菌株 Aeromonas veronii DYU Too-19 分解幾丁質之最適培養探討

盧沛誼、吳淑姿

E-mail: 381992@mail.dyu.edu.tw

摘 要

幾丁質為自然界中之高分子醣類，分佈廣泛，其存量僅次於纖維素。在結構上，幾丁質是以N-乙醯葡萄醣胺聚合而成，當被分解成聚合度較低之N-乙醯幾丁寡醣時，具多重用途。N-乙醯幾丁寡醣以幾丁質分解酵素製備時比強鹼處理更佳。因此本研究從彰化縣大村鄉篩選出具幾丁質分解酵素之菌株Aeromonas veronii DYU-Too 19為對象，探討菌株分解幾丁質之最適條件。以 chitin broth (CB) 培養基培養，首先以一次一因子，探討不同碳、氮源對還原醣生成量、幾丁質分解?活性及pH值影響，並進行中心混成試驗，探討菌株分解幾丁質之最適培養條件。結果顯示，以β-幾丁質為碳源時，可生合成最多還原醣，於β-幾丁質含量4%時，有最高還原醣產量，約15.4 g/L；在氮源方面以peptone為氮源時，還原醣產量高於以其他氮源培養，peptone濃度為0.5 g/L時，還原醣產量較高，為12.5 g/L。本研究進一步以反應曲面法，探討菌株A. veronii DYU-Too 19生合成還原醣之最適化培養條件，兩因子中β-幾丁質含量 與peptone含量，對還原醣生成量達到顯著水準 ($p < 0.05$)，由反應曲面法尋得最適培養基組成分為：4.42% β-幾丁質粉末與0.55 g/L peptone，可預估得到最大還原醣產量，為20.52 g/L。經由發酵培養驗證最適化條件，還原醣產量，為20.24 g/L，即，實際的觀測值與回應模式的預測值比較，並無太大差異，表示此一回應模式能適切預測菌株A. veronii DYU-Too 19生產還原醣之產量。

關鍵詞 : 幾丁質分解?、還原醣、反應曲面法、最適培養條件

目錄

參考文獻

1. 林榮耀 (總校閱)。2008。Stryer ’ s 生物化學第六版。藝軒圖書。台北。 2. 洪哲穎、陳國誠。1992。回應曲面實驗設計法在微生物酵素生產上之應用。化工專論 39(2):3-18。 3. 洪碧霙。2003。蜡蚧輪枝菌幾丁聚醣?之特性分析及應用研究。朝陽科技大學應用化學所碩士論文。台中。 4. 莊榮輝。2000。酵素化學實驗。國立台灣大學農業化學系生物化學研究室。台北。 5. 連德昇。2002。以本土菌株分解幾丁質生產N-乙醯幾丁寡醣之研究。大葉大學食品工程研究所碩士論文。彰化。 6. 陳嘉芬。2006。細胞生物學。藝軒圖書。台北。 7. 張讚昌、邱南昌、尤封陵、施玟玲、朱敬儀、謝雅如、王瑜琦、李文珍。2003。實用微生物學(含免疫學)。華格那發行。台中。 8. 張玉瓏、徐乃芝、許素菁。2008。生物技術。新文京開發。新北市。 9. 張育祥。2011。菌株 Bacillus sp. DYU-Too17 生產N-乙醯幾丁寡醣之最適培養條件。大葉大學生物產業學系研究所論文。彰化。 10. 謝伊金。2007。N-乙醯幾丁寡醣生產菌之篩選與幾丁質分解?之特性分析。大葉大學生物產業學系研究所論文。彰化。11. 蕭正明。2009。限氮條件下添加有機酸對菌株Yu-3生和成PHBV之影響。大葉大學生物產業學系研究所論文。彰化。12. Adrangi, S., Mohammad. A. F., Ahmad, R. S, Zargham Sepehrizadeh. 2010. Purification and characterization of two extracellular endochitinases from Massilia timonae. Carbohydrate Research., 345:402-407. 13. Bing-Lan Liu., Pao-Min Kao., Yew-Min Tzeng., Kuo-Ching Feng. 2003. Production of chitinase from Verticillium lecanii F091 using submerged fermentation. Enzyme and Microbial Technology ., 33:410 – 415. 14. Blanes, M., Pilar, M. D. and Jesus, F. 2007. Inmunosupresion e infeccion en el paciente trasplantado. Enfermedades Infecciosas y Microbiologia Clinica., 27(2):143-154. 15. Bhattacharya, D., Nagpure, A., Gupta, R.K. 2007. Bacterial chitinases: properties and potential. Crit. Rev. Biotechnol., 27: 21 – 28. 16. Chre ’ tiennot-Dinet M-J., Giraud-Guille M-M., Vaulot, D., Putaux, J-L. and Chanzy, H. 1997.The chitinous nature of filament ejected by Phaeocystis (Prymnesiophycae). J Phyco., 33:666 – 672. 17. Chang, K. L., Lee, J. and Fu, W. R. 2000. HPLC analysis of N-acetyl-chito-oligosaccharides during the Acid Hydrolysis of Chitin. J. Food Drug Anal. 8(2):75-83. 18. Choi, J.H., Choi, B.K., Kim, K.Y., Yoo, Y.J., Oh, S.J. 2001. In vitro antimicrobial activity of a chitooligosaccharide mixture. International Journal of Antimicrobial Agents., 18:553 – 557. 19. Chen, C.T., Chien-Jui Huang., Yi-Huei Wang, and Chao-Ying Chen. 2004 .Two-step purifcation of Bacillus circulans chitinase A1 expressedin Escherichia coli periplasm. Protein Expression and Purifcation, 37: 27 – 31. 20. Fenice, M., Selbmann, L., Giambattista, RD., Federici, F. 1998 .Chitinolytic activity at low temperature of an Antarctic strain (A3) of Verticillium lecanii. Res Microbiol., 149: 289 – 300. 21. Felse,PA., Panda, T. 1999 .Self-directing optimization of parameters forextracellular chitinase production by Trichoderma harzianum in batch mode. Process Biochem., 34: 563 – 566. 22. Fiandra, L., Maro, A. D., Terracciano, I., Sticco, L., Ruocco, M., Corrado, G., Parente, A., Rosa, R. 2010. Journal of Biotechlogy., 147:1-6. 23. Frandberg, E., Schnurer, J. Chitinolytic properties of Bacillus pabuli K1. 1994 . J Appl Bacteriol ., 76: 361 – 367. 24. Hadwiger, L. A., Kendra, D. F., Fristensky, B. W., and Wagoner, W. 1985. Chitosan both activates genes in plants and inhibits RNA synthesis in fungi. In chitin in Nature and Technology. p. 209-222. 25. Hart, P.J., Pfluger, H.D., Monzingo, A.F., Hollis, T., Robertus, J.D. 1995. The refined crystal structure of an endochitinase from Hordeum vulgare L. seeds at 1.8 A resolution. J. Mol. Biol., 248: 402 – 413. 26. Hackman, R. H. 1960. Studies on chitin. IV. The nature of α- and β-chitin. J.Biol. Sci. 18: 935. 27. Hirano, S. and Midorikawa, T. 1998. Novel method for the preparation of N-acylchitosan fiber and N-acylchitosan – cellulose fiber. Biomaterials. 19:293 – 7. 28. Huang, R., Niranjan, R. and Se-Kwon., K. 2006. Structural factors affecting radical scavenging activity of chitooligosaccharides (COS) and its derivatives. Carbohydr. Polymers., 63:122-129. 29. Izabela, S. Santos., Maura Da Cunha, Olga L.T. Machado., Valdirene, M. Gomesa. 2004. A chitinase from Adenanthera pavonina L. seeds: purification,characterisation and immunolocalisation. Plant Science.167: 1203 – 1210. 30. Jang, M.-K., Kong, B.-G., Jeong, Y.-I., Lee, C. H., & Nah, J.-W. 2004. Physicochemical characterization of a-chitin, b-chitin and c-chitin separated from natural resources. Journal of Polymer Science: Part A: Polymer Chemistry, 42:3423 – 3432.31. Jeuniaux, c. 1966. Chitinases. Methods Enzymol., 8: 644-650. 32. Jie, F., Luhang, Z., Qiqi, Y. 2004. Receptor-mediated stimulatory effect of oligochitosan in macrophages. Biochem. biophys. res. commun. 317: 414-420. 33. Kawada, M., Hachiya, Y., Arihiro, A., Mizoguchi, E. 2007. Role of mammalian chitinases in inflammatory conditions. Keio J. Med., 56:21-27. 34. Keyhani, NO., Roseman, S. 1996 .The chitin catabolic cascade in the marine bacterium Vibrio furnissii. Molecular cloning, isolation, and characterization of a periplasmic b-N-acetylglucosaminidase. J Biol Chem. 271:425 – 432. 35. Krajewska, B. 2004.Application of chitin- and chitosan-based materials for enzyme immobilizations: a review. Enzyme Microbiol Technol., 35:126 – 139. 36. Kumar, R., Majeti, N. V. 2000. A review of chitin and chitosan applications. Reactive and functional polymers 46 :1-27. 37. Marguerite, Rinaudo. 2006. Chitin and chitosan:Properties and applications. Prog. Polym. Sci. 31:603-632. 38. Mahadevan, B., Crawford, DL. 1997 .Properties of the chitinase of the antifungal agent Streptomyces lydicuis WYEC108. Enzyme Microb Technol., 20:489 – 493. 39. Mathur, NK., Narang, CK. 1990.Chitin and chitosan, versatile polysaccharides from marine animals. J Chem Educ., 67:938 – 942. 40. Matahira, Y., Ito, M. and Sakai, K. 1998. The application of chitin-chitosan to bone filling materials. Kichin Kitosan Kenkyu: Publ. Nippon Kitin, Kitosan Gakkai. 4:142-143. 41. Maro, A.D., Terracciano, I. Sticco, L., Fiandra, L., Ruocco, M., Corrado, G., Parente, A.,Rosa R 2010. Purification and characterization of a viral chitinase active against plant pathogens and herbivors from transgenic tobacco. Journal of Biotechlogy147:1-6.42. McKay, G., Blair, H.S. and

Gardner, J.R. 2000. Adsorption of dyes on beads and microgranules of chitosan, Biomaterials 21 chitin. I. Equilibrium studies, J. Appl. Polym. Sci., 27:1115- 3043. 43. Molinari, L. M., Raissa, Bocchi, Pedroso., Denise, de Oliveira Scoaris , Tania Ueda-Nakamura., Celso, Vataru Nakamura., Benedito, Prado Dias Filho. 2007 .Identification and partial characterisation of a chitinase from Nile tilapia, Oreochromis niloticus Comparative Biochemistry and Physiology., Part B 146:81 – 87. 44. Ohishi, K., Yamagishi, M., Ohta, T., Suzuki, M., Izumida, H., Sano, H. et al. 1996 . Purification and properties of two chitinases from Vibrio alginolyticus H-8. J Ferment Bioeng., 82:598 – 600. 45. Patel, A. K., Vijay, Kumar Singh., Ravi, Prakash Yadav., Arthur, J.G. Moir, Medicherla. V. Jagannadham. 2010. Purification and characterization of a new chitinase from latex of Ipomoea carnea. Process Biochemistry.,45 :675 – 681. 46. Patil, RS., Ghormade, V. 2000. Deshpande MV. Chitinolytic enzymes: an exploration. Enzyme Microb Technol., 26:473 – 483. 47. Pera, LM., Infante, Majoll?, MV., Baigor?, MD. 1997. Purification and characterization of a thermostable and highly specific b-N-acetyl-Dglucosaminidase from Aspergillus niger 419. Biotechnol Appl Biochem.,26:183 – 187. 48. Pillai, C. K. S., W. Paul and C. P. Sarma, 2009. Chitin and chitosan polymers: Chemistry,solubility and fiber formation. Progress in Polymer Science. 34:641-678. 49. Rajapakse, N., Moon, M. K., Eresha, M., Ronghua, H. and Se K. K. 2006. Carboxylated chitooligosaccharides (CCOS) inhibit MMP-9 expression in human fibrosacoma cells via down-regulation of AP-1. Biocmica Biophysica et Acta., 1760:1780-1788.50. Rinaudo, M. 2006. Chitin and chitosan: Properties and applications. Prog. Polym. Sci. 31:603-632. 51. Rogalski, J., Krasowska, B., Glowiak, G., Wojcik, W., Targonski, Z. 1997. Purification and some properties of extracellular chitinase produced by Trichoderma viride F-19. Acta Microbiol Pol., 46:363 – 375. 52. Tagawa, K., Okazaki, K. 1991. Isolation and some cultural conditions of Streptomyces species which produce enzymes lysing Aspergillus niger cell wall. J Ferment Bioeng., 71:230 – 236. 53. Tao, Y. Yan, Z-Z., Sun, J-W., Cheng, X-L., Zhang, S-Z. 1990 .Purification and characterization of b-N-acetylhexosaminidase from Aspergillus tamarii. Acta Microbiol Sin., 30:259 – 266. 54. Takayanga, T., Ajisaka, K., Takiguchi, Y., Shimahara, K. 1991 .Isolation and characterization of thermostable chitinase from Bacillus licheniformis X-7U. Biochem Biophys Acta., 1078:404 – 410. 55. Thamthiankul, S., Suan-Ngay S., Tantimavanich, S., Panbangred, W. 2001 .Chitinase from Bacillus thuringiensis subsp. pakistani. Appl Microbiol Biotechnol., 56:395 – 401. 56. Tokoro , A., Suzuki, K., Mikami, T., Okawa, Y., Suzuki, S. and Suzuki, M. 1989. Antitumor effect of hexa-N-acetylchitohexaose and chitohexaose. Carbohydr. Res. 51(15): 403-408. 57. Tsigos, I., Aggeliki, M., Dimitris, K and Vassilis, B. 2000. Chitin deacetylases: new, versatile tools in biotechnology. Trends Biotechnol. 18:(7):305-312. 58. Varma, R. S., George, K.J., Balaji, S. and Parthasarathy,V. A. 2009. Differential induction of chitinase in Piper colubrinum in response to inoculation with phytophthora capsici,the cause of foot rot in black pepper. Saudi Journal of Biological Sciences., 16:11 – 16. 59. Tweddell, RJ., Jabaji-Hare SH., Charest, PM. 1994 .Production of chitinaseand _-1,3-glucanse by Stachybotrys elengans, a mycoparasite of Rhizoctonia solani. Appl Environ Microbiol., 60:489 – 498. 60. Usui, T., Hayashi, Y., Nanjo, F., Ishido, Y. 1984 . Transglycosylation reaction of a chitinase purified from Nocardia orientail. Biochem Biophys Acta., 923:302 – 309. 61. Vyas, P., Deshpande, MV. 1989 .Chitinase production by Myrothecium verucaria and its significance in fungal mycelia degradation. J Gen Microbiol., 35:343 – 350. 62. Van, Aalten, D.M., Komander, D., Synstad, B., Gaseidnes, S., Peter, M.G., Eijsink, V.G. 2001. Structural insights into the catalytic mechanism of a family 18 exochitinase. Proc. Natl. Acad. Sci.USA., 98:8979-8984. 63. Wipa, S., Chomphunuch, S., Supansa, P., Adeleke, H. A., Robinson, R. C. 2008. Crystal structures of Vibrio harveyi chitinase A complexed with chitooligosaccharides: Implications for the catalytic mechanism J. Struct. Biol. 62:491 – 499. 64. Yusof, NL., Wee, A., Lim, LY., Khor, E. 2003 .Flexible chitin filmsas potential wound-dressing materials: wound model studies. J Biomed Mater Res A., 66A:224 – 232. 65. Young, ME., Bell, RL., Carroad, PA. 1985 . Kinetics of chitinase production. I. Chitin hydrolysis. Biotechnol Bioeng., 27:769 – 775. 66. Young, ME., Bell, RL., Carroad, PA. 1985 .Kinetics of chitinase production. II. Relation between bacterial growth, chitin hydrolysis, and enzyme synthesis. Biotechnol Bioeng., 27:776 – 780.