。Fungal Science 18(1,2):23-31。 7. 何志煌。1998。植物二次代謝產物的生產。生物技術的應用。九州圖書。台北。pp151-163。 8. 林弘裕。2002。液化澱粉芽孢桿菌胜?抗生物質之分析與回收純化探討。國立東華大學生物技術研究所 碩士論文。花蓮。 9. 徐泰浩、曾耀銘。1994。生物界面活性劑生產技術之開發與應用。化工。41:42-56 10. 徐泰浩、曾耀銘。1994。生物界面活性劑發酵產程與分離純化。生物產業。5:55-65 11. 郭?烈，郭慶華。1996。新型發酵蛋白飼料。北京:科學技術出版社。147-352 12. 陳俊位。1999。生物農藥枯草桿菌在植物病害防治上之應用。台中區農業專訊。26(8):19。 13. 陳洪章、徐建。2004。現代固態發酵原理及應用。化學工業出版社。北京。 14. 黃達明、吳其飛、陸建明、管國強。2003。固態發酵技術及其設備的研究進展。江蘇大學生物工程研究所。鎮江。第29卷第6期。 15. 陳俊位。2003。迎接生物性農藥的新時代。豐年。53(19):34-39。 16. 楊紹榮。1992。農業廢棄物處理與再利用。台南區農業改良場。 17. 楊盛行。2002。固態發酵在農工業上之應用。科學農業。50(1,2):156-167 18. 謝奉家。2005。植物病害的殺手明星－枯草桿菌。科學發展月刊第391期，p.18-21 19. 謝建元、高穗生。2002。生物性農藥固態發酵量產開發，化工技術，10(4)，166-175。 20. 陳加忠、曹之祖。1977。兩種食品之水活性測定與適用模式之評估。農林學報。46(3):73-90 21. 陳惠婷、余世宗、?瑞澤。2001。pH 與碳源對Penicillium chrysogenum發酵生產青黴素V之影響。大葉學報 第十卷 第二期。 22. 蘇照堂。2002。利用反應曲面法對Saccharomyces cerevisiae菌株在實驗培養基中生長控制的探討。國立中興大學食品科技研究所碩士論文。台中。 英文部份 1. Adinarayana T., Prabhakar V., Srinivasulu M., Anitha Rao P. ,Jhansi Lakshmi,P. Ellaiah 2003. Optimization of process parameters for cephalosporin C production nunder solid state fermentation from Acremonium chrysogenum Process Biochemistry 39:171-177 2. Agger, T., Petersen, J.B., Connor, S.M., Murphy, R.L., Kelly, J.M.,Nielsen, J. 2002. Physiology characterization of recombinant Aspergillus nidulans strains with creA genotypes expressing A. oryzae α-amylase. Journal of Biotechnology 92:279 – 285. 3. Ahimou, F., Jzcques, P. and Deleu, M. 2000. Surfactin and Iturin A on Bacillus subtilis surface hydrophobicity. Enzyme Microb. Technol. 27: 749 4. Aijun, Z., Hongzhang, C., Zuohu. L., 2005. Air pressure pulsation solid state production of alkaline protease by Bacillus pumilus 1.1625. Process Biochemistry 40:1547-1551. 5. Aikat K, Bhattacharyya BC. 2000. Protease extraction in solid state fermentation of wheat bran by a local strain of Rhizopus oryzae and growth studies by the soft gel technique. Process Biochem. 35:907-14. 6. Alazard, D. and M. Raimbault. 1981. Europ. Appl. Microbiol. Biotechnol., 12:113-117 7. Andre ’ s Corona, Doris Sa ’ ez, Eduardo Agosin. 2005. Effect of water activity on gibberellic acid production by Gibberella fujikuroi under solid-state fermentation conditions.Process Biochemistry 40:2655 – 2658 8. Anne-Laure Moyne, Thomas E. Cleveland, Sadik Tuzun, 2004. Molecular characterization and analysis of the operon encoding the antifungal lipopeptide bacillomycin D 9. Aon T., Kobayashi A. and Shoda M. 1990. Transformation of B. subtilis NB22 with treatment by alkali cations. Biotechnol. Lett. 12 pp.99-105. 10. Balakrishna K,Pandey A. Production of biologically active secondary metabolites in solid state fermentation. J Sci Ind Res 1996;55:365-72. 11. Besson, F., Peypoux, F. and Michel, G. 1979. Antifungal activity upon Saccharomyces cerevisiae of iturin A, mycosubtilin. 12. Besson, F., Peypoux, F., Michel, G. and Delcambe, L. 1978. Identification of antibiotics of iturin group in various strains of Bacillus subtilis. J. antibiot. 31: 284. 13. Brown, M. R. W., and Richards, R. M. E. 1964. Effect of polysorbate（Tween 80）on the resistance of Pseudomonas aeruginosa to chemical inactivation. J. Pharmacol. 16:51-55. 14. Box and Wilson. 1951. On the experiment attainment of optimum condition. JRSS-B, 13, 1-45. 15. Carlsen, M., Nielsen, J., Villadsen, J., 1996. Growth and α-amylase production by A. oryzae during continuous cultivations. Journal of Biotechnology 45:81 – 93. 16. Chakraborty R, Srinivasan MJ. 1993. Production of a thermostable alkaline protease by a new Pseudomonas sp. by solid substrate fermentation. J Microb Technol. 8:7-16. 17. Chundakkadu Krishna. 1999. Production of bacterial cellulases by solid state bioprocessing of banana wastes. Bioresource Technology 69:231-239. 18. Delcambe, L. and Devignat, R. 1957. L ’ iturine,nouvel antibiotique d ’ origine congolaise. Acad. R. Sci. Coloniales. 6: 1. 19. Fiechter, A. 1992. Biosurfactants: moving towards industrial application.Tibtech. 10: 208. 20. Fikret Uyar , Zubeyde Baysal . 2004. Production and optimization of process parameters for alkaline protease production by a newly isolated Bacillus sp. under solid state fermentation Process Biochemistry 39:1893 – 1898. 21. Gautam, P., Sabu, A., Pandey, A., Szackacs, G., Soccol, C.R., 2002.Microbial production of extra-cellular phytase using polystyrene as inert support. Biores. Technol. 83, 229-233. 22. Hajjaj,H.,Blane, P, J.,Groussac, E., Goma, G., Uribelarrea, J. L. and Loubiere, P. 1999. Improvement of red pigment/citrinin production ration as a function of environmental conditions by Monascus rubber. Biotechnol Bioeng. 64:497-501. 23. Heins, S. D., Manker, D. C., Jmenez, D. R., McCoy, R. J., Marrone, P. G. and Orjala, J. E. 2000. Compositions and methods for controlling plant pests. U.S.Patent 6,103,228. 24. Hwang, W. I. 1993. Biosurfactant production from Klebsiella Oxytoca. J.Chinese Arg. Chem. Soc. 31: 466. 25. Kaur S, Vohra RM, Kapoor M, Beg QK, Hoondal GS. 2001. Enhanced production and characterization of a highly thermostable alkaline protease from Bacillus sp. P-2. World J Microbiol Biotechnol. 17(2):125 – 9. 26. Kota KP, Sridhar P. 1999. Solid state cultivation of Streptomyces clavuligerus for cephamycin C production. Process Biochem. 34:325-/8. 27. Krishnan Roopesh a, Sumitra Ramachandran a,1, K. Madhavan Nampoothiri a,George Szakacs b, Ashok Pandey a,. 2005.Comparison of phytase production on wheat bran and oilcakes in solid-state fermentation by Mucor racemosus.Bioresource Technology. 28. Lin, S. C., Sharma, M. M. and Georgiou, G. 1993. Production and deactivation of biosurfactant by Bacillus licheniformis JF-2. Biotechnol. Prog. 9:138. 29. Magan N. 2001. Physiological approaches to improving the ecological fitness of fungal biocontrol agents. In: Bull T, Jackson C, Magan N, editors. Fungal biocontrol agents: progress, problems and potential. CAB International. 30. Medvedevan, G., Meissel, M., and Volkova, T. 1969. Antonie van Leeuwenhoek J. Microbiol. Serol.35:27-28. 31. Monica Dominguez,Armando Mejfa and Javier Barrios-Gonzalez. 2000. Respiration Studies of Penicillin Solid state Fermentation。 Journal of Bioscience and Bioengineering 89(5):409-413. 32.

Morikawa, M.,Ito,M. And Imanaka,T. 1993. Isolation of a new surfactin producer Bacillus pumilus A-1, and cloning and nucleotide sequence of the regulator gene,psf-1.J.Ferment.Bioeng.74:255-261. 33. Murthy MVR, Mohan EVS, Sadhukhan AK. 1999. Cyclosporin A production by Tolypocladium inflatum using solid state fermentation.Process Biochem. 34:269-80. 34. Nascimento, A. E., Lima, M. A. B. and Campos-Takaki, G. M. 2000. A cytochemical study of acid carbohydrates on the surface of candida lipolytica grown in tween80-containing medium. Brazilian Journal of Microbiology. 31:30-36. 35. Nigam P. 1990. Investigation of some factors important for solid state fermentation of sugarcane bagasse for animal feed production. Enzyme Microb Technol. 12:805 – 11. 36. Nutan D. Mahadik, Ulka S. Puntambekar, Kulbhushan B. Bastawde, Jayant M. Khire,Digambar V. 2002. Gokhale.Production of acidic lipase by Aspergillus niger in solid state fermentation Process Biochemistry 38:715-721. 37. Ohno A, Ano T, Shoda M. 1992. Production of antifungal antibiotic,iturin in solid state fermentation by Bacillus subtilis NB 22 using wheat bran as substrate. Biotechnol Lett. 14:817-22. 38. Ohno, A. Ano, T. and Shoda, M.1995. Effect of Temperature on Production of Lipopeptide Antibiotics,Iturin A and Surfactin by a Dual Producer, Bacillus subtilis RB14, in Solid-State Fermentation. Journal of fermentation and Bioengineering 5:517-519. 39. Ohno, A. Ano, T. and Shoda, M. 1996. Use of soybean curd residue, okara, for the solid state substrate in the production of lipopeptide antibiotic,iturin A, by Bacillus subtilis NB22. Process biochem. 31: 801. 40. Pandey, A., Selvakumar, P., Soccol, C.R., Nigam, P., 1999. Solid state fermentation for the production of industrial enzymes. Current Science 77 (1), 149 – 162. 41. Pandey, A., Soccol, C.R., Nigam, P., Brand, D., Mohan, R., Roussos,S., 2000. Biotechnological potential of coffee pulp and coffee huskfor bioprocesses. Biochem. Eng. J. 6, 153 – 162. 42. Pascual S, Melgarejo P, Magan N. 1999. Production of the fungal biocontrol agent Epicoccum nigrum by sold substrate fermentation: effect of water activity on accumulation of compatible solutes. Mycopathologia.146:83 – 9. 43. Park, J. P.,Kim, Y. M., S.W., Hwang, H. J.,Cho, Y. J.,Lee, Y. S., Song, C. H. and Yun, J. W. 2002. Effect of agitation intensity on the exo-biopolymer production and mycelial morphology in Cordyceps militaris. Lett Appl. Microbial.34（6）:433-438. 44. Pandey, A., Szakacs, G., Soccol, C.R., Jose, A., Rodriguez, L., Soccol,V.T. 2001. Production, purification and properties of microbial phytase. Biores. Technol. 77, 203 – 214. 45. Pandey, A., Soccol, C.R., Rodriguez-Leon, J.A., Nigam, P., 2001. Solid-state Fermentation in Biotechnology. Asiatech Publishers Inc., New Delhi, p. 221. 46. Paunesen, E., A. Ciolac-Negoescu, and Piscu, G. 1964. The effect of Tween 80 and penicillin on the physiochemical properties of the cell wall in mycobacteria. Acad. Requb. Pop. Rom. Stud. Cercet. Biochem. 7:184-190. 47. Pandey, A., Soccol, C.R., Nigam, P., Brand, D., Mohan, R., Roussos,S., 2000. Biotechnological potential of coffee pulp and coffee husk for bioprocesses. Biochem. Eng. J. 6, 153 – 162. 48. Phae, C. C. and Shoda, M.1991. Investigation of Optimal conditions for form separation of iturin, an antifungal peptide produced by Bacillus subtilis. J.Ferment Bioeng. 71: 118. 49. Prakasham, Ch. Subba Rao, P.N. Sarma Green gram husk—an inexpensive substrate for alkaline protease production by Bacillus sp. in solid-state fermentation Bioresource Technology 50. Puri S, Beg QK, Gupta R. 2002. Optimization of alkaline protease production from Bacillus sp. by response surface methodology. Curr Microbiol. 44:286 – 90. 51. Ramesh MV, Lonsane BK. 1990. Critical importance of moisture content of the medium in alpha-amylase production by Bacillus licheniformis M27 in a solid state fermentation system. Appl Microbiol Biotechnol. 33:501 – 5. 52. Ramesh, M.V., Lonsane, B.K. 1987. Solid-state fermentation for production of alpha amylase by Bacillus megaterium 16M.Biotechnology Letters 9, 323-328. 53. Ramachandran S., Patel, A. K., Nampoothiri, M. Francis, F., Nagy, V., Szakacs, G., Pandey, A., 2004. Coconut oil cake- a potential raw material for the production of α -amylase Bioresource Technology 93:169-174. 54. Regine Maget-Dana and Peypoux F.. 1994. Iturins, a special class of pore-forming lipopeptides: biological and physicochemical properties. Toxicology. 87: 151 55. Regine Maget-Dana, Thimon, L., Peypoux, F. and Ptak, M. 1996. Surfactin/iturin A interactions may explain the synergistic effect of surfactin on the biological properties of iturin A. Biochime. 74: 1047. 56. Ronald, w. w. and William, E. M. L.1975. Effectiveness of various unsaturated fatty acids in supporting growth and respiration in Saccharomyces cerevisiae.The Journal of Biological Chemistry.250(23):9121-9129. 57. Sandrin, C., Peypoux, F. and Michel, G. 1990. Coproduction of surfactin and iturin A, lipopeptides with surfactant and antifungal properties,by Bacillus subtilis. Appl. Biochem. 12: 370. 58. Sen S. 1995. Alkaline protease of a moderate thermophile Bacillus licheniformis S40. Ph.D. Thesis, University of Delhi. 59. Sekhar C, Rajasekar VW, Balaraman K. 1997. Production of cyclosporine A by solid state fermentation. Bioprocess Eng.17:257-9. 60. Sphor, A., Carleen, M., Nielsen, J., Villadsen, J., 1998. a-Amylase production in recombinant A. oryzae during fed-batch and continuous cultivation. Journal of Fermentation and Bioengineering 86 (1), 49 – 56. 61. Valik L, Pieckova E. 2001. Growth of hat-resistant fungi: the effect of water activity. Int J Food Microbiol.63:11 – 7. 62. Winkelmann, G. 1983. Iturin AL-a new long chain iturin A possessing an unusual high content of C16-s-amino acids. J. antibiot. 36: 1451. 63. Yang, F. C., Ke, Y. F., Kuo, S. S., 2000. Effect of fatty acids on the mycelial growth and polysaccharide formation by Ganoderma lucidum in shake flask cultures.Enzyme and microbial technology,27:295-301. 64. Yoo, Y.J., Cadman, T.W., Hong, J., Hatch, T., 1988. Kinetics of alpha amylase from Bacillus amyloliquefaciens. Biotechnology and Bioengineering 31, 357 – 365. 65. Yu, G. Y. 1998. Biocontrol of Rhizoctonia solani on soybean with Bacillus amyloliquefaciens producing iturin A. Illinois thesis. 66. Zu··beyde Baysal, Fikret Uyar, 2003. etin Aytekin a Solid state fermentation for production of α -amylase by a thermotolerant Bacillus subtilis from hot-spring water.Process Biochemistry 38:1665-1668.