

果膠分解? 高甲氧基果膠降解之酵素活性探討

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摘要

本研究利用果膠分解酶(Pectinase; pectolytic enzymes; PLEs)進行對高甲氧基果膠降解之酵素活性探討。實驗結果顯示,對高、低甲氧基果膠之降解而言,PLEs之最適反應溫度皆為60℃,最佳反應時間為前30分鐘,最適反應pH值則相對分別為5.0和4.0,PLEs酵素活性將隨著反應溫度及pH值增加而下降。對PLEs而言,其酵素最佳熱穩定溫度在30~40℃之間,並在室溫下試驗,其酵素最適穩定性之緩衝液pH值範圍在3.0~5.0之間,而且可維持大約原有的80~90%活性,PLEs在4℃下儲存一週後,其酵素活性雖略為下降,但無明顯的改變,可維持大約原有的80%活性;在30℃儲存前三天時,其酵素活性可。在不同溫度(4℃與30℃)下進行PLEs儲存安定性試驗,結果顯示維持大約原有的95%活性,但在第四天的酵素活性即明顯下降至原有的40%以下。對PLEs之酵素動力學而言,若以高甲氧基果膠為受質時,其酵素動力學常數Km及Vm值分別為0.16mg/mL及2.31mg/mL min,若以低甲氧基果膠為受質時,其Km及Vm值分別為1.53mg/mL及7.71mg/mL min。

關鍵詞:果膠分解酶、果膠、酵素活性、還原糖;動力學

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參考文獻

中文部分 1. 王姿惠。2001。益生菌於含果寡糖之培養環境中生長情形之探討:23-26。中興大學碩士論文。台中。 2. 李穎儒。1994。機能性寡糖應用於畜牧生產上。飼料營養雜誌12:89-92。 3. 周君蘭。1997。健康新主張—富力得健康活寡糖。食品資訊141:57-58。 4. 林瑩禎。1996。保健食品之簡介。食品工業28(11):31-36。 5. 林正彥。1976。果膠。食品工業8(4):18-22。 6. 林欣榜。1995。植物多醣體之應用—果膠。食品工業27(11):46-55。 7. 吳祝如。1994。果膠質及其在食品之應用。食品資訊1:32-34。 8. 香紅星、董仲華、劉亞力。2001。功能性寡糖的研究應用進展(上)。飼料研究7:9-11。 9. 翁志華。1996。果膠的基本特性與應用。食品資訊122:24-30。 10. 許清森。1991。利用酵素合成法由蔗糖生產低聚果糖之研究:20-25。台灣大學博士論文。台北。 11. 張珍田。2001。木寡糖之特性與生理功能。食品工業33(6):10-18。 12. 張振忠。1993。高壓下高甲氧基果膠之凝膠形成與品質特性:4-5。中興大學碩士論文。台中。 13. 郭文怡。1995。漫

談果膠類產品—果醬、果凍及果糕。烘焙科學60:13-16。14. 陳怡芳。2005。篩選分解蒴菌菌株與其發酵液之酵素活性探討:52。大葉大學碩士論文。彰化。15. 黃怡雯。2001。以纖維素水解?幾丁聚醣所得之水溶性產物對腸內細菌組成之影響:20。海洋大學碩士論文。基隆。16. 裘仲元。1996。高壓下高甲氧基果膠凝膠作用中機能性代糖使用的研究:24-25。中興大學碩士論文。台中。17. 羅淑卿、林木連。2001。認識果膠。農業試驗所技術服務45:30-33。18. 譚靜芬。1999。機能性寡糖之介紹。食品工業31(7):1-8。19. 譚靜芬。1992。利用酵素合成法由蔗糖生產巴拉金糖之研究:23。台灣大學碩士論文。台北。20. 川端晶子。1990。植物多醣體 構造 機能。食品科學。pp.111-122。朝倉書局。東京。日本。21. 草地道一。1984。食品工業 應用。食品工業11(2):44-55。22. 黃海三雄。1988。食品 應用 技術的諸問題。食品工業3(2):59-65。23. 渡邊浩、岩瀨弘士郎。1994。利用。食品 開發29(2):17-19。24. 機能性甘味料 市場動向。1997。食品 開發32(11):39-44。英文部分 1。

Acuna-Arguelles, M. E., Gutierrez-Rajas, M., Viniegra-Gonzalez, G. and Favela-Toress, E. 1995. Production and properties of three pectinolytic activities produced by *A. niger* in submerged and solid state fermentaion. *Appl. Microbiol. Biotechnol.* 43:808-814. 2. Alana, A., Alkorta, I., Dominguez, J. B., Llama, M. J. and Serra, J. L. 1990. Pectin lyase activity in a *Penicillium italicum* strain. *Appl. Environ. Microbiol.* 56(12): 3755-3759. 3. Al-Obaidi, Z. S., Aziz, G. M. and Al-Bakir, A. Y. 1987. Screening of fungal strains for polygalacturonase production. *J. Agric. Water Resour. Res.* 6:125-182. 4. Arcamone, F., Casinelli, G., Ferni, G., Penco, S., Pennella, P. and Pol, C. 1970. Ergotamine production and metabolism of *Claviceps purpurea* strain 275 FI in stirred fermenters. *Can. J. Microbiol.* 16:923-931. 5. Barfod, N. M. and Pedersen, K. S. 1990. Determining the setting temperature of high-methoxyl pectin gels. *Food Technol.* 44:139. 6. Bazzarre, T. L., Wu, S. L. and Yubes, J. A. 1983. Total and HDL- cholesterol concentration following yogurt and calcium supplementation. *Nutr. Rep. Int.* 28:1225-1232. 7. Bealing, F. J. and Bacon, J. S. D. 1953. The action of mold enzymes on sucrose. *Biochem. J.* 53:277-285. 8. Be Miller, J. N. 1986. An introduction to pectins: Structure and properties. In: Fishman, M. L., Jem, J. J. (Eds.), *Chemistry and Functions of Pectin*, ACS Symposium Series 310. American Chemical Society, Washington, DC. 9. Bernal, V. M., Smajda, J. L., Smith, J. L. and Stanley, D.W. 1987. Interactions in protein/polysaccharide/calcium gels. *J. Food Sci.* 52:112. 10. Bohdziewicz, J. and Bodzek, M. 1994. Ultrafiltration preparation of pectinolytic enzymes from citric acid fermentation broth. *Proc. Biochem.* 29:99-107. 11. Borin, M. D. F., Said, S. and Fonseca, M. J. V. 1996. Purification and biochemical characterization of an extracellular endo- polygalacturonase from *Penicillium frequentans*. *J. Agric. Food Chem.* 44:1616-1620. Brawman, J. W. 1981. Application of enzymes in fruit juice tech- nology. In: Brich, G. G., Blakcorough, N. and Barker, J. K. (Eds.), *Enzymes and food processing*. p. 247-261. Applied Science, London. 12. Bruhlmann, F., Kim, K. S., Zimmerman, W. and Fiechter, A. 1994. Pectinolytic enzymes from actinomycetes for the degumming of ramie bast fibers. *Appl. Environ. Microbiol.* 60(6):2107-2112. 13. Cao, J., Zheng, L. and Chen, S. 1992. Screening of pectinase producer from alkalophilic bacteria and study on its potential application in degumming of rammie. *Enz. Microbiol. Technol.* 14:1013-1016. 14. Carbonell, E., Costell, E. and Duran, L. 1991. Fruit content influence on gel strength of strawberry and peach jams. *J. Food Sci.* 56:1384. 15. Channe, P. S. and Shewal, J.G. 1995. Pectinase production by *Sclerotium rolfsii*: Effect of culture conditions. *Folia Microbiol.* 40:111-117. 16. Charley, V. L. S. 1969. Advances in food processing using pectic and other enzyme. *Chem. Ind.* 20:635-641. 17. Chesson, A. and Codner, R. C. 1978. Maceration of vegetable by a strain of *Bacillus subtilis*. *J. Appl. Bacteriol.* 44:347-364. 18. Christensen, S. H. 1986. Pectins. In Glicksman, M. (ed.), *food Hydrocolloids*, Vol. III. pp.205-212. CRC Press, Inc., Florida. 19. Darvill, A., Augur, C., Bergmann, C., Carlson, R. W., Cheong, J. J., Eberhard, S., Hahn, M. G., L and oacute, V. M., Marf and agrave, V., Meyer, B., Mohnen, D., O ' Neill, M. A., Spiro, M. D., van Halbeek, H., York, W. S. and Albersheim, P. 1992. Oligosaccharins- oligo- saccharides that regulate growth, development and defence responses in plants. *Glycobiology* 2:181-198. 20. Dave, B. A. and Vaughn, R. H. 1971. Purification and properties of a polygalacturonic acid trans-eliminase produced by *Bacillus pumilus*. *J. Bacteriol.* 108:166-174. 21. Deuel, H. and Stutz, E. 1958. Pectic substances and pectic enzyme. *Advances in Enzymology* 20:341. 22. Dickerson, A. G. 1972. -D-fructofuranosidase from *Claviceps purpurea*. *Biochem. J.* 129:263-272. 23. Duan, K. J., Chen, J. S. and Sheu, D. C. 1994. Kinetic studies and mathematical model for enzymatic production of fructooligo- saccharides from sucrose. *Enzyme Microb. Technol.* 16:334-339. 25. Dumville, J. C. and Fry, S. C. 2000. Uronic acid-containing oligo- saccharins: Their biosynthesis, degradation and signaling roles in non-diseased plant tissues. *Plant Physiology and Biochemistry.* 38(12):125-140. 26. Fogarty, M. V. and Kelly, C. T. 1983. Pectic enzymes. In: Fogarty, M. W. (Ed.), *Microbial Enzymes and Biotechnology*. p.131-182. Applied Science Publishers, London. 27. Fogarty, W. M. 1973. Bacteria, enzymes and wood permeability. *Proc. Biochem.* 8:30-34. 28. Fujita, K., Hara, K., Hashimoto, H. and Kitahara, S. 1990. Purification and some properties of -fructofuranosidase from *Arthrobacter* sp. K-1. *Agric. Biol. Chem.* 54:913-919. 29. Glicksman, M. 1969. Pectins. In Glicksman, M. (ed.), *Gum Technology in the Food Industry*. p. 159-190. Academic Press, New York. 30. Gummadi, S. N. 2003. Purification and biochemical properties of microbial pectinases – a review. *Process biochemistry.* 38:987-996. 31. Gupta, A. K. and Bhatia, I. S. 1982. Glucofructosan biosynthesis in *Fusarium oxysporum* regulation and substrate specificity of fructosyl transferase and invertase. *Phytochemistry* 21:1249-1253. 32. Hankin, L. and Meintype, J. L. 1977. Production of kestose(fructosyl sucroses) by *phytophthora parasitica* var. *nicotianae*. *Appl. Environ. Microbiol.* 33:522-524. 33. Hata, Y., Hara, T., Oikawa, T., Yamamoto, M., Hirose, N., Nagashima, T., Torihama, N., Nakajima, K., Watabe, A., and Yamashita, M. 1983. The effects of fructooligosaccharides against hyperlipidemia. *Geriatr. Med.* 21:156-167. 34. Hayashi, S., Imada, K., Kushima, Y. and Ueno, H. 1989. Observation of the chemical structure of fructooligosaccharide produced by an enzyme from *Aureobasidium* sp. ATCC 20524. *Curr. Microbiol.* 19:175-177. 35. Hayashi, S., Nonokuchi, M., Imada, K. and Ueno, H. 1990. Production of fructosyl- transferring enzyme by *Aureobasidium* sp. ATCC 20524. *J. Ind. Microbiol.* 5:395-400. 36. Hayashi, S., Ito, K., Nonoguchi, M., Takasaki, Y. and Imada, K. 1991. Immobilization of a fructosyl- transferring enzyme from *Aureobasidium pullulans* sp. on Shirasu porous glass. *J. Ferment. Bioeng.* 72:68-70. 37. Hershonhorn, J., Manulis, S. and Barash, I. 1990. Poly- galacturonase associated with infection of

Valencia orange by *Penicillium italicum*. *Phytopathology* 80:1374-1376. 38. Hidaka, H. 1985. The role of intestinal flora in nutrition. *Biseibutsu(Microbe)*1:32-40. 39. Hidaka, H., Hirayama, M. and Sumi, N. 1988. A fructooligo- saccharide producing enzyme from *Aspergillus niger* ATCC 20611. *Agric. Biol. Chem.* 52:1181-1187. 40. Hidaka, H., Eida, T. and Saitoh, Y. 1989. Industrial production of fructooligosaccharides and its application for human and animals. *Nippon Nogeikagaku Kaishi.* 61:915-923. 41. Hirota, T. 1990. The health maintenance effects of lactic acid bacteria being used in milk products. *New Food Ind.* 32(10):9-12. 42. Horikoshi, K. 1990. Enzymes of alkalophiles. In: Fogarty, W. M., Kelly, C. T. (Eds.), *Microbial Enzymes and Biotechnology*, second ed. p. 275-294. Elsevier Applied Science, London. 43. Hughes, J. B. and Hoover, D. G. 1991. Bifidobacteria: Their potential for use in American dairy products. *Food Technol.* 45:74-83. 44. Ikeda, T., Kurita, T., Hidaka, H., Michalek, S. M. and Hirasawa, M. 1990. Low-cariogenicity of the tetrasaccharide nystose. *Gen. Pharmacol.* 21:9-175. 45. Ishii, S. and Yokotshuka, T. 1971. Maceration of plant tissues by pectin transeliminase. *Agricultural and Biological Chemistry* 35:1157-1162. 46. Joseph, G. H. and Baier, W. E. 1949. Methods of determining the firmness and setting time of pectin test jellies, *Food Technology* 3:18-21. 47. Joslyn, N. A., Mist, S. and Lambart, E. 1952. Clarification of apple juice by fungal PG preparations. *Food Technol.* 6:133-139. 48. Jung, K. H., Lim, J. Y., Yoo, S. J., Lee, J. H. and Yoo, M. Y. 1987. Production of fructosyltransferase from *Aureobasidium pullulans*. *Biotechnol. Lett.* 9:703-708. 49. Jung, K. H., Yun, J. W., Kang, K. R., Lim, J. Y. and Lee, J. H. 1989. Mathematical model for enzymatic production of fructooligo- saccharides from sucrose. *Enzyme Microb. Technol.* 11:491-494. 50. Kanbe, M. 1992. Chp.17: Cancer control and fermented milk. p. 377. Nakazawa and Hosono. 51. Karbassi, A. and Vaughn, R. H. 1980. Purification and properties of polygalacturonic acid trans-eliminase from *Bacillus stearothermophilus*. *Can. J. Microbiol.* 26:377-384. 52. Kashyap, D. R., Chandra, S., Kaul, A. and Tewari, R. 2000. Production purification and characterization of pectinase from a *Bacillus* sp. DT7. *World J. Microbiol. Biotechnol.* 16:277-282. 53. Kashyap, D. R., Vohra, P. K., Chopra, S. and Tewari, R. 2001. Applications of pectinases in the commercial sector: a review. *Bioresour Technol.* 77:216-285. 54. Kawabata, A., Sawayama, S., Nakahara, H. and Kamata, T. 1981. Mechanism of association of various demethylated pectins by calcium ions. *Agricultural and Biological Chemistry* 45(4):965-974. 55. Keijbets, M. J. H. and Pilnik, W. 1974. -elimination of pectin in the presence of anions and cations. *Carbohydrate Research* 33:359-365. 56. Kida, M., Yoshikawa, T., Senda, T. and Yoshihiro, Y. 1988. Formation of fructooligosaccharides from sucrose catalyzed by immobilized -fructofuranosidase originated from *Aspergillus oryzae*. *Nippon Kagaku Kashi.* 11:1830-1835. 57. Kilara, A. 1982. Enzymes and their uses in the processed apple industry: A Review. *Proc. Biochem.* 23:35-41. 58. Kramer, K. J. and Muthukrishnan, S. 1997. Insect chitinases: Molecular biology and potential use as biopesticides. *Insect Biochemistry and Molecular Biology.* 27(11):887-900. 59. Lang, C. and Dornenburg, H. 2000. Perspectives in the biological function and the technological application of polygalacturonases. *Appl. Microbiol. Biotechnol.* 53:366-375. 60. Lopez, A. and Li, L. H. 1968. Low-methoxyl pectin apple gels. *J. Food Technol.* 22:91. 61. Magro, P., Varvaro, L., Chilosi, G., Avanzo, C. and Balestra, G. M. 1994. Pectinolytic enzymes produced by *Pseudomonas syringae* pv. *Glycinea*. *FEMS Microbiol. Lett.* 117:1-6. 62. Marcus, L., Barash, I., Sneh, B., Koltin, Y. and Finker, A. 1986. Purification and characterization of pectolytic enzymes produced by virulent and hypovirulent isolates of *Rhizoctonia solani* KUHN. *Physiol. Mol. Plant Pathol.* 29:325-336. 63. Maruyama, Y. and Onodera, K. 1979. Production and some properties of invertase isozymes of *Fusarium oxysporum*. *J. Gen. Appl. Microbiol.* 25:361-366. 64. Masai, T., Wada, K., Hayakawa, K., Yoshihara, L. and Mitsuoka, T. 1987. Effects of soybean oligosaccharides on human intestinal flora and metabolic activities. *Japan. J. Bacteriol.* 42(1):313. 65. McCready, R. M., Owen, H. S. and Maclay, W. D. 1944. Alkali hydrolyzed pectins are potential industrial products. *Food Ind.* 16:69. 66. Mckellar, R. C. and Modler, H. W. 1996. Metabolism of fructo- oligosaccharides by bifidobacterium spp. *Appl. Microbiol. Biotechnol.* 31:537-541. 67. Muhammad, E. and Hiroshi, T. 1996. Lineweaver-burk analysis for the blocking effects of mammalian dopamine receptor antagonists on dopamine-induced currents in achatina giant neurones. *Gen. Pharmac.* 27(7):1209-1213. 68. Muramatsu, M., Kainuma, S., Miwa, T. and Nakakuki, T. 1988. Structures of some fructooligosaccharides produced from sucrose by mycelia of *Aspergillus sydowi* IAM 2544. *Agric. Biol. Chem.* 52:1303-1304. 69. Mutai, M. 1978. Characteristics of " Mil-Mil ", a Bifidobacterium fermented milk. *New Food Ind.* 20(8):17. 70. Muting, D., Escherich, D. and Mayer, J. P. 1968. The effect of *Bifidobacterium bifidum* on intestinal bacterial flora and toxic protein metabolites in chronic liver diseases. *Am. J. Protol.* 19:336-342. 71. Nagel, C. W. and Vaughn, R. H. 1961. The characterisitic of a polygalacturonase produced by *Bacillus polymyxa*. *Arc. Biochem. Biophys.* 93:344-352. 72. Naidu, G. S. N. and Panda, T. 1998. Production of pectolytic enzymes: a review. *Bioproc. Eng.* 19:355-361. 73. Nasumo, S. and Starr, M. P. 1967. Polygalacturonic acid trans- eliminase of *Xanthomonas compestris*. *Biochem. J.* 104:178-184. 74. Ohtsuka, K., Tsuji, K. Y., Nakagawa, H., Ueda, O., Ozawa, T. and Ichikawa, T. 1990. Availability of 4 ' -galactosyllatose (O-beta-D-galactopyranosyl-(1-4)-D-glucopyranose) in rats. *Journal of nutritional science and vitaminology.* 36:265-276. 75. Oku, T. and Nakamura, S. 2002. Digestion, absorption, fermentation, and metabolism of functional sugar substitutes and their available energy. *Pure Appl. Chem.* 74:1253-1261. 76. Olsen, A. G. 1993. Pectin studies III: General theory of pectin jelly formation. *J. Physical Chem.* 38:919. 77. Padival, R. A., Ranganna, S. and Manjrekar, S. P. 1979. Mechanism of gel formation by low methoxyl pectins. *J. Food Technol.* 14:277. 78. Patel, V., Saunders, G. and Bucke, C. 1994. Production of fructooligosaccharides by *Fusarium oxysporum*. *Biotechnol. Lett.* 11:1139-1144. 79. Pazur, J. H. 1952. Transfructosidation reactions of an enzyme of *Aspergillus oryzae*. *J. Biol. Chem.* 199:217-225. 80. Perez, S. M., Rodriguez-Carvajal, A. and Doco, T. 2003. A complex plant cell wall polysaccharide: rhamnogalacturonan II. A structure in quest of a function. *Biochimie.* 85:109-121. 81. Rambouts, F. M. and Pilnik, W. 1980. Pectic enzyme. In: Rose, A. H. (ed), *Economic microbial enzymes and bioconversions.* p. 227-282. Academic Press, New York. 82. Reid, I. and Ricard, M. 2000. Pectinase in papermaking solving retention problems in mechanical pulps bleached with hydrogen peroxide. *Enzyme Microbiol. Technol.* 26:115-123. 83. Rijssel, M. W., Gerwig, J. G. J. and Hausen, T. A. 1993. Isolation and characterization of an extracellular glycosylated protein complex from *Clostridium thermosaccharolyticum* with pectin methylesterase and

polygalacturonate hydrolase activity. *Appl. Environ. Microbiol.* 59(3):828-836. 84. Rolin, C. and Vries, J. D. 1990. Pectin. In Harris, P. (Ed), *Food Gels*. p. 422-423. Elsevier Science Publishing Co, Inc., New York. 85. Sakai, T., Sakamoto, T., Hallaert, J. and vandamme, E.I. 1993. Pectin, pectinase and protopectinase: production, properties and application. *Adv. Appl. Microbiol.* 39:213-294. 86. Smith, J. M., Grove, D., Luenser, S. J. and Park, L. 1980. Process for the production of fructose transferase enzyme. U.S. patent 4:309-505. 87. Straathof, A. J. J., Kieboom, A. P. G. and van Bekkum, H. 1986. Invertase-catalysed fructosyl transfer in concentrated solutions of sucrose. *Carbohydr. Res.* 146:154-159. 88. Takabe, H., Otsuki, Y. and Aoki, S. 1968. Isolation of tobacco mesophyll cells in intact and active state. *Plant Cell Physiol.* 9:115. 89. Thakur, B. R., Singh, R. K. and Handa, A. K. 1997. Chemistry and uses of pectins-a review. *Critical Reviews in Food Science and Nutrition* 37(1):47. 90. Takasoye, M., Inoue, N. and Knuma, C. 1990. Clinical investi- gation of feces improvements by soybean oligosaccharides. *Rinsho tohkenkyu(Clinics and Research)*. 67:304-310. 91. Towel, G. A. and Christensen, O. 1973. Pectin. In Whistler, R. L. and Be Miller, J. N. (eds), *Industrial Gums*. p. 429-461. Academic Press, New York. 92. Usami, S., Ishii, T., Kirimura, K., Uehara, K. and Chen, J. 1991. Production of -fructofuranosidase showing fructose-transferring activity by penicillium frequentans(P. glabrum). *J. Ferment. Bioeng.* 72:303-305. 93. Vaccari, G., Lodi, G., Tamburini, E., Dosi, E. and Mantovani, G. 2000. Planar chromatography applied to the analysis of oligo- saccharides in sugar product. *Association Andrew Van Hook 7th Symposium*: p. 28-38. 94. Van Buren, J. P. 1979. The chemistry of texture in fruits and vegetables. *Journal of Texture Studies* 10:1. 95. Wada, K., Watabe, J., Mizutani, J., Suzuki, H., Kiri, N., Hayakama, K. and Yamaguchi, C. 1991. Effect of soybean oligosaccharides in- take on fecal microflora, enzyme activity, ammonia and frequency of evacuation in elderly person. *Bifidus*. 4:135-140. 96. Ward, O. P. and Young, M. M. 1989. Enzymatic degradation of cell wall and related plant polysaccharides. *Crit. Rev. Biotechnol.* 8:237-274. 97. Watanuki, M., Wada, Y. and K. Matsumoto. 1996. Digestibility and physiological heat of combustions of 1-4 and 1-6 galacto- oligosaccharides on vitro. *Annual Reports of the Yakult Central Institute for Microbiological Research*. 16:1-12. 98. Yamashita, K., Kawai, K. and Itakura, M. 1984. Effects of fructooligosaccharides on blood glucose and serum lipids in diabetic subjects. *Nutrition Research* 4:961-965. 99. Yun, J. W. 1996. Fructooligosaccharide-Occurrence,preparation, and application. *Enzyme and Microbial. Technol.* 19:107-117. 100. Yun, J. W., Jung, K. H., Oh, J. W. and Lee, J. H. 1990. Semibatch production of fructo-oligosaccharides from sucrose by immobilized cells of *Aureobasidium pullulans*. *Appl. Biochem. Biotechnol.* 24(25):299-308. 101. Yun, J. W., Jung, K. H., Jeon, Y. J. and Lee, J. H. 1992. Continuous production of fructo-oligosaccharides from sucrose by immobilized cells of *Aureobasidium pullulans*. *J. Microbiol. Biotechnol.* 2:98-101. 102. Yun, J. W., Jeon, Y. J., Lee, M. G. and Song, S. K. 1993. Production of fructo-oligosaccharides by the fructosyltransferase immobilized onto a ion-exchange resin. *Korean J. Biotechnol. Bioeng.* 8:307-312.