Production and characterization of the proteases from a bacteria strain LCF007

劉錦芳、王三郎 ; 吳芳禎

E-mail: 9300034@mail.dyu.edu.tw

ABSTRACT

LCF007 was isolated from soils collected at central part in Taiwan. The culture supernatant has protease to the growth. Maximum protease activity was obtained when LCF007 was grown aerobically at 30°Cfor 60hr in a medium consisting 0.3%Monascus power 、0.1%Sucrose、0.1% Tris-aminomethan、0.1% Cellulose、0.1% K2HPO4、0.05% MgSO4．7H2O at the pH 8.4. The proteases (T1 & T2) were not thermostable. Two proteases were purified in a three-step procedure involving ammonium sulfate precipitation, DEAE-Sepharose CL-6B ionic exchange chromatography and Sephacryl S-200 gel permeation chromatography. The enzymes (T1 & T2) were shown to have a relative molecular weight of 30and 43 kDa by SDS-PAGE. The optimum pH and temperature of T1 and T2 were shown to be 9.0, 8.0 and 45℃, 30℃, respectively. The pH stability were analyzed and exhibited more stable at pH 9.0~11.0 and pH 8.0. T1 and T2 were estimated to be 5.45 and 5.49 by chromatofocusing, respectively. T1 and T2 proteases were strongly inhibited by the metal chelator 0.5mM EDTA and 1mM PMSF. Expect that the proteases could apply to the Monascus sp. in the future and assay the produced.

Keywords : Monascus sp. ; protease

Table of Contents

REFERENCES

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）:1-8。 3.王三郎 (2002) 應用微生物學，高立圖書有限公司。 4.田蔚城主編(2001) 生物技術的發展與應用，九州圖書文物有限公司。 5.李佩玲(2002) Pseudomonas aeruginosa M-1001 所生產抗菌成分 之研究，私立大葉大學食品工程學系研究所碩士論文，台灣彰化縣。 6.呂鋒洲、林仁混 (1987)基礎酵素學，經聯出版事業公司，臺北。 7.邱麗君(1994) 蛋白酵素催化酯化反應，私立大葉工學院食品工 程學系研究所碩士論文，台灣彰化縣。 8.林子傑(2000) 嗜水性產氣單胞桿菌Aeromonas hydrophila絲氨 酸蛋白?之特性及基因分析，國立台灣大學農業化學研究所碩士論文，台灣臺北市。 9.林讚峰（1982）紅麴研究發展之演進。製酒科技專論彙編，第四期:66-77。 10.林讚峰（1983）紅麴菌的鑑定及實用分類法。製酒科技專論彙編，第五期:104-113。 11.林讚峰（1986）紅麴菌次級代謝物的經濟性評估及增產策略。製酒科技專論彙編，第八期:81-99。 12.林讚峰（1992）紅麴研究發展之演進。科學農業，40 (3-4): 193-198。 13.林讚峰(1995)紅麴菌培養工藝及紅麴應用之演進。製酒科技專論彙編，第十七期:156-168。 14.徐茂輝（1999）傳統紅麴及功能紅麴的研究開發與進展。食品資訊No.162:14-19。 15.徐泰浩、曾耀銘編譯(1993)生物技術概論，藝軒圖書出版社。 16.莊榮輝(1985)水稻蔗糖合成?之研究，國立台灣大學農業化學研究所博士論文，台灣台北市。 17.張文智(1996)蝦蟹殼加工之廢棄物回收與再利用，私立大葉大 學食品工程學系研究所碩士論文，台灣彰化縣。 18.陳自珍編譯(1988)食品酵素學，復文書局。 19.陳彥霖（2000）紅麴與高血壓。科學與技術，第32卷第12期:54-59。 20.黃顯宗（1985）紅麴菌研究的回顧與展望。真菌學之最近發展（曾聰徹、陳瑞青主編）。國科會生物科學研究中心專刊（臺北），第十二集:109-124。 21.楊政國(1999)利用枯草菌進行蝦蟹殼去蛋白之研究，私立大葉 大學食品工程學系研究所碩士

論文，台灣彰化縣。 22.楊鵑華(1998)紅麴食品素材的機能和利用。烘焙工業，82:44-46。 23.歐靜枝譯(1988)酵素，復漢出版社。 24.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，台灣台中市。 25.賴滋漢、賴業超(1994)食品科技辭典，富林出版社。 26.蕭維仁(2001)以紅麴發酵蝦蟹殼粉生產抗菌幾丁質?之研究，私立大葉大學食品工程學系碩士論文，台灣彰化縣。 27.蘇遠志(2001)紅麴製品介紹及國內研究現況。機能性發酵製品研討會論文集，台北。 28.山內吉彥、近藤英彥、清水彌生、蓮見惠司、遠藤章，1986.???????合成阻害劑ML-236B(Compactin)???血中??????低下作用????動物種差。2p-16，日本農藝化學大會論文報告。 29.玉田英明，1988.紅麴?各種調味料??應用。食品?科學，July:96-99。 30.田邊伸和(1998)紅麴?食品素材??? ?機能?利用。食品?開發33（2）:18。 31.佐藤喜吉（1936）東洋產Monascus屬分類?對??-考察。日農化會誌，12:583-586。 32.野畸紘一（1979）食品加工?利用???天然色素????。New Food Ind., 21（5）:2-7。 33.鈴木秀昭（1988）加工食品????????利用????。New Food Ind., 30（4）:20-24。 34.遠藤章，1985.紅麴?紅麴菌???為歷史?最近?動向。發酵?工業，43（6）:544-552。 35.樽井壓一（1993）紅麴素材?開發?利用。食品?開發，28（1）:47-50。 36.?啟介、市川富夫、河村雅子、中川靖枝，1986.紅???（Monascus pilosus）?血壓降下作用。2Q-20，日本農藝化學大會論文報告。 37.?啟介等（1992）紅麴食品?高血壓自然發症????血壓?及??影響。日食工會誌，39（10）:919-924。 38.稻垣長典、大橋晉（1940）果汁製品關研究（第2報）。日本農藝化學會誌16（11）:1089-1097。 39.Abdallah A. A. Magboul, Lotte B. Larsen, Paul L.H. McSweeney, Alan L. Kelly. (2001) Cystein protease activity in bovine milk. International Dairy Journal. 11:865-872. 40.A. Dayanandan, J. Kanagaraj, Lesley Sounderraj, R. Govindaraju, G.Suseela Rajkumar. (2003) Application of an alkaline protease in leather processing: an ecofriendly approach. Journal of Cleaner Production. 11:533-536. 41.Barnard, E. L. (1987)A new species of Monascus from pine tissues in floride. Mycologia. 79（3）:479-484. 42.Boonyaras Sookkheo, Supachok Sinchaikul, Suree Phutrakul, and Shui-Tein Chen. (2000) Purification and characterization of the highly thermostable proteases from Bacillus stearothermophilus TLS33. Protein Expression and Purification. 20:143-151. 43.Bruce W. Elliott, Jr. and Carolyn Cohen. (1986) Isolation and characterization of a Lysine-specific protease from Pseudomonas aeruginosa. J. of Bio. Chem. 261:11259-11265. 44.Cannon, P. F., Abdullah, S. K., and Abbas, B. A.（1995）Two new species of Monascus from Iraq, with a key to know, species of the genus. Mycol. Res. 99（6）:659-662. 45.C. G. Kumar, M.P. Tiwari, K.D. Jany. (1999) Novel alkaline serine proteases from alkalophilic Bacillus spp.: purification and some properties. Process Biochemistry. 34:441-449. 46.Claudia Toma, Yoshio Ichinose, Masaaki Iwanaga. (1999) Purification and characterization of an Aeromonas caviae metalloprotease that is related to the Vibrio cholerae henagglutinin/protease. FEMS Microbiology Letters. 170:237-242. 47.David J. Fairbairn and Barry A. Law. (1986) Purification and characterization of the extracellular proteinase of Pseudomonas fluorescens NCDO 2085. J. of Dairy Res. 53:457-466. 48.Endo, A. (1979) Monacolin K, a new hypocholesterolemic agent produced by a Monascus species. J. Antibiot. 32:852-854. 49.Endo, A. (1980) Monacolin K, a new Hypocholesterolemic Agent that Sepcifically Inhibits 3-Hydooxy-3-Methylglutraryl Coenzyme A Reductase. J. Antibiotics 3:334-337. 50.Eui Ichishima, Makoto Ojima, Youhei Yamagata, Satoshi Hanzawa and Tetsuo Nakamura. (1995) Molecular and enzymatic properties of an aspartic proteinase from Rhizopous hangchow. Phytochemistry. 38(1):27-30. 51.Frank Seeber. (2000) An enzyme-release assay for the assessment of the lytic activities of complement or antimicrobial peptides on extracellular Toxoplasma gondii. Journal of Microbiological Methods. 39:189-196. 52.Hartley, B. S. 1960. Proteolytic enzymes. Ann. Rev. Biochem, 29:45. 53.Haruto Kumura, Katsuhiko Mikawa and Zenichi Saito (1993) Purification and some properties of proteinase from Pseudomonas fluorescens No.33. J. of Dairy Res. 60:229-237. 54.Hawksworth, D. L. and J. I. Pitt. (1983）A new taxonomy of Monascus species based on cultural and microscopical characters. Aust. J. Bot. 31:51-61. 55.Hector M. Fernindez Lahore, Maria V. Miranda, Elda R. Fraile, Mirtha J. Biscoglio de Jimknez Boninoh & Osvaldo Cascone. (1995) Partition behaviour and purification of a Mucor bacilliformis acid protease in aqueous two-phase systems. Process Biochemistry. 7:615-621. 56.Hee-Jung Kim, Yoshiaki Tamanoue, Gwang-Ho Jeohn, Akihiro Iwamatsu, Akira Yokota, Yong-Tae Kim, Takayuki Takahashi, and Kenji Takahashi. (1997) Purification and characterization of an extracellular metalloprotease from Pseudomonas fluorescens. J. Biochem. 121:82-88. 57.Hideto Takami, Teruhiko Akiba, and Koki Horikoshi. (1989) Production of extremely thermostable alkaline protease from Bacillus sp. no. AH-101. Appl. Microbiol Biotechnol. 30:120-124. 58.Iizuka, H. and C. F. Lin. (1980）On the genus Monascus of Asia and its specific characteristics. Adv. Biotech. 2:555-561. 59.Jean-Pierre Chessa, Ioan Petrescu , Mostafa Bentahir , Jozef Van Beeumen , Charles Gerday. (2000) Purification, physico-chemical characterization and sequence of a heat labile alkaline metalloprotease isolated from a psychrophilic Pseudomonas species. Biochimica et Biophysica Acta. 1479:265-274. 60.Jen-Kuo Yang, Ing-Lung Shih, Yew-Min Tzeng, San-Lang Wang. (2000) Production and purification of protease from a Bacillus subtillus that can deproteinize crustacean wastes. Enzyme and Microbial Technology. 26:406-413. 61.Johannes P. T. W. van den Hombergy, Peter J.I. van de Vondervoort, Laurence Fraissinet-Tachet and J. Visser. (1997) Aspergillus as a host for heterologous protein production: the problem of proteases. Tibtech July. 15:256-263. 62.Jose Escobar and Stanley Barnett. (1995) Synthesis of Acid Protease from Mucor miehei: Integration of Production and Recovery. Process Biochemistry. 8:695-700. 63. Kaustav Aikat and Bimal Chandra Bhattacharyya. (2000) Protease extraction in solid state fermentation of wheat bran by alocal strain of Rhizopus oryzae and growth studies by the soft gel technique. Process Biochemistry. 35(9):907-914. 64.Kaustav Aikat, Bimal Chandra Bhattacharyya. (2001) Protease production in solid state fermentation with liquid medium recycling in a stacked plate reactor and in a packed bed reactor by a local strain of Rhizopus oryzae. Process Biochemistry. 36:1059-1068. 65.K. L. Kohlmann, S. S. Nielsen, and M. R. Ladisch. (1991) Purification and characterization of an extracellular protease produced by Pseudomonas fluorescens M3/6. J. Dairy Sci. 74: 4125-4136. 66.Kono, I. and Himeni, K.（2000）Changes in γ-aminobutyric acid content during beni-koji making. Biosci. Biotechnol. Biochem. 64（3）:617-619. 67.Laemmli. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature Aug 15. 227 (259): 680-685. 68.Lahl, W.J. and Braun, S.D. 1994. Enzymatic production of protein hydrolysates for food use. Food Technol. 48(10): 68-71. 69.L. C. Wu and Y. D. Hang. (2000) Acid protease production from Neosartorya

fischeri. Lebensm-Wiss. u.-Technol. 33:44-47. 70.Leslie T. Mathaba, Catherine H. Pope, Jason Lenzo, Maria Hartofillis, Helen Peake, Robert L. Moritz, Richard J. Simpson, Andreas Bubert, Philip J. Thompson, Geoffrey A. Stewart. (2002) Isolation and characterization of a 13.8-kDa bacteriolytic enzyme from house dust mite extracts: homology with prokaryotic proteins suggests that the enzyme could be bacterially derived. FEMS Immunology and Medical Microbiology. 33: 77-88. 71.Lilik Ikasari and David A. Mitchell. (1996) Leaching and characterization of Rhozipus oligosporus acid protease from solid-state fermentation. Enzyme and Microbial Technology. 19:171-175. 72.Loffler, A.(1986) Proteolytic enzymes: sources and application. Food Technol. 40(12): 63-70. 73.Maria de Fatima Silva Lopes, Ana Lucia Leitao, J.J. Figueiredo Marques, Manuel Jose Teixeira Carrondo, Maria Teresa Barreto Crespo. (1999) Processing of extracellular lipase of Lactobacillus plantarum:involvement of a metalloprotease. FEMS Microbiology Letters. 00:483-487. 74.Maria Papagianni, Murray Moo-Young. (2002) Protease secretion in glucoamylase producer Aspergillus niger cultures: fungal morphology and inoculum effects. Process Biochemistry. 37:1271—1278. 75.Marie-Claude Jobin, Daniel Grenier. (2003) Identification and characterization of four proteases produced by Streptococcus suis. FEMS Microbiology Letters. 220:113-119. 76.Marianna Turkiewicz, Ewa Gromek, Halina Kalinowska, Maria Zielin ’ ska. (1999) Biosynthesis and properties of an extracellular metalloprotease from the Antarctic marine bacterium Sphingomonas paucimobilis. Journal of Biotechnology. 70:53—60. 77.Martha L. Diaz-Torres, Roy R.B. Russell. (2001) HtrA protease and processing of extracellular proteins of Streptococcus mutans. FEMS Microbiology Letters. 204:23-28. 78.M.J. Zapelena, I. Astiasaran, J. Bello. (1999) Dry fermented sausages made with a protease from Aspergillus oryzae and/or a starter culture. Meat Science 52:403-409. 79.Nathalie Durand-Poussereau, Michel Fevre. (1996) Characterization and a protease deficient strain of Penicillium roqurforti generated by heterologous plasmid integration: potential us for protein production. Journal of Biotechnology. 51:97-105. 80.Nora Muller and Frank Bordusa. (2000) Assay of diverse protease activities on the basis of a small synthetic substrate. Analytical Biochemistry. 286:86-90. 81.Pirkko Helisto, Gleb Aktuganov, Nailia Galimzianova, Alexander Melentjev, Timo Korpela. (2001) Lytic enzyme complex of an antagonistic Bacillus sp. X-b: isolation and purification of components. Journal of Chromatography B. 758:197-205. 82.Price, N. C. and Steven, L. (1999) Fundamentals of enzymology, pp. 184-185. Oxford University Press Inc., New York. 83.Rao, M. B., Tankale, A. M., Chatge, M. S. and Deshpande, V. V. (1998) Molecular and Biotechnological aspects of microbial proteases. Microbiol. Mol. Biol. Rev. 62(3): 597-635. 84.R. K. Malik and D. K. Mathur. (1984) Purification and characterization of a heat-stable protease from Pseudomons sp. B-25. J. Dairy Sci. 67:522-530. 85.R. Triveni, T. R. Shamala. (1999) Clarification of xanthan gum with extracellular enzymes secreted by Trichoderma koningii. Process Biochemistry. 34:49-53. 86.Sandro Germano, Ashok Pandey, Clarice A. Osaku, Saul N. Rocha, Carlos R. Soccol. (2003) Characterization and stability of proteases from Penicillium sp. produced by solid-state fermentation. Enzyme and Microbial Technology. 32:246—251. 87.S. E. Jensen, L. Phillippe, J. Teng Tseng, G. W. Stenmke, and J. N. Campbell. (1979) Purification and characterization of exocellular proteases produced by a clinical isolate and a laboratory strain of Pseudomonas aeruginosa. Can. J. Microbiol. 26:77-86. 88.Shaoliang Li, Shigemi Norioka, and Fumio Sakiyama. (1997) Purification, staphylolytic activity, and cleavage sites of α-Lytic protease from Achromobacter lyticus. J. Biochem. 122:772-778. 89.Shin-ichi Miyoshi, Sumio Shinoda. (2000) Microbial metalloproteases and pathogenesis. Microbes and Infection. 2:91-98 90.Suresh Shastry, M. S. Prasad. (2002) Extracellular protease from Pseudomonas sp. (CL 1457) active against Xanthomonas campestris. Process Biochemistry. 37:611-621. 91.Su, Y. C. (1976) Purification and some properties of amylase produced by Monascus anka V-2. Memoris of the college of Agriculture, National Taiwan University. 16（1）:93-103. 92.S. W. Budi, D. van Tuinen, C. Arnould, E. Dumas-Gaudot, V. Gianinazzi-Pearson, S. Gianinazzi. (2000) Hydrolytic enzyme activity of paenibcillus sp. strain B2 and effects of the antagonistic bacterium on cell integrity of two soil-borne pathogenic fungi. Applied Soil Ecology. 15:191-199. 93.Thomas Aalbaek, Morten Reeslev, Bo Jensen, Susanne H. Eriksen. (2002) Acid protease and formation of multiple froms of glucoamylase in batch and continuous cultures of Aspergillus niger. Enzyme and Microbial Technology. 30:410-415. 94.T. Kobayashi, Y. Hakamade, J. Hitomi, K. Koike, S. Ito. (1996) Purification of alkaline proteases from a Bacillus strain and their possible interrelationship. Appl. Mocrobiol Biotechnol. 45:63-71. 95.Toma, C. (1999) Purification and characterization of an Aeromonas caviae metalloprotease that is related to Vibrio cholerae hemagglutinin/ protease. FEMS Microbiol. Lett. 170: 237-242. 96.T. R. Patel, D. M. Jackman, and F. M. Bartlett. (1983) Heat-stable protease from Pseudomonas fluorescens T16: Purification by affinity column chromatography and characterization. Appl. Environ. Microbiol. 46:333-337. 97.Van Tieghem, M.（1884）Monascus genre nouveaude I, ondre des Ascomycetes. Bull. Soc. France . 31:231-256. 98.Wong, H. C. and Bau,Y. S.（1977）Pigment and antibacterial activity of fast neufron and X-ray induced strains of Monascus purpureus. Plant Physiol. 60:578. 99.Wong, H. C. and Y. S. Bau（1978）Morphology and photoresponses of fast neutron and X-ray induces strains of Monascus purpureus. Mycologia. 70（3）:645-659. 100.Wong, H. C.（1986）Monascus pilosus . Applied and Environ. Microbio. 52:1147. 101.Young, E. M.（1930）:Physiological studies in relation to the taxonomy of Monascus species. Trans. Wis. Acad. Sci. Arts and Letters, 227-244.