

# 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

## ABSTRACT

In recent years, development of the molecular diagnostic methods for bacteria identification has been noteworthy. Conventional methods for detection of LAB are laborious and time consuming and may require 1 week. Of the molecular detection methods, when many LAB species are subjected to identification, the biochip method could be used. To establish the online monitoring system for the detection of the LAB cells in beers during brewing, in my thesis work, the immunomagnetic beads method specific for LAB species in beers will be developed for the collection and concentration of lactic acid bacteria cells including *Lactobacillus brevis*, *L. casei*, *L. plantarum* and *Pediococcus damnosus*. These four lactic acid bacteria may spoil the beer and should be detected online during the brewing processes. The antibody to these LAB species was obtained from immunized rabbit and then purified. The purified antibody was linked to magnetic bead. After obtaining the immunomagnetic bead, the LAB cells in beer were captured and detected using the biochip from Dr. Chip Company. By this way, the online monitoring system for detection of LAB during the beer brewing process could be established.

Keywords : immunomagnetic separation(IMS) , *Lactobacillus* spp, Beer chip

## Table of Contents

|            |     |              |    |                       |   |                  |    |                   |     |                   |    |                   |     |                     |   |                     |   |                         |   |                             |   |   |   |                                  |   |   |    |                 |    |                   |    |                   |    |                      |    |                      |    |                  |    |               |    |             |    |               |    |               |    |               |    |                 |    |               |    |             |    |                   |    |                   |    |                   |    |                 |    |                  |    |                 |    |                   |    |                    |    |                    |    |                          |    |                             |    |                   |    |                   |    |  |    |                     |    |                                   |    |                         |    |  |    |                                     |    |                       |    |                       |    |                                   |    |                             |    |                    |    |                    |    |                      |    |            |    |                   |    |                              |    |                                      |    |  |    |   |    |   |    |                                  |    |                                 |    |            |  |
|------------|-----|--------------|----|-----------------------|---|------------------|----|-------------------|-----|-------------------|----|-------------------|-----|---------------------|---|---------------------|---|-------------------------|---|-----------------------------|---|---|---|----------------------------------|---|---|----|-----------------|----|-------------------|----|-------------------|----|----------------------|----|----------------------|----|------------------|----|---------------|----|-------------|----|---------------|----|---------------|----|---------------|----|-----------------|----|---------------|----|-------------|----|-------------------|----|-------------------|----|-------------------|----|-----------------|----|------------------|----|-----------------|----|-------------------|----|--------------------|----|--------------------|----|--------------------------|----|-----------------------------|----|-------------------|----|-------------------|----|--|----|---------------------|----|-----------------------------------|----|-------------------------|----|--|----|-------------------------------------|----|-----------------------|----|-----------------------|----|-----------------------------------|----|-----------------------------|----|--------------------|----|--------------------|----|----------------------|----|------------|----|-------------------|----|------------------------------|----|--------------------------------------|----|--|----|---|----|---|----|----------------------------------|----|---------------------------------|----|------------|--|
| 授權書.....   | iii | 中文摘要.....    | iv | 英文摘要.....             | v | 誌謝.....          | vi | 目錄.....           | vii | 圖目錄.....          | xi | 表目錄.....          | xiv |                     |   |                     |   |                         |   |                             |   |   |   |                                  |   |   |    |                 |    |                   |    |                   |    |                      |    |                      |    |                  |    |               |    |             |    |               |    |               |    |               |    |                 |    |               |    |             |    |                   |    |                   |    |                   |    |                 |    |                  |    |                 |    |                   |    |                    |    |                    |    |                          |    |                             |    |                   |    |                   |    |  |    |                     |    |                                   |    |                         |    |  |    |                                     |    |                       |    |                       |    |                                   |    |                             |    |                    |    |                    |    |                      |    |            |    |                   |    |                              |    |                                      |    |  |    |   |    |   |    |                                  |    |                                 |    |            |  |
| 1. 前言..... | 1   | 2. 文獻整理..... | 2  | 2.1 啤酒釀造過程乳酸菌的影響..... | 2 | 2.2 乳酸菌菌種簡介..... | 4  | 2.2.1 乳酸菌的定義..... | 4   | 2.2.2 乳酸菌的分類..... | 5  | 2.3 鑑定乳酸菌的方法..... | 6   | 2.3.1 傳統生理生化鑑定..... | 7 | 2.3.1.1 革蘭氏染色法..... | 7 | 2.3.1.2 Catalase試驗..... | 7 | 2.3.1.3 API 50CHL 菌種鑑定..... | 7 | 2.3.2 聚合-連鎖反應(polymerase chain reaction ; PCR)..... | 8 | 2.3.3 生物晶片法(Biochip method)..... | 9 | 2.3.4 免疫磁珠分離法(immunomagnetic separation ; IMS)..... | 10 | 2.4 抗體免疫原理..... | 11 | 2.4.1 何謂免疫系統..... | 11 | 2.4.1.1 基本特性..... | 11 | 2.4.1.2 抗體抗原的結構..... | 12 | 2.4.1.3 抗體的運作方式..... | 12 | 2.4.2 抗體的種類..... | 13 | 3. 材料與方法..... | 15 | 3.1 材料..... | 15 | 3.1.1 菌種..... | 15 | 3.1.2 動物..... | 15 | 3.1.3 藥品..... | 15 | 3.1.4 試驗材料..... | 17 | 3.1.5 儀器..... | 17 | 3.2 方法..... | 18 | 3.2.1 菌株收集培養..... | 19 | 3.2.2 抗原乳劑處理..... | 19 | 3.2.2.1 抗原處理..... | 19 | 3.2.2.2 免疫..... | 20 | 3.2.3 抗血清製備..... | 20 | 3.2.3.1 採血..... | 20 | 3.2.3.2 血清製備..... | 20 | 3.2.4 抗血清力價測試..... | 21 | 3.2.5 免疫球蛋白純化..... | 22 | 3.2.6 IgG抗體蛋白片段大小鑑定..... | 22 | 3.2.6.1 SDS-PAGE電泳膠片配製..... | 22 | 3.2.6.2 電泳步驟..... | 23 | 3.2.6.3 膠體染色..... | 24 | 3.2.7 利用FPLC(Fast Protein Liquid Chromatography) 製備抗體蛋白..... | 25 | 3.2.7.1 蛋白精製純化..... | 25 | 3.2.7.2 AKTA purifier儀器清洗、保養..... | 25 | 3.2.8 免疫磁珠表面抗體接合反應..... | 26 | 3.2.8.1 國產免疫磁珠(Tanbead U-118、U-128)..... | 26 | 3.2.8.2 進口免疫磁珠(Dynabead M-280)..... | 26 | 3.2.9 免疫磁珠表面菌體分離..... | 27 | 3.2.9.1 標準菌液磁珠吸附..... | 27 | 3.2.9.2 模擬工廠末端製備完成啤酒液微量菌磁珠吸附..... | 27 | 3.2.10 免疫磁珠表面菌體分離液研究分析..... | 28 | 3.2.10.1 菌量測定..... | 28 | 3.2.10.2 菌種測定..... | 28 | 3.2.11 免疫磁珠清洗保存..... | 28 | 4. 結果..... | 30 | 4.1 乳酸菌的培養條件..... | 30 | 4.2 利用紐西蘭長耳兔所製備抗體力價測試結果..... | 30 | 4.3 利用AKTA purifier純化抗體蛋白質IgG結果..... | 31 | 4.4 個別設計四種乳酸菌的特異性引子，並利用標準乳酸菌株，以測試引子的靈敏度與專一性..... | 32 | 4.5 免疫磁珠與標準菌液(108 cfu/ml)作用後，利用PCR 引子擴增法測試結果..... | 32 | 4.6 免疫磁珠與標準菌液(108 cfu/ml)作用後，利用生化 塗菌方法分析結果..... | 33 | 4.7 利用市售生啤酒，模擬工廠端之乳酸菌微量測試結果..... | 33 | 4.8 直接利用兔子的抗血清與免疫磁珠結合與測試結果..... | 34 | 4.9 測試免疫磁珠 |  |

|                          |    |            |    |                |    |
|--------------------------|----|------------|----|----------------|----|
| 可否重複使用，並使用於檢測同種乳酸菌株..... | 34 | 5. 討論..... | 35 | 6. 結<br>論..... | 37 |
|                          |    | 參考文獻.....  |    |                | 66 |

## REFERENCES

- 許勝傑。2009。博士論文。中興大學大學食品營養科技學系。
- 李承隆。2008。碩士論文。弘光科技大學食品營養科技學系。
- 楊韻潔。2008。碩士論文。弘光科技大學食品營養科技學系。
- 劉輝、朱正美。2002。簡明免疫學。科學出版社。
- 廖啟成。1998。乳酸菌之分類及應用。食品工業月刊.30:1-9.
- 宋華聰、林茂勇。1995。禽病檢查手冊。藝軒圖書出版社。
- 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*.
- 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)
- 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)
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- Hohmann, E.L. 2001. Nontyphoidal salmonellosis. *Clin. Infect. Dis*. 15;32(2): 263-9.
- Johnson, T.R., Case, C.L. 1995. Laboratory experiments in Microbiology 4th edition. The Benjamin / Cummings Publishing Co. Inc.
- 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 -
- 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.
- 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.
- 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.
- Popoff, M.Y., Bockemuhl, J., Gheesling, L.L. 2004. Supplement 2002 (no. 46) to the Kauffmann-White scheme. *Res Microbiol*. 155(7):568-70.
- 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.
- 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.
- 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.
- 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.
- Skjerve, E. and Olsvik, O. 1991. Immunomagnetic separation of *Salmonella* from foods. *Int. J. Food Microbiol*. 14:11-17.
- 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.
- 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*.