Bacillus subtilis W-118所產幾丁質?之研究

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摘 要

由台灣北部土壤分離出一株在pH6.0 、30℃能以3%的蝦蟹殼粉為主要碳源之幾丁質?生產菌，Bacillus subtilis W-118。利用3 %蝦蟹殼粉、0.1%K2HPO4、0.05%MgSO4．7H2O，用磷酸緩衝液（pH6）培養體積為100mL加入於250mL之三角錐瓶中，在30℃之培養箱培養三天，可獲得最大之幾丁質分解?活性。 利用Bacillus subtilis W-118之幾丁質?最適生產條件生產幾丁質?，經硫酸銨沉澱、透析後，進行DEAE-Sephacel離子交換層析、Sephacryl S-200膠體層析及chromatofocusing一連串純化分離的步驟。所純化分離出之幾丁質?純度約為未純化分離前的7倍左右。經純化分離出來之幾丁質?在50℃、pH 5～7之內酵素都能保持穩定狀態，而最適反應溫度及最適反應pH值分別為37℃及pH6；進行protein 200 chip分析可知其分子量約為20.6 k Da左右。 懸浮態幾丁質經過Bacillus subtilis W-118所產幾丁質?酵素水解之後，可產生聚合度1至6之N-乙醯幾丁寡醣。乙睛與水之體積比在30分鐘之內由70:30線性降低至55:44 之梯度沖提，可於30分鐘內得到聚合度1至6的N-乙醯幾丁寡醣之最佳解析與鑑定效果。 利用Bacillus subtilis W-118分解懸浮態幾丁質產生之N-乙醯幾丁寡醣處理人類血癌細胞株K562後，經WST-1偵測後發現N-乙醯幾丁寡醣具有抑制細胞增殖率的作用，並以顯微鏡觀察N-乙醯幾丁寡醣處理後之細胞形態，發現細胞會產生皺縮的現象。利用Bacillus subtilis W-118分解懸浮態幾丁質產生之N-乙醯幾丁寡醣處理植物病原性真菌Fusarium oxysporum，經顯微鏡觀察後，發現Fusarium oxysporum 之菌絲呈不完整之菌絲外觀，與控制組的菌絲於外觀上有極大的差異。

關鍵詞 : 枯草桿菌、幾丁質?、蝦蟹殼粉、N-乙醯幾丁寡醣、K562

目錄

參考文獻

1. 王三郎 (1996) 水產資源利用學 , 高立圖書出版社. 2. 王三郎 (1998) 應用微生物學 , 高立圖書出版社. 3. 王三郎 (1999) 海洋未利用生物資源之回收再利用-幾丁質及幾丁聚醣 , 生物資源 生物技術,1:1-8 . 4. 王三郎 (2000) 生物技術 , 高立圖書出版社. 5. 王中和，陸順娟，胡海生，等(1997)低分子殼多糖對癌症放療患者免疫功能的影響.首都醫科大學學報.，18:80 6. 王啟浩 (1999) 利用細菌發酵農水產廢棄物生產生物製劑之研究. 大葉大學食品工程研究所碩士論文. 7. 杜昱光，白雪芳，虞星炬，等(1997)寡聚糖類物質生理活性的研究.中國生化藥物雜誌.18:268 8. 呂明洲 (1994) Pseudomonas aeruginosa K-181 所產幾丁質分 解酵素之探討.大葉工學院食品工程研究所碩士論文. 9. 邱少華（1997）利用綠膿桿菌K187發酵蝦蟹殼廢棄物生產幾丁質?之應用及量產條件之研究.大葉工學院食品工程研究所碩士論文. 10. 吳秀蓉, 林錦幸, 張珍田, 宋賢一 (1991) 淡水大蝦肝臟幾丁質酵素之純化與性質利用.中國農化學會會誌, 29:154-161. 11. 梁慈雯（2000）Bacillus subtilis V656所生產微生物抑制物質之研究.大葉工學院食品工程研究所碩士論文. 12. 章瑩(1996)幾丁質應用於腫瘤治療的研究現狀，國外醫學腫瘤分冊.23:346. 13. 陳榮輝、金曉珍(1999)水產甲殼類廢棄物開發高經濟價值之幾丁質、幾丁聚醣、幾丁寡醣研究之規劃報導，科學發展月刊，23:550-562. 14. 陳福智(1998)幾丁寡醣之高效能液相層析分離及葡萄胺糖苯甲醯化之探討。國立台灣海洋大學食品科學所碩士論文. 15. 張珍田(2000)幾丁質?及幾丁聚醣?之研究，生命科學簡訊，14:3-8. 16. 黃安德(1999)利用部分純化之Amycolatopsis orientalis 細胞外N-乙醯葡萄糖胺?製備N-乙醯幾丁寡醣。國立台灣海洋大學食品科學所碩士論文. 17. 張虎，杜昱光，虞星炬(1999)幾丁寡糖與殼寡糖的製備和功能.中國生化藥物雜誌，20:99. 18. 蔡國珍，吳冠政，詹淑玲(2002)幾丁寡醣之抗菌及免疫活性，幾丁質幾丁聚醣研討會論文輯. 19. 劉瓊淑(1994)幾丁質、幾丁聚醣極其相關酵素之特性與應用，食品工業，26:26-36. 20. 蕭瑞昌(1997)利用水溶液性幾丁聚醣以薄膜超過濾法去除微量之金屬離子，碩士論文，私立元智工學院. 21. Angel, Z. H. and Angel, A. B. (1983) Chitinolyticactivity from Neurosproa crassa. J. Gen. Microbiol., 129:3319-3321. 22. Araki, Y. and Ito, E. (1974) A pathway of chitosan formation in Mucor rouxii : enzymatic deacetylation of chitin, Biochem. Biophys. Res. Commun., 56 :669-674. 23. Bassler, B. L., Yu, C., Lee, A. M. and Roseman, S. (1991) Chitin utilization by marine bacteria, degradation and cata-bolism of chitin oligosacchides by Vibrio funissii. J. Bio. Chem.,266:24276-24281. 24. Bihari-Varga, M. , Sepulchre, C. and Moczar, E.（1975）Thermoanalytical studies on Protein polysaccharide complex of connective tissue. J. Thermal Anal. , 7:675-679. 25. Chae Gun Phae., Makoto Shoda., Nobuhiro Kita., Mituyuki Nakano., and Kinji UshiyamA. (1992) Biological control of crown and root rot and bacterial wilt of tomato by Bacillus subtilis NB22. Ann. Phytopath. Soc. 58:329-339. 26. Corroad, D. A. and Tom, R. A. (1978) Bioconversion of s hellfish chitin waste : process conception and selection of microorganism. J. Food Sci., 43:1158-1164. 27. Cosio, I. G. , Fisher, R. A. and Carroad , D. A. (1982) Bioconversion of shellfish chitin waste : waste pretretement , enzyme production, process design, and economic analysis. J . Food Sci., 47:901-905. 28. Deshpande, M. V. (1986) Enzymatic degradation of chitin & its biological application. J. Sci. Ind. Res., 45 :273-277. 29. Defaye, J., Gadelle, A., and Pedersen, C. (1989). Chitin and chitosan oligosaccharides. In Chitin and Chitosan, pp.415-429. 30. Felix. Mauch., Brigitte Mauch-Mani., and Thomas Boller (1988) Antifungal hydrolase in pea tiussue . Plant Physiol. 88:936-942. 31. Hasegawa, M., Isogi, A. and Onabe, F. (1993). Preparation of low molecular weight chitosan using phosphoric acid. Carbohydr. Polym., 20:279-283. 32. Hoitink,H.A.J.and P.C.Fahy. (1986) Basis for the control of soil-borneplant pathogen swithcxomposts. Ann. Rev. Phytopathol., 24:93-114. 33. Hadwiger L. A., Kendra, D. F. (1986) Chitosan both activates genes in plantsan inhibits RNA synthesis in fungi. PlenumPerss, new York, USA.209-21. 34. Hyun-Ock Pae, Won-Gil Seo. (2001)Induction of granulocytic differentiation in acute promyelocytic leukemia cells (HL-60) by water-soluble chitosan oligomer, Leukemia Res, 25: 339-346. 35. Imoto, T. and Yagishita, K. (1971) A sample activity measurement of lysozyme. Agric .Biol. Chem., 35 : 1154. 36. Izume, M., Nagae, S., Kawagishi, H., Mitsutomi, M., and Ohtakara, A. (1992). Action pattern of Bacillus sp. No. 7-M chitosanase on partially N-acetylated chitosan. Biosci. Biotech. Biochem., 56:448-453. 37. Kendra, D. F., Christian, D. and Hadwiger, L. A. (1989) Chitosan oligomers from Fusarium solani pea interactions, chitinase／β-glucanase digestion of sporelings and from fungal wall chitin actively inhibit fungal growth and enhance disease resistance. Physiol. Mol. Plant Pathol. 35:215-230. 38. Kobayashi M, Watanabe T, Suzuiki S, (1990) Effect of N-acetylchitohexaose agains Candida albicans infection of tumor-bearing mice. Microbiol Immunol, 34(5):413. 39. Kang, C. D., Do, I. R., Kim, K. W., Ahn, B. K., Kim S. H., Chung, B. S., Jhun, B. H. and Yoo, M. A. (1999) Role of Ras/ERK-dependent pathway in the erythroid differentiation of K562 cells. Experi Mol Medicine, 31: 76-82. 40. Knorr, D.（1984）Use of chitinous polymer in food.

Food Technol. , 1:85-89. 41. Koga D., Sueshige, N., Orikono, K., Utsumi, T., Tanaka, S., Yamada, Y, and Ide, A. (1988) Efficiency of chitinolytic enzyme in the formation of tricoderma matsutake protoplasts. Agric. Boil. Chem., 52:2091-2094. 42. Koga D., Sueshige, N., Usumi, T. and Ide, A. (1989) Kinetics of chitinase fromyam, Dioscorea oppsita thumb. Agric. Biol. Chem., 53:3121-3127. 43. Mitsutomi, M., Ohtakara, A., Fukamizo, T. and Goto, S. (1990) Action pattern of Aeromonas hydrophila chitinase on partially N-acetylated chitosan. Agric. Biol. Chem. 54:871-877. 44. Lorito M., Di Pietro A., Hayes C. K., Woo S. L., Harman G. E. (1993) Antifungal,synergistic interation between chitinlytic enzymes from Trichoderma harzianum and Enterobacter cloacae. Mol Plant Pathol. 83:721-728. 45. Lorito M., Harman G. E., Hayes C. K., Broadway R. M., Tronsmo A. (1993) Chitinolytic enzyme produced by Trichoderma harzianum :Antifungal activity of purified endochitinase andhitobiosidase. Mol Plant Pathol. 83:302-307. 46. Oxford. O'brine, M. and Colwell, R. R.（1987）Arapid test for hitinase activity that uses4-methylumbelliferyl-N-acetyl- - D -glucosamine. Appl Envion Microbiol 53:1718-1724. 47. Negro M.J., Solano M.L., Ciria P., Carrasco J.(1999) Composting of sweet sorghum bagasse with other wastes. Biore Techno, 67:89-92 48. Ohtakara, A., Matsunaga, H. and Mitsutomi, M. (1990) Action pattern of Streptomyces grisous chitinase on partialy N-acetylated chitosan. Agric. Biol. Chem. 54:3191-3199. 49. Robert, W. K. and Selitrennidkoff, C. P. (1988) Plant and bacterial chitinase differ in antifungal activity. J. Biol Chem.134 :. 169-171. 50. Ghatak S., Misra S., and Toole B. P.. (2002) Hyaluronan oligosaccharides inhibit anchorage-independent growth of tumor cells by suppressing the phosphoinositide-3- kinase/Akt cell survival pathway. J Biol Chem. 11;277:38013-38020. 51. Wang S. L., Shin I. L., Wang C. H. (2002) Production of antifungal compounds from chitin by Bacillus subtilis. Enzyme Microb.Technol. 31:321-328 52. Wang S. L., Shin I. L.(2002) Purification and characterization of two antifungal chitinase exteacellularly produced by Bacillus amyloliquefaciens V656 in a shrimp and ahell powder medium. J. Agric. Food Chem 50:2241-2248 53. Stanley, W. L. , Watters, G. G. , Chan, B. G. and Kelly, S. H.（1976）Immbolization of glucose isomerase on chitin with glutaraldehyde and by simple adsorption. Biotech. andBioeng. 18:439-442. 54. Stanley, W. L. , Watters, G. G. , Chan, B. G. and Marcer, J. M.（1975）Lactose and other enzymes bound to chitin with glut-aldehyde. Biotech. and Bioeng. , 17:315-319. 55. Suzuki K, Tokro A, Okawa Y. (1985) Enhancing effects of N-acetyl-chito-oligosaccharides on the active oxygengenerating and microbicidal activities of peritoneal exudatecells in mice. Chem Pharm Bull, 33:886-888 56. Suzuki K Tokoro A, Okawa Y. (1986) Effect of N-acetylchitooligosaccharides on activation of phagocytes. MicrobiolImmunol, 30:777-787 57. Suzuki K, Mikami T, Okawa Y.(1986) Antitumor effect of hexa-N-acetylchitohexaose and chitohexaose. Carbohydrate Res, 151:403-408. 58. Suzuki S, Okawa Y, Suzuki K, (1987) Candidacidal effect of peritoneal exudae cells in mice administered with chitin or chitosan:the role of seine protease on the mechanism of oxygen-independent candidacidal effect. MicrobiolImmunol ,31:375-383. 59. Tokor A, Suzuki K, Suzuki S (1988) Growth-inhibitory effect of N-cetylchitohexaose and chitohexaose against Meth-Asolid tumor.Chem. Pharm. Bull.36:784-790. 60. Tokoro A, Kobayashi M, Tatewaki N.(1989) Protective effect of N-acetyl chitohexaose on Listeria monocytogenes infection in mice. Microbiol Immunol, 33:357-367. 61. Tsutomu, T., Kasumi, A., Yasuyaki, T. and Venzo, S. (1991) Isolation and characterization of thermostable chitinase from Bacillus licheniformis. Biochim. Biophys. Acta., 1078 :404-411. 62. Usui, T., Hayashi, Y., Nanjo, F., Sakai, K. and Ishido, Y. (1987) Transglycoylation reaction of chitinase purified from Nocardio orientalis. Biochim. Biophys. Acta., 9923 : 302-309. 63. Yamasaki Y., Hayashi I., Nakagawa T.(1993) Purifiaction and mode of action of chitosanolytic enzyme from Enterobactersp.G-1.Biosci. Biotech. Biochem., 57:444-449