Bacillus subtilis W-118所生產蛋白?之純化及定性

何瑞元、王三郎 ; 顏裕鴻

E-mail: 9318489@mail.dyu.edu.tw

摘 要

本研究以低成本之水產廢棄物－“ 蝦蟹殼粉 ”作為菌株產生蛋 白?所需之主要營養源研究中，Bacillus subtilis W-118最適培養基為0.1% K2HPO4、0.05% MgSO . 7H2O、1%之蝦蟹殼粉，培養條件為30℃ 、 pH6 ，經振盪培養48小時可得最高活性。經由DEAE -Sepharose CL-6B及 Sephacry S-200 Gel filtration的純化，最終純化倍率為2.31，回收率為2%，經純化出之酵素在37oC以下、pH7~9之內，酵素都能保持穩定狀態，而最適反應溫度及pH值分別為50oC及pH8，由於蛋白?受到PMSF的抑制，因而推測此蛋白?係屬一種絲胺酸型蛋白?。酵素等電點為8.7，分子量約為17kDa。酵素應用在植物生長促進劑方面，將具有蛋白?活性之醱酵液做為植物生長促進劑，針對小白菜促進生長研究中，以不同天數發酵液稀釋10倍後，每天添加100 ml於小白菜，發現到蛋白?活性較高的醱酵液對小白菜有較顯著的促進生長效果。將促進劑與只有加水的空白組比較，其株重及株長的相對增加比例增加為原來的156%及133%。

關鍵詞 : Bacillus subtilis ; 蝦蟹殼粉 ; 蛋白& ; #37238 ; 純化

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 (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）: 3.王三郎 (2002) 應用微生物學，高立圖書出版社。 4.楊政國(1999) 利用枯草菌進行蝦殼去蛋白之研究。私立大葉大學 食品工程研究所碩士論文，彰化。 5.陳自珍編譯 (1978) 食品酵素學。復文書局。 6.陳國誠(1989) 微生物酵素工程學。藝軒圖書出版社。 7.張文重(1977) 蛋白質分解酵素。環球書社，台北。 8.張文智 (1996) 蝦蟹殼加工之廢棄物回收與再利用，私立大葉大學食品工程系研究所碩士論文，台灣彰化縣。 9.賴威安 (2000) Bacillus sp. P-6 中蛋白?的生產與性質分析，國立中興大學食品科學研究所碩士論文，台中。 10.Adil Anwar and Mohammed Saleemuddin (1998) Alkaline protease : a review, Biores. Technol. 64 : 175~183 11. Amare Gessesse, Rajni Hatti-Kaul, Berhanu A. Gashe, Bo Mattiasson. (2003) Novel alkaline protease from alkaliphilic bacteria grown on chicken feather. Enzyme Microb. Technol. 32:519~524 12.Anson, M. L. 1938. The estimation of pepsin, trypsin, papain and cathepsin with haemoglobin. J. Gen. Physiol. 22, 79?89. 13.A.Dayanandan (2003) Application of an alkaline protease in leather processing:an ecofriendly approach. Journal of Cleaner Production. 11:533-536 14.Aiyappa,P.S. , Harris, J. O. 1976. The extracellular metalloprotease of Serratia marcescens: I.purification and characterization. Mol. Cell. Biochem. 13:95-100 15.Anwar, A. , Saleemuddin, M. 1998. Alkaline protease: a review.Biosource Technol. 64: 175-1156. 16.Babe, L.M. , Schmidt, B. 1998. Purification and biochemical analysis of WprA, a 52-kDa serine protease secreted by B. subtilis as an active complex with its 23-kDa propeptide. Biochim. Biophys. Acta. 1386:211-219. 17.Barett, A. J. 1994. Proteolytic enzymes:serine and cystein peptidases. Methods Enzymol. 244:1-15. 18.Beynon, R. J. , Bond, J. S. 1989. Proteolytic enzymes: a piratical approach. pp. 57-65. IRL Press. 19.Bollag, D. M., Rozycki, M. D., Edelstein, S. J. 1996. Protein methods. Wiley-Liss Inc, USA, New York. 20.Boyce, C. O. L. 1986. NOVO'S handbook of practical biotechnology. A Publication of NOVO Industry A/S Enzymes Division Bagsvaerd, Denmark. 21.Boonyaras Sookkheo ., (2000) Purification and characterization of the highly thermostable protease from Bacillus stearothermophilus TLS33. Protein Expression and Purification.20:143-151 22.Bruce W. Elliott, Jr. , Carolyn Cohen. (1986) Isolation and characterization of a Lysine-specific protease from Pseudomonas aeruginosa. J. Bio. Chem. 261:11259-11265 23.C. G. Kumar, (1999) Novel alkaline serine proteases from alkalophilic Bacillus spp. Purification and some properties. Process Biochemistry. 34:441-449 24.Claudia Toma (1999) Purification and characterization of an Aeromonas caviae metalloprotease that is related to the Vibrio cholerae henagglutinin/protease. FEMS Microbiology Letters. 170:237-242 25.Haruto Kumura (1993) Purification and some proterties of proeinase from Pseudomonas fluorescens No. 33. J. of Dairy Res. :229-23760 26.Hee-Jung Kim (1997) Purification and characterization of an extracellular metalloprotease from Pseudomonas fluorescens. J. Biochem. 121:82-88. 27.Hageman, J. H. Shankweiler, G.W. , Wall, P.R. , Quinones, C. 1984. Single chemically defined sporulation and extracellular protease production. J. Bacteriol. 30:41-62. 28.Jen-Kuo Yang et (2000) Production and purification of protease from a Bacillus subtilis that can deproteinize crustacean wastes. Enzyme Microb Technol. 26:406-413 29.Jose Escobar , Stanley Barnett. (1995) Synthesis of Acid Protease from Mucor miehei: Integration of Production and Recovery. Process Biochem. 8:659-700. 30.Kawamura, F. , Doil, R. H. 1984. Construction of a Bacillus subtilis double mutant deficient in extracellular alkaline and neutral proteases. J. Bacteriol. 160 : 442-444 31.K. L. Kohlmann (1991) Purification and characterization of an extracellular protease produced by Pseudomonas fluorescens M3/6. J. Dairy Sci. 74:4125-4136 32.Kunitz, M. 1947. Crystalline soybean trypsin inhibitor. J. Gen. Physiol. 30, 291?310. 33.Loffler, A. 1986. Proteolytic enzymes: sources and applications. Food Technol. 40:63-70. 34.Lilik Ikasari (1996) Leaching

and characterization of Rhozipus oligosporus acid protease from solid-state fermentation. Enzyme Microb Technol. 19:171-175. 35. L. C. Wu , Y. D. Hang. (2000) Acid protease production from Neosartorya fischeri. Lebensm-Wiss. U.-Rechnol. 33:44-47 36.Marianna Turkiewicz (1999) Biosynthesis and properties of anExtracellular metalloprotease from the Antarctic marine bacterium Sphingomonas paucimobilis. Journal of Biotechnology 70:53-60 37.Moracova, J. , Chaloupka, J. 1984. Repression of the synthesis of exocellular and intracellular proteinases in Bacillus megaterium. Folia Microbiol. 29:273-281. 38.Nathalie Durand-Poussereau (1996) Characterization and a protease deficient strain of Penicillium roqurforti generated by heterologous plasmid intergration:potential us for protein production. J. Biotechnol. 51:97-105 39.Nora Muller , Frank Bordusa.(2000) Assay of diverse protease activities on the basis of a small synthetic substrate. Anal Biochem. 286:86-90 40.Piggot, P. J. , Coote, J. G. 1976. Genetic aspects of bacterial endospore formation. Bacteriol. Rev. 40:908-962. 41.Price, N. C. , Steven, L. 1999. Fundamentals of enzymology, pp. 184-185. Oxford university Press Inc., New York. 42.Priest, F. G. 1977. Extracellular enzyme synthesis in the genus Bacillus. Bacteriol. Rev. 41: 711-753. 43.Rao, M. B., Tanksale, A. M., Chatge, M. S. , Deshpande, V. V. 1998. Molecular and Biotechnological aspects of microbial proteases. Microbiol. Mol. Biol. Rev. 62 : 597-635 44.R. K. Malik , D. K. Mathur.(1984) Purification and characterization of a heat-stable protease from Pseudomonas sp. B-25.J. Dairy Sci. 67:522-530 45.Sandro Germano(2003) Characterization and stability of proteases from Penicillium sp. Produced by solid-state fermentation. Enzyme Microb. Technol. 32:246-251. 46.Schaeffer, P. 1969. Sporulation and the production of antibiotics. Exoenzymes and Exotoxins Bacteriol. Rev. 33:48-71. 47.Susan Long, M. A., Mothibeli, F. T., Robb, F. T. , Wods, D. R. 1981. Regulation of extracellular alkaline protease activity by histidine in a collagenolytic Vibrio alginolyticus strain. J. Gen. Microbiol.127: 193-199. 48.Suresh Shastry, M. S. Prasad. (2002) Extracellular protease from Pseudomonas sp. (CL 1457) active against Xanthomonas campestris. Process Biochem. 37:611-621 49.Takami, H., Nakamura, S., Aono, R., Horikoshi, K. 1992. Degradation of human hair by a thermostable alkaline protease by alkalophilic Bacillus Sp. AH-101. Biosci. Biotechnol. Biochem. 56: 1667-1669. 50.Thomas Aalbaek, (2002) Acid protease and formation of multiple froms of glucoamylase in batch and continuous cultures of Aspergillus niger. Enzyme , Microbial Technology.30:410-415. 51.T. Kobayashi(1996) Purification of alkaline proteases from a Bacillus strain and their possible interrelationship. Appl. Mocrob. Biotechnol. 52.Walter, H. E. 1981. Method with haemoglobin, casein and azocoll as substrate. In : Methods of Enzymatic Analysis. (5) pp. 270?275. (ed. H. U. Bergmeyer). Verlag Chemie GmbH. D-6940 Weinheim 53.Yasuda, M., Aoyama, M., Sakaguchi, M., Nakachi, K. , kobarnoto, N. 1999. Purification and characterization of a soybean-milk-coagulating enzyme from Bacillus pumilus TYO-67. Appl. Microbiol. Biotechnol. 51:474-479.