

以批次醣酵槽生產聚麴胺酸及其抗凍性之研究

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摘要

本研究係以醣酵槽探討*B.licheniformis* CCRC 12826生產聚麴胺酸最佳條件，採用逐步固定法之方式，探討曝氣量、攪拌速度、pH值對聚麴胺酸產量影響，並於培養期間探討碳源消耗，溶氧，黏度，細胞生長及聚麴胺酸產量變化，以找出聚麴胺酸之最適生產條件。結果顯示在pH=6.0時最適合菌的生長，而在pH=6.5時則較適合聚麴胺酸之生合成。於攪拌速度之探討中發現在100rpm時，由於基質質傳速率慢且菌量生長緩慢，導致聚麴胺酸的產量不高，而在300rpm時，雖然其基質消耗快速，但是可能因轉速快而造成聚合酶不易產生或是易被破壞，因此產量相對較低，200rpm最適合聚麴胺酸的聚合。於曝氣量的變化中，以曝氣量(3L/min)的產量最高，其產量較曝氣量(2L/min)多了33%。總之，本研究發現當*B.licheniformis* CCRC 12826培養於培養基(NH₄Cl 7.0(g/L), K₂HPO₄ 0.5(g/L), MgSO₄ · 7H₂O 0.5(g/L), FeCl₃ · 6H₂O 0.04(g/L), CaCl₂ · 2H₂O 0.15(g/L), MnSO₄ · 4~6H₂O 0.104(g/L), Citric acid 22(g/L), Glutamic acid 65(g/L), Glycerol 170(g/L))，於pH=6.5，攪拌速率200rpm，曝氣量(3L/min)之10L醣酵槽中，所得之最佳產率為25.93g/L，較搖瓶培養時增加了23%。除運用醣酵槽生產聚麴胺酸之外，所得之聚麴胺酸則進行抗凍性之研究。我們製備不同的光學異構，不同分子量及各種金屬鹽(鈉、鉀、鎂、鈣)之聚麴胺酸並探討其分子結構與抗凍活性之關係。結果顯示不同光學異構組成之聚麴胺酸之抗凍活性是相似的，顯然光學異構不影響聚麴胺酸抗凍活性。在不同分子量之聚麴胺酸方面，分子量越小者其抗凍活性越高，而在分子量為15,151時，其抗凍活性(AF)達到了5.57。在不同金屬鹽之聚麴胺酸中，其抗凍活性為Mg > Na ?Ca > K，這趨勢與無機金屬鹽之抗凍性相似，即高離子電荷具高抗凍性，然而如何解釋分子結構與抗凍性之反應機構則尚待研究。

關鍵詞：聚麴胺酸；抗凍性

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參考文獻

- 參考資料 1.Adamson J.G.,Hoang T., Crivici A.,Lajoie G.A., “ Use of Marfey ’ s Reagent to Quantitate Racemization upon Anchoring of Amino Acids to Solid Supports for peptide Synthesis ” , Analytical Biochemistry 202,p.210-214,(1992). 2.Akinori M, Masata M, Shigeru Toba, Masao M, “ Antifreeze activities of Various Food Components ” , J.Agric.Food Chem., 45, p.14~18, (1997). 3.Arai, S., & Watanabe, M., “ Freeze texturing of food materials by ice-nucleation with the bacterium with the bacterium Erwinia ananas ” , Journal of Biological Chemistry, 50(1), p.169~175, (1986). 4.Cheng C., Asada Y., Aida T., “ production of -polyglutamic acid by bacillus licheniformis A35 under denitrifying conditons ” , Agric.Biol.Chem., 53(9), p.2369-2375, (1989). 5.Crevel R.E.R. et al., “ antifreeze proteins: characteristics, occurrence and human exposure ” , Food and Chemical Toxicology, 40, p.899-903, (2002). 6.Cromwick A.M., Gross R.A., “ Effects of manganese(II) on bacillus licheniformis ATCC 9945A physiology and -poly(glutamic acid) formation ” , J.Biol.Macromol, 17(5), p.259-267, (1995). 7.Cromwick, A., Gross, A., “ Effects of pH and Aeration on -Poly(glutamic acid) Formation by Bacillus licheniformis in Controlled Batch Fermentor Cultures ” , Biotechnology and Bioengineering , 50, p.222~227, (1996). 8.Feeney, R.E. and Yeh, Y., “ antifreeze proteins: properties mechanism of action and possible applications ” , Food Technology (January), 90, p.82-88, (1993). 9.Feeney R.E. and Yeh Y., “ antifreeze proteins: current status and possible food uses ” , Trends in food Science&Technology, 9, p.102-106, (1998). 10.Feeney R.E. and Osuga D.T., “ Polar fish proteins ” , Trends in Biochem.Sci., 2, p.269-271, (1977). 11.Feeney, R.E., “ Inhibition and promotion of freezing: fish antifreeze proteins and ice-nucleating proteins ” , Comments Agric. Food Chem., 1(3), p.147-181, (1988). 12.Fletcher, G.L., Goddard, S.V., Wu, Y., “ Antifreeze proteins and their genes: from basic research to business opportunity ” , Chemtech., 30, p.17~28, (1999). 13.Graciela P., Francisco C., “ Biosynthesis and Ultrasonic Degradation of Bacterial Poly(-Glutamic acid) ” , Biotechnology and bioengineering, 63(1),April 5, p.110-115, (1999). 14.Gregory A.B., Cromwick A.M., Gross R.A., “ -Poly(glutamic acid) formation by Bacillus licheniformis 9945a:physiological and biochemical studies ” , int.J.Biol.Macromol, 16(5), p.265-275, (1994). 15.Hew, C.L., Yang, D.S.C., “ protein interaction with ice ” , European Journal of Biochemistry, 203, p.33-42.(1992). 16.Ito Y., Tanaka Y., Ohmachi T., Asada Y., “ Glutamic acid independent production of poly(-glutamic acid) by Bacillus subtilis TAM-4 ” , Biosci. biotech. biochem., 60(8), p.1239-1242, (1996). 17.Jin, H.D., “ Effect Recovery of -Poly(glutamic acid) From Highly Viscous Culture Broth ” , Biotechnology and Bioengineering , 76(3), p.219~223, (2001). 18.Kambourova M., Tangeney M., Priest F.G., “ Regulation of Polyglutamic Acid Sythesis by Glutamate in Bacillus licheniformis and Bacillus subtilis ” , Applied and Environmental Microbiology, 67, 2, p.1004-1007, (2001). 19.Kawahara H, “ The structures and functions on ice crystal- controlling proteins from bacteria ” , J. Bioscience and Bioengineering, 94(6), p.492-496, (2002). 20.Kochhar S., Christen P., “ Amino Acid Analysis by High- Performance liquid Chromatography after Derivatization with 1-Fluoro-2,4-dinitrophenyl-5-L-alanine Amide ” , Analytical Biochemistry, 178 , p.17-21, (1989). 21.Kunioka M., “ Biosynthesis and chemical reactions of poly(amino acid)s from microorganisms ” ,Appl Microbiol Biotechnol, 47, p.469-475, (1997). 22.Kunioka M., Choi H.J., “ Hydrolytic degradation and mechanical properties of hydrogels prepared from microbial poly(amino acid)s ” , polymer degradation and stability,59,p.33-37,(1998). 23.Kubota H., Matsunobu T., Uotani K., Takebe H., Satoh A., Tanoka T., Taniguchi M., “ production of poly(-glutamic acid) by Bacillus subtilis F-2-01 ” , Biosci.biotech.biochem., 57(7), p.1212-1213, (1993). 24.Kunioka M., Goto A., “ Biosynthesis and Hydrolysis of Poly(-glutamic acid) from Bacillus subtilis IFO3335 ” , Biosci. Biotech. Biochem., 56(7), p.1031-1035, (1992). 25.Kunioka M., Goto A., “ Biosynthesis of poly(-glutamic acid) from L-glutamic acid , citric acid, and ammonium sulfate in Bacillus subtilis IFO3335 ” , Appl. Microbiol Biotechnol., 40, p.867-872, (1994). 26.Kunioka M., “ Biosynthesis of poly(-glutamic acid) from L-glutamine, citric acid and ammonium sulgate in Bacillus subtilis IFO3335 ” , Appl. Microbiol Biotechnol., 44, p.501-506, (1995). 27.Li B., Sun D.W., “ Novel methods for rapid freezing and thawing of foods a review ” , J.Food Engineering, 54, p.175-182, (2002). 28.Li, J.,& Lee, T.C., “ Bacterial ice nucleation and its potential application in the food industry ” , Trends in Food Science and Technology, 6, p.259~265, (1995). 29.Li, J., & Lee, T.C., “ Bacterial extracellular ice nucleator effects on freezing of foods ” , Journal of Food Science, 63(3), p.375~381, (1998). 30.Madura J.D.et al., “ the dynamics and binding of a Type III antifreeze protein in water and on ice ” , J.Molecular Structure (Theochem), 388, p.65-77, (1996). 31.Matsumoto J.J., “ Denaturation of fish Muscle Proteins During Frozen storage ” , American Chemical Society, 180, p.205-224, (1979). 32.Mitsuki M., Mizuno A., et al., “ Isolation and characterization of High Antifreeze Active Peptides in Enzymic Digest of Hemoglobin ” , cryobiology and cryotechnology, 42(2), p.102-107, (1996). 33.Nagan T., Iton Y., ” Characterization of a Generalized Transducing Phage of Poly- -Glutamic Acid—Producing Bacillus subtilis and Its Application for Analysis of Tn917-Ltv1 Insertional Mutants Defective in Poly- -Glutamic

Acid production ” , Applied and environmental microbiology , 63(10), p.4087-4089, (1997). 34.Ogawa, Y., Yamaguchi, F., “ Effect Production of -Polyglutamic Acid by Bacillus subtilis (natto) in Jar Fermenters ” , Biosci. Biotech. Biochem., 61(10), p.1684~1687, (1997). 35.Oppermann F.B., Pickartz S., Steinbiichel A., “ Biodegradation of polyamides ” , polymer degradation and stability, 59, p.337-344, (1998). 36. Parducci L.G., Duckworth R.B., “ Differential thermal analysis of frozen food systems ” , J.Fd .Technol., 7, p.423-430, (1972). 37.Payne, S.R., Sandford, D., Harris, A.,& Young, O.A., “ The effects of antifreeze proteins on chilled and frozen meat ” , Meat Science, 37, p.429~438, (1994). 38.Payne, S.R. and Young, O.A., “ Effects of Pre-slaughter Administration of Antifreeze Proteins on Frozen Meat Quality ” , Meat Sci., 41, p.147~155, (1995). 39ichard, A., Margaritis, A., “ Rheology , Oxygen Transfer , and Molecular Weight Characteristics of Poly(glutamic acid) Fermentation by Bacillus subtilis ” , Biotechnology and Bioengineering, 82(3), p.299~305, (2003). 40.Sato Y., Noguchi S, “ Evaluation of Bound water in Water-Souble Dietary Fivers by the Pulsed NMR and Isotherm Methods ” , J.Home Econ.Jpn., 44(3), p.185-189, (1993). 41.Seguro K., Tamiya T. et al., “ Cryoprotective Effect of Sodium Glutamate and Lysine-Hcl on Freeze Denaturation of Lactate Dehydrogenase ” , cryobiology, 27, p.70-79, (1990). 42. Shiio H., “ Ultrasonic Interferometer Measurements of the Amount of Bound Water. Saccharides ” , J.Am.Chem.Soc., 80, p.70-73, (1957). 43.Sikorski Z., Olley J. and Kostuch S., “ Protein changes in frozen fish ” , CRC Crit. Rev. Food Sci.Nutr., 8, p.97-129, (1976). 44.Suzuki E., Nagashima N., “ Freezing-Thawing Hysteresis Phenomena of Biological Systems by the New Method of Proton Magnetic Resonance ” , Bull.Chem.Soc.Jpn., 55, p.2730-2733, (1982). 45.Tanimoto H, Mori M, Motok M, Torii K, Kadokawa M, Noguchi T, “ Natto mucilage containing poly- -glutamic acid increase soluble calcium in the rat small intestine ” Biosci. Biotechnol. Biochem. 65, 516-521(2001). 46. Toledo R., Steinberg M.P.,Nelson A.I., “ Quantitative Determination of Bound Water By NMR ” , J.Food Science, 33, p.315-317, (1968). 47.Troy F.A., “ Chemistry and biosynthesis of the poly(-D-glutamyl) capsule in bacillus licheniformis ” , J.Biological Chemistry, 248(1), p.305-315, (1973). 48.Uraji T., Kohno H. et al., “ Freezing point depression of polyol-Aqueous solutions in the high concentration Range ” , Food Sci. Technol., Int., 2(1), p.38-42, (1996). 49.Wakamatu Y., Sato Y., “ Determination of Unfreezeable water in Sucrose, Sodium Chloride and Protein Solutions by Differential Scanning Calorimeter ” , Nippon Nogeikagaku Kaishi, 53(12), p.415-420, (1979). 50.Warren, G. and Wolber, P., “ Molecular aspects of microbial ice nucleation ” , Molecular Microbiology, 5, p.239~243, (1991). 51.Watanabe, M., Watanabe, J., Kumeno, K., Nakahama, N., & Arai, S., “ Freeze concentration of some foodstuffs using ice nucleation-active bacterial cells entrapped in calcium alginate gel ” , Journal of Biological Chemistry, 53(10), p.2731~2735, (1989). 52.Yamamoto K, Kumagai H, Suzuki A, Arai S, “ inhibitory activity of oligo-and poly-L-glutamic acids against calcium phosphate iusolubilization and calcium binding with special relevanu to their molecular weight dependence ” , Biosci.Biotechnol.Biochem. 58, 1662-1665(1994). 53.Young, H.K., “ Effects of Glucose and Glycerol on -Poly (glutamic acid) Formation by Bacillus licheniformis ATCC9945a ” , Biotechnology and Bioengineering, 57(4), p.430~437, (1998). 54.Yoon, S.H., “ Production of poly- -glutamic acid by fed-batch culture of Bacillus licheniformis ” , Biotechnology Letters, 22, p.585~588, (2000). 55.賀孝雍。儀器分析。曉園出版社。1991年。 56.黃錦城。冷凍食品之安全性。食品工業。32卷12期 , p.20~27。 57.劉廷英。冷凍食品之原理與加工。食品工業月刊社。 58.加藤舜郎。食品冷凍?理論??用。株式?社 光琳。1966年 59.朝倉邦造。最新冷凍食品事典。株式?社 朝倉書店。1987年 60.高橋雅弘。冷凍食品?知識。株式?社 幸書房。1982年 61.蔡祐輔。抗凍醣蛋白。Chemistry(the Chinese chem.soc., Taipei), 58(2), p.317-322. (2000). 62.范宜琮。以苔蘚桿菌生產聚麩胺酸之研究。碩士論文。2001年