開發快速抓取啤酒中乳酸菌之免疫磁珠分離法 = Development of immunoseparation method for the rapid capture of LAB in beers

葉哲宏、李世傑 李泰林 曾浩洋

E-mail: 9806539@mail.dyu.edu.tw

摘 要

近年來已陸續發展出許多種鑑定乳酸菌的方法，如利用生化、培養形態等方法，然而傳統生化學原理所發展出來之乳酸菌檢驗方法。以往在食品及生物產業用來檢測其中所含微生物的方法，大都以傳統方法鑑定，但此種方法在檢測時必需先花費相當時間，以LAB言，可能需要一星期去培養所要檢測的微生物。其中光花在培養微生物的時間上，就已經佔去了大半的實驗時間。 在已知之分子檢測法中，對於大量菌株具有同時檢測之能力者，則以生物晶片為首選。本碩士論文的工作，即利用晶宇生技 公司的生物晶片，去檢測啤酒中的造成品質敗壞的乳酸菌，包括Lactobacillus中的brevis、case、plantarum 以及Pediococcus spp.etc. 且為建立線上監控系統 ，我們採用免疫磁珠方法吸附濃縮 這些菌株，進而以生物晶片檢測之。我們先以兔子免疫，生產上述 乳酸菌的抗體。這些抗體純化後，再與磁珠(magnetic beads) 結合，形成免疫磁珠(Immunomagnetic bead)；我們再使用這些免疫磁珠，運用於這些啤酒中特定LAB的吸附、濃縮。進而將吸附的 LAB 以生物晶片檢測，如此，完成啤酒晶片生產線上監督之監督系統。

關鍵詞 : 免疫磁珠、乳酸菌、啤酒晶片

目錄

參考文獻

1.許勝傑。2009。博士論文。中興大學大學食品營養科技學系。 2.李承隆。2008。碩士論文。弘光科技大學食品營養科技學系。 2.楊韻潔。2008。碩士論文。弘光科技大學食品營養科技學系。 3.劉輝、朱正美。2002。簡明免疫學。科學出版社。 4.廖啟成。1998。乳酸菌之分類及應用。食品工業月刊.30:1-9. 5.宋華聰、林茂勇。1995。禽病檢查手冊。藝軒圖書出版社。 6. Kye, M. C., Renukaradhya, K. M., Shah-Md, A. I., Woo, J. L., Su, Y. H., Jong, M. K., Myoung, G. Y., Ji-Joong Cho and Han-Dae Yun. 2009. Novel multiplex PCR for the detection of lactic acid bacteria during kimchi fermentation. Molecular and Cellular Probes. 7. Chiang, Y. C., W. Y. Pai, C.Y. Chen, P.C. Lee, and H.Y. Tsen. 2008. PCR detection of Mastitis pathogens,Streptococcus agalactiae, Streptococcus uberis, Streptococcus bovis and Staphylococcus aureus in milk samples using new primers designed from the hsp70, hsp40, hsp10, and htr gene sequences. Molecular & Cellular Probes. (SCI) (Accepted, in press) 8. Chiang, Y. C., Chih, M. F., Wan. W. L., Chieu-Ku Lin and Hau-Yang Tsen. 2007. Real-time PCR detection of Staphylococcus aureus in milk and meat using new primers designed from the heat shock protein gene htrA sequence.J. Food Protection. 70(12):2855-9. (SCI) 9. Coleman, D. J., Chick, K. E. and Nye, K. J. 1995. An evaluation of immunomagnetic separation of the detection of Salmonellas in raw chicken carcasses. Lett. Appl. Microbiol. 21:152-154. 10. Cudjoe, K. S. and Krona, R. 1997. Detection of Salmonella from raw food samples using Dynabeads anti-Salmonella and a conventional reference method. Int. J. Food Microbiol. .37:55-62. 11. Cudjoe, K. S., Hagtvedt, T., and Dainty, R. 1995. Immunomagnetic separation of Salmonella from foods and their detection using immunomagnetic particle (IMP)-ELISA. Int. J. Food Microbiol. 27:11-25. 12. Cudjoe, K. S., Krona, R. and Olsen, E. 1994. IMS: a new selective enrichment technique for detection of Salmonella in foods. Int. J. Food Microbiol. 23:159-165. 13. Dziadkowiec, D., Mansfield, L.P. and Forsythe, S. J. 1995. The detection of Salmonella in skimmed milk powder enrichments using conventional methods and immunomagnetic separation. Lett. Appl. Microbiol. 20:361-364. 14. Edwards, M. C., and Gibbs, R. A.1995. Multiplex PCR, p.157-171. In C. W. Dieffenbach and Dveksler, G. S.(ed.), PCR primer a laboratory manual, CSHL Press, Plainview, New York. 15. Fleet, G.H., Lafon-Laforcade, S. and Ribereau-Gayon P. 1984.Evolution of Yeasts and Lactic Acid Bacteria During Fermentation and Storage of Bordeaux Wines. Appl. Environ. Microbiol. 48, 1034-1038. 16. Herrera-Leon, S, Ramiro, R, Arroyo, M, Diez R, Usera MA, Echeita MA.（2007）Blind comparison of traditional serotyping with three multiplex PCRs for the identification of Salmonella serotypes. Res.Microbiol. 158(2):122-7. 17. Hohmann, E.L. 2001. Nontyphoidal salmonellosis. Clin. Infect. Dis. 15;32(2): 263-9. 18. Johnson, T.R., Case, C.L.1995. Laboratory experiments in Microbiology 4th edition. The Benjamin / Cummings Publoshing Co.Inc. 19. Kardos, G., Farkas, T., Antal, M., Nogrady, N., Kiss, I. 2007. Novel PCR assay for identification of Salmonella enterica serovar Infantis. Lett Appl Microbiol. 45(4):421-5. - 67 - 20. LABAN P., FAVRE C., RAMET F. and LARPENT J.P. 1978. Lactobacilli isolated from French saucisson (Taxonomic Study). Zbl. Bakt. Parasitenk. 1 ATB-B 166, 105-111. 21. March, C, Manclus, JJ, Abad, A, Navarro, A, and Montoya, A. 2005. Rapid detection and counting of viable beer-spoilage lactic acid bacteria using a monoclonal chemiluminescence enzyme immunoassay and a CCD camera. J Immunol Methods. 303(1-2):92-104. 22. Mauch, H., Kumel, G., Hammer, H.J. 1980. Large-scale purification of IgM from human sera. Comparison of three optimized procedures utilizing protein A chromatography. Res Exp Med (Berl).177(1):33-41. 23. Popoff, M.Y., Bockemuhl, J., Gheesling, L.L. 2004. Supplement 2002 (no. 46) to the Kauffmann-White scheme. Res Microbiol. 155(7):568-70. 24 . Purcifull, D. E., and D. L. Bstchelor. 1977. Immunodiffusion Test with Sodium Dodecyl Sulfate (SDS)-treated Plant Viruses and Plant Viral Inclusions. Florida Agric. Expt. Sta. Bull. No. 78, 39 pp. 25. Rijpens, N., Herman, L., Vereecken, F., Jannes, G., Smedt, J. D. and Zutter, L. D. 1999. Rapid detection of stressed Salmonella spp. in dairy and egg products using immunomagnetic separation and PCR. Int. J.Food Microbiol. 46:37-44. 26. Salehi, T.Z., Tadjbakhsh H, Atashparvar N, Nadalian MG, Mahzounieh MR. 2007. Detection and identification of Salmonella Typhimurium in bovine diarrhoeic fecal samples by immunomagnetic separation and multiplex PCR assay. Zoonoses Public Health. 54(6-7):231-6. 27. Shaw, S. J., Blais, B. W. and Nundy, D. C. 1998. Performance of the Dynabeads anti-Salmonella system in the detection of Salmonella species in foods, animal feeds, and environmental samples. J. Food Prot.61:1507-1510. 28. Skjerve, E. and Olsvik, O. 1991. Immunomagnetic separation of Salmonella from foods. Int. J. Food Microbiol. 14:11-17. 29. Wattiau, P., Weijers, T., Andreoli, P., Schliker, C., Veken, H.V., Maas, H.M., Verbruggen, A.J., Heck, M.E., 2008. Evaluation of the Premi Test Salmonella, a commercial low-density DNA microarray system intended for routine identification and typing of Salmonella enterica. 23(3):293-8. 30. Wannet, W.J., Imberechts, H., and Vos, P. 2008. Evaluation of the Premi (R) Test Salmonella, a commercial low-density DNA microarray system intended for routine identification and typing of Salmonella enterica. Int J Food Microbiol. 123(3):293-8. 31. Yang, H, Qu L, Wimbrow AN, Jiang X and Sun Y. 2007. Rapid detection of Listeria monocytogenes by nanoparticle-based immunomagnetic separation and real-time PCR. 15;118(2):132-8 32. Pfannebecker, Jens. and Frohlich, Jurgen.。 2008。International Journal of Food Microbiology 。 33. Neil A. Campbell。1999。Biology。