

# Studies on the Preservation of Starter Activity of *Lactobacillus acidophilus*

王彩娟、陳鴻章

E-mail: 9318618@mail.dyu.edu.tw

## ABSTRACT

Cell suspensions of *Lactobacillus acidophilus* prepared by the addition of 5~15% of gelatin, gum arabic or lecithin were frozen at -20, -40 or -80 °C, then freeze-dried. The water contents of the freeze-dried products were in the range of 3.04~3.86%. The survival rate of the lyophilized culture varies with the type and concentration of the protectants added and the prefrozen temperatures. Reference to the protectant type, the survival rate was in the order of gelatin > gum arabic > lecithin. Reference to the protectant concentration, it is in the order of 15% > 10% > 5%. Reference to the freezing temperature, it was in the order of -80 °C > -40 °C > -20 °C. The survival rates of all lyophilized cultures treated with protectants were higher than that of untreated. The specific activity of b-galactosidase of the culture increased both after frozen and freeze-dried, which was likely a result caused by mechanical damage of cell membrane during freezing and freeze-drying. The lyophilized cultures of *L. acidophilus* were stored at different temperatures for 3 months. Reference to the storage temperature, the storage stability of the culture was in the order of 0 °C > 25 °C > 50 °C. Reference to the protectant type, it was in the order of gelatin > gum arabic > lecithin during storage at 0 °C and 25 °C, however, the gum arabic had the best protective effect during storage at 50 °C. Reference to the freezing temperature, it was -80 °C > -40 °C > -20 °C. Regardless of protectant type and concentration, the specific activity of b-galactosidase increased with storage time. The lyophilized culture of *L. acidophilus* added with lecithin had a higher b-galactosidase specific activity than those added with gelatin or gum arabic.

Keywords : *Lactobacillus acidophilus* ; lactic acid bacteria ; culture preservation ; freeze-drying ; lecithin ; gelatin ; gum arabic ; b-galactosidase specific activity

## Table of Contents

封面內頁 簽名頁 授權書 iii	中文摘要 iv	英文摘要 v	誌謝 vii	目錄 viii	圖目錄 xiii	表目錄 xvii																																																																										
第一章 緒論 1	1.1 研究背景 1	1.2 研究動機 1	1.3 研究大綱 2	第二章 文獻回顧 4	2.1 益生菌 (Probiotics) 之定義 4	2.1.1 益生菌之特性 4	2.2 乳酸菌之定義與分類 5	2.2.1 乳酸菌之應用 8	2.3 嗜酸乳酸桿菌 ( <i>L. acidophilus</i> ) 11	2.4 菌種保存方法 11	2.4.1 定期接種法 (繼代培養) 12	2.4.2 以石蠟或礦物油封存菌株及培養基 12	2.4.3 冷凍 14	2.4.4 乾燥保存 14	2.4.5 其他保存方法 18	2.5 影響冷凍乾燥菌體存活的因素 18	2.5.1 菌種之種類 19	2.5.2 菌體的收集 19	2.5.3 菌體的濃度 20	2.5.4 生長培養基的影響 20	2.5.5 菌體保護劑懸浮液的影響 21	2.5.6 細胞懸浮液的影響 25	2.5.7 冷凍及解凍的速率及溫度 27	2.6 乾燥菌粉之保存 28	2.6.1 含氧量 28	2.6.2 含水量 28	2.6.3 儲存溫度 29	2.6.4 保護劑的種類 29	2.6.5 其他 29	2.7 本研究所用之保護劑 30	2.7.1 卵磷脂 (Lecithin) 30	2.7.2 明膠 (Gelatin) 31	2.7.3 阿拉伯膠 (Gum arabic) 34	第三章 材料與方法 38	3.1 材料 38	3.1.1 菌株 38	3.2 實驗藥品 38	3.2.1 培養基 38	3.2.2 培養基添加劑及添加濃度 38	3.2.3 菌體保護劑 38	3.2.4 測定 b-galactosidase 活性所用之試劑及濃度 40	3.2.5 配製 pH 7.0 之 0.01M Sodium Phosphate Buffer 所用之試劑及濃度 40	3.3 儀器 40	3.4 實驗流程 41	3.4.1 培養基製備 41	3.4.2 仿 MRS broth 培養基製備 41	3.4.3 實驗菌株之活化與保存 41	3.4.4 實驗菌株之製備 41	3.4.5 實驗菌株之批式饋料培養 43	3.4.6 菌液的收集 44	3.4.7 菌體懸浮液之製備 44	3.4.8 冷凍及冷凍乾燥 44	3.4.9 凍乾 <i>L. acidophilus</i> 菌體之儲存 45	3.4.10 凍乾 <i>L. acidophilus</i> 菌體粉末回溶 45	3.5 分析方法 45	3.5.1 菌體濁度的測定 45	3.5.2 存活菌數之測定 45	3.5.3 b-galactosidase 活性測定 47	3.5.4 統計分析 47	第四章 結果與討論 48	4.1 冷凍及凍乾後 <i>L. acidophilus</i> 的水含量 48	4.2 預冷凍及凍乾後 <i>L. acidophilus</i> 的存活率及 b-galactosidase 比活性之變化 48	4.2.1 預冷凍後 <i>L. acidophilus</i> 的存活率 48	4.2.2 凍乾後 <i>L. acidophilus</i> 的存活率 51	4.2.3 b-galactosidase 比活性於 <i>L. acidophilus</i> 冷凍及冷凍乾燥後之變化 54	4.3 <i>L. acidophilus</i> 冷凍乾燥菌於儲存期間存活率與 b-galactosidase 比活性之變化 58	4.3.1 冷凍溫度對 <i>L. acidophilus</i> 冷凍乾燥菌於儲存安定性與 b-galactosidase 比活性之影響 59	4.3.2 保護劑種類對 <i>L. acidophilus</i> 菌於儲存安定性與 b-galactosidase 比活性之影響 68	4.3.3 保護劑濃度對 <i>L. acidophilus</i> 冷凍乾燥菌於儲存安定性與 b-galactosidase 比活性之影響 76	4.3.4 儲存溫度對 <i>L. acidophilus</i> 冷凍乾燥菌於儲存安定性與 b-galactosidase 比活性之影響 83	第五章 結論 92	參考文獻 94	圖目錄 圖 2.1 水替換假說示意圖 26	圖 2.2 磷脂質之種類與結構 32	圖 3.1 實驗流程 42	圖 4.1 預冷凍溫度對未添加保護劑之 <i>L. acidophilus</i> 冷凍乾燥菌於不同溫度下儲存三個月期間存活率之影響 60	圖 4.2 預冷凍溫度對卵磷脂添加不同濃度之 <i>L. acidophilus</i> 冷凍乾燥菌於不同溫度下儲存三個月期間存活率之影響 61	圖 4.3 預冷凍溫度對明膠添加不同濃度之 <i>L. acidophilus</i> 冷凍乾燥菌於不同溫度下儲存三個月期間之存活率之影響 62	圖 4.4 預冷凍溫度對阿拉伯膠添加不同濃度之 <i>L. acidophilus</i> 冷凍乾燥菌於不同溫度下儲存三個月期間存活率之影響 63	圖 4.5 預冷凍溫度對未添加不同濃度之 <i>L. acidophilus</i> 冷凍乾燥菌於不同溫度下儲存三個月期間

— galactosidase比活性之影響 64 圖4.6 預冷凍溫度對卵磷脂添加不同濃度之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間 — galactosidase比活性之影響 65 圖4.7 預冷凍溫度對明膠添加不同濃度之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間 — galactosidase比活性之影響 66 圖4.8 預冷凍溫度對阿拉伯膠添加不同濃度之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間 — galactosidase比活性之影響 67 圖4.9 濃度5%之各種保護劑對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間存活率之影響 69 圖4.10 濃度10%之各種保護劑對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間存活率之影響 70 圖4.11 濃度15%之各種保護劑對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間存活率之影響 71 圖4.12 濃度5%之各種保護劑對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間之影響 — galactosidase 比活性 73 圖4.13 濃度10%之各種保護劑對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間之影響 — galactosidase 比活性 74 圖4.14 濃度15%之各種保護劑對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間之影響 — galactosidase 比活性 75 圖4.15 卵磷脂濃度對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月存活率之影響 77 圖4.16 明膠濃度對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月存活率之影響 78 圖4.17 阿拉伯膠濃度對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月存活率之影響 79 圖 4.18 卵磷脂濃度對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間 — galactosidase比活性之影響 80 圖 4.19 明膠濃度對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間 — galactosidase比活性之影響 81 圖 4.20 阿拉伯膠濃度對不同溫度預冷凍之 *L. acidophilus* 冷凍乾燥菌?於不同溫度下儲存三個月期間 — galactosidase 比活性之影響 82 圖4.21 儲存溫度對添加卵磷脂及不同預冷溫度之 *L. acidophilus* 冷凍乾燥菌?於儲存三個月期間存活率之影響 84 圖4.22 儲存溫度對添加明膠及不同預冷溫度之 *L. acidophilus* 冷凍乾燥菌?於儲存三個月期間存活率之影響 85 圖4.23 儲存溫度對添加阿拉伯膠及不同預冷溫度之 *L. acidophilus* 冷凍乾燥菌?於儲存三個月期間存活率之影響 86 圖4.24 儲存溫度對未添加保護劑及不同預冷溫度之 *L. acidophilus* 冷凍乾燥菌?於儲存三個月期間 —galactosidase比活性之影響 87 圖4.25 儲存溫度對添加卵磷脂及不同預冷溫度之 *L. acidophilus* 冷凍乾燥菌?於儲存三個月期間 — galactosidase比活性之影響 88 圖4.26 儲存溫度對添加明膠及不同預冷溫度之 *L. acidophilus* 冷凍乾燥菌?於儲存三個月期間 — galactosidase 比活性之影響 89 圖4.27 儲存溫度對添加阿拉伯膠及不同預冷溫度之 *L. acidophilus* 冷凍乾燥菌?於儲存三個月期間 — galactosidase 比活性之影響 90 表目錄 表2.1 乳酸菌之分類 6 表2.2 乳酸菌所適用之培養基種類 13 表2.3 細菌保存於液體石蠟中的存活率 15 表2.4 冷凍乾燥保存所用之保護劑 22 表2.5 常見膠類之來源 35 表3.1 仿MRS broth培養基之組成分 39 表 4.1 菌體懸浮液冷凍前之含水量 49 表 4.2 保護劑種類及濃度對 *L. acidophilus* 於不同溫度下預 冷凍再經冷凍乾燥後含水量影響 50 表 4.3 保護劑種類及濃度對 *L. acidophilus* 於不同溫度下預 冷凍後存活率之影響 52 表 4.4 保護劑種類及濃度對 *L. acidophilus* 於不同溫度下預 冷凍再經冷凍乾燥後存活率影響 53 表 4.5 添加不同濃度之各式保護劑之 *L. acidophilus* 懸浮液 經冷凍及冷凍乾燥後 — galactosidase比活性之變化 55

## REFERENCES

- 1.久保道徳 (1992) 卵磷脂:生命的基本物質。青春出版社,台北。
- 2.小崎道雄 (1992) 乳酸菌實驗????。朝倉書店,東京。P.161。
- 3.井口信義、那須野精一 (1978) 醬油釀造?微生物。發酵工程56:645-655。
- 4.王宗燦 (2001a) 乳酸菌與人體健康。食品資訊183:93-96。
- 5.王逢興 (1994) 腸內乳酸菌之應用。雜糧與畜產253:2-6。
- 6.王學堯 (2001b) 乳酸菌在食品加工上的應用。食品資訊184:54-57。
- 7.台灣阿拉伯膠公司 (2001) 纖維膠-阿拉伯膠天然膳食纖維與其對人體益菌產生的功效。食品資訊187:68-69。
- 8.朱燕華 (1995) 卵磷脂技術發展現狀及應用。食品工業12:8-12。
- 9.江晃榮 (1985) 微生物菌種保存法。華香園出版社,台北。
- 10.宋文杰 (2001) 食用膠在烘焙產品的應用。烘焙工業96:41-49。
- 11.李文齡、趙世彬、蔡東璣、陳河吉、周大中 (1996) 生物化學。藝軒圖書出版,台灣,台北。
- 12.李敏雄 (1997) 卵磷脂之製備及應用。食品科學24 (6) :728-735。
- 13.李福臨 (1989) 食品加工上乳酸菌之利用。食品工業21 (12) :32-38。
- 14.李福臨 (1998) 乳酸菌之保存。食品工業30 (2) :54-63。
- 15.李寶珠 (1985) 嗜酸性乳酸菌 (*Lactobacillus acidophilus*) 之分離與鑑定。台南師專學報18:43-49。
- 16.周繼發、陳金輝 (1993) 自豬骨萃取消明膠之研究。中畜會誌, 12:7-14。
- 17.林燦漲 (2001) 淺談菌種冷凍乾燥保護劑的保護機制。菌種保存及研究簡訊,財團法人食品工業發展研究所,台灣,新竹p.9-11。
- 18.邵志中 (1995) 卵磷脂之產品概觀與產業調查。食品工業12:13-24。
- 19.邱健人 (1979a) 膠在食品工業上的應用 (一)。食品工業10 (12) :36-42。
- 20.邱健人譯 (1979b) 膠在食品工業上之應用 (三)。食品工業11 (2) :25-33。
- 21.邱健人 (1979c) 膠在食品工業上之應用 (四)。食品工業11 (4) :40-46。
- 22.邱雪惠 (2004) 乳酸菌之抗癌機制。食品工業36 (3) :27-33。
- 23.柯文慶 (1996) 食品中之玻璃態轉移。食品工業12:38-48。
- 24.神戶千幸、內田金治 (1984) 醬油乳酸菌 *Pediococcus halophilus* ?還原力?醬油諸味?酸化還原電位????。農業化學會誌58:487-490。
- 25.張天鴻 (1978) 細菌菌種保存簡論。食品工業10:11-16。
- 26.張為憲 (1984) 高等食品化學。華香園出版,台北, p.229-230。
- 27.淺野行藏、修司吉川、吉史田村 (1998) 乳酸菌流動層乾燥技術及其應用。生物產業9 (4) :26-30。
- 28.許南茵、林慶文、陳明汝 (2000) 原生菌於乳製品上之應用。食品資訊171:45-51。
- 29.陳秀華 (2000) 冷凍乾燥對 *Streptococcus salivarius* ssp. *thermophilus* 儲存安定性及產酸能力之影響。國立中興大學畜產學系碩士論文。
- 30.陳俊成 (2000) 發酵乳常用乳酸菌之生理。食品資訊171:52-59。
- 31.陳俊成 (2002) 動物膠在食品中之功能性與應用。食品資訊188:52-58。
- 32.陳俊成 (2003) 乳酸菌之益生作用。食品資訊196:52-58。
- 33.陳美菁 (1999) 乳酸菌之冷凍乾燥。食品工業31(10):52-62。
- 34.陳慶源、林富美 (2004) 益生菌之保健功效,食品工業36 (3) :1-3。
- 35.森地敏樹、山里一英、鈴木正敏、高野光男、根井外喜男 (1982) 微生物?保存法。東京大學出版會,東京。p.30-80

- 。 36.黃守潔 (1993) 高壓下明膠及果膠凝膠化反應之熱力學性狀及熱效應的探討。國立中興大學食品科學研究所碩士論文。 37.黃加成 (1991) 乳酸菌之特性與利用。雜糧與畜產221:21-28。 38.楊媛綸 (1996) *Lactobacillus acidophilus* 之降膽固醇及抗癌性。食品資訊38:67-75。 39.廖啟成 (1998) 乳酸菌之分類及應用。食品工業30(2):1-10。 40.劉怡菁 (2001) 不同保護劑的添加對 *Lactobacillus acidophilus* CCRC 10695 菌株在貯藏過程中的影響。國立中興大學食品科學系碩士論文。 41.劉淑美 (1985) 菌種保存的方法。食品工業17 (8) :36-40。 42.劉淑美 (1986) 細菌冷凍乾燥保存之原理。食品工業18 (3) :36-41。 43.蔡英傑 (1998a) 乳酸菌與益生菌。生物產業9(2):26-32。 44.蔡英傑 (1998b) 乳酸菌應用綜論。生物產業9(4):50-56。 45.賴永裕 (2000) 乳酸菌的深度應用。食品資訊178:49-52。 46.賴滋漢、金安兒、柯文慶 (1992) 「食品加工學 (方法篇)」。精華出版, 台中。p.326-328。 47. Anon. (1992) Yoghurt and probiotics. *Choice*.70:32-5。 48.Banno, I. and Sakane, T. (1981) Prediction of prospective viability of L-dried cultures of bacteria after long-term preservation. *IFO Res.* 10:33-38。 49.Baumann, D. P. and Reinbold, G. W. (1966) Freezing of lactic cultures. *J. Dairy Sci.* 49:259-263。 50.Beal, C., Fonseca, F., and Corrieu, G. (2001) Resistance to freezing and frozen storage of *Streptococcus thermophilus* is related to membrane fatty acid composition. *J. Dairy Sci.* in press。 51.Bozoglu, T. F., Ozilgen, M., and Bakir, U. (1987) Survival kinetics of lactic acid starter culture during and after freeze drying. *Enzyme Microb. Technol.* 9:531-537。 52.Brennan, M., Wanismail, B., Johnson, M. C., and Ray, B. (1986) Cellular damage in dried *Lactobacillus acidophilus*. *J. Food Prot.* 49:47。 53.Broadbent, J. R. and Lin, C. (1999) Effect of heat shock or cold shock treatment in the resistance of the *Lactococcus lactis* to freezing and lyophilization. *Cryobiol.* 39:88-102。 54.Champagne, C. P., Gardner, N., Brochu, E., and Beaulieu, Y. (1991) The freeze-drying of lactic acid bacteria. A review. *J. Inst. Can. Sci. Technol. Aliment.*24 (3/4) 118。 55.Champagne, C. P., Mondou, F., Raymond, Y., and Brochu, E. (1996) Effect of immobilization in alginate on the stability of freeze-dried *Bifidobacterium longum*. *Biosci. Microflora.* 15 (1) :9。 56.Chavarri, F. J., De Paz, M., and Nunez, M. (1988) Cryoprotective agents for frozen concentrated starters from nonbitter *Streptococcus lactis* strains. *Biotechnol. Lett.* 10:11-16。 57.Choi, S. S. and Regenstien, J. M. (2000) Physicochemical and sensory characteristics of fish gelatin. *J. Food Sci.* 65:194-199。 58.Chou, C.C., Lian, W. C., and Hsiao, H. C. (2002) Survival of *Bifidobacteria* after spray-drying. *Int. J. Food Microbiol.* 74:79-86。 59.Couture, C. P., Gagne, D., and Champagne C. P. (1991) Effect de drivers additives sur la survie a la lyophilisation de *Lactobacillus lactis*. *J. Inst. Can. Sci. Technol. Alim.* 5:224。 60.Crow, J. H., Crow, L. M., Carpenter, J. F., Rudolph, A. S., Wistrom, C. A., Spargo, B. J., and Anchoroguy, T. J. (1988) Interactions of sugars with membranes. *Biochim. Biophys.* 947:367-384。 61.Crow, L. M., Crowe, J. H., and Chapman, D. (1985) Interaction of carbohydrates with dry dipalmitoylphosphatidylcholine. *Arch. Biochem. Biophys.* 236:289-296。 62.Dashiell, G. L. (1989) Lecithin in food processing application. Szuhaj, editor. *Lecithins:sources, manufacture & uses.* American Oil Chemists' Society, AOCS. p.213-224。 63.De Urraza, P. and De Antoni, G. (1997) Induced cryotolerance of *Lactobacillus delbrueckii* subsp. *bulgaricus* LGG by preincubation at suboptimal temperatures with fermentable sugar. *Cryobiol.* 35:159-164。 64.Fernanda, F., Catherine, B., and Georges, C. (2001) Operating conditions that affect the resistance of lactic acid bacteria to freezing and frozen storage. *Cryobiol.* 43:189-198。 65.Ferry, J. D. (1948) Mechanical properties of substances of high molecular weight. *Adv. Protein Res.* 4:2244-2249。 66.Font de Valdez, G. de Giori, G. S. de Ruiz Holgado, A. P., and Oliver, G. (1983) Comparative study of the efficiency of some additives in protecting lactic acid bacteria against freeze-drying. *Cryobiol.* 20:560。 67.Font de Valdez, G. de Giori, G. S. de Ruiz Holgado, A. P., and Oliver, G. (1985a) Effect of drying medium on residual moisture content and viability of freeze-dried lactic acid bacteria. *Appl. Environ. Microbiol.* 49:413。 68.Font de Valdez, G. de Giori, G. S. de Ruiz Holgado, A. P., and Oliver, G. (1985b) Effect of the rehydration medium on the recovery of the freeze-dried lactic acid bacteria. *Appl. Environ. Microbiol.* 50:1339。 69.Foschino, R., Fiori, E., and Galli, A. (1996) Survival and residual activity of *Lactobacillus acidophilus* frozen cultures under different condition. *J. Dairy Res.* 63:295-303。 70.Franks, F. (1990) Freeze-drying:from empiricism to predictability. The significance of glass transitions. *Development Biological Standardization.* 74:9-19。 71.Fuller, R. (1989) Probiotics in man and animals. *J. of Appl. Bact.*66:365-378。 72.Gilliland, S. E. and Rich, C. N. (1990) Stability during frozen and subsequent refrigerated storage of *Lactobacillus acidophilus* grown at different pH. *J. Dairy Sci.* 73:1187-1192。 73.Goldberg, I. and Eschar, L. (1977) Stability of lactic acid bacteria to freezing as related to their fatty acid composition. *Appl. Environ. Microbiol.* 33:489-496。 74.Gomez Zavaglia, A., Disalvo, E. A., and De Antoni, G. L. (2000) Fatty acid composition and freeze-thaw resistance in lactobacilli. *J. Dairy Res.* 67:241-247。 75.Greiff, D. (1971) Protein structure and the effects of residual moisture on freeze-drying. *Cryobiol.* 8:145。 76.Hanafusa, J. V. (1985) The hydration water and protein cryoprotectant. In: *Fundamentals and applications of freeze-drying to biological materials, drugs and foodstuffs.* International Institute of Refrigeration. Paris. p.59。 77.Hayashi, A. and Oh, S. —C. (1983) Gelation of gelatin solution. *Agric. Biol. Chem.* 47 (8) 1711-1716。 78.Heckly, R. J. (1976) Free radicals in dry biological systems. in W. S. Pryor, ed., *Free radicals in Biology,* Academic Press, New York. 2:135-158。 79.Heckly, R. J. and Dimatteo, J. (1975) Rhythmic changes in dry heat resistance of *Bacillus subtilis* spores after rapid changes in pH. *Appl. Microbiol.* 29:565-566。 80.Ishibashi, N., Tatematsu, T., Shimamura, S., Tomita, M., and Okonogi, S. (1985) Effect of water activity on the viability of freeze-dried bifidobacteria and lactic acid bacteria. In: *Fundamentals and applications of freeze-drying to biological materials, drugs and foodstuffs.* Inter. institute of Refrigeration. Paris. p.227。 81.Ishibashi, N. and Shimamura, S. (1993) *Bifidobacteria:Research and development in Japan.* *Food Technol.* 47 (126) 129-134。 82.Jos, H. J., Huis, I. V., and Havemaar, R. (1992) Probiotics and health in man and animal. *European J. Chem.Nutrition.* 1:29-31。 83. Karlsson, J. O. M. and Toner, M. (1996) Long-term preservation of tissues by cryopreservation:Critical issues. *Biomaterials.* 17:243-256。 84.Kearney, L., Upton, M., and Das, N. K. (1976) Effect of cryoprotection agents on freeze-drying and storage on lactic cultures. *Cult. Dairy Prod. J.* 11 (May) :8。 85.Kilara, A., Shahani, K. M., and Das, N. K. (1976) Effect of cryoprotective agents on freeze-drying and storage of lactic culture. *Cult. Dairy Prod. J.* 11 (2) :8-11。 86.Kim, W. S. and Dunn, N. W. (1997) Identification of a cold shock gene in lactic acid bacteria and storage and

the effect of cold shock on cryotolerance. *Curr. Microbiol.* 35:59-63. 87.King, V. A. —E., and Lin, H. —J. ( 1995 ) Studies on the effect of protectants on *Lactobacillus acidophilus* strain dehydrated under controlled low-temperature vacuum dehydration and freeze-drying by using response surface methodology. *J. Sci. Food Agric.* 68:191. 88.King, V. A. —E., Zall, R. R., and Ludington, D. C. ( 1989 ) Controlled low-temperature vacuum dehydration-a new approach for low-temperature and low pressure food drying. *J. Food Sci.* 54 ( 6 ) :1573-1579,1593. 89.Ledward, D. A. ( 1986 ) Gelation of gelatin. In " Function properties if food macromolecules " , Elsevier Science Publishing Co, Inc., New York. 90.Leslie S. B., Israeli, E., Lighthart, B., Crowe, J. H., and Crowe, L. M. ( 1995 ) Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. *Appl. Environ. Microbiol.* 61:3592-3597. 91.Lian, W. C., Hsiao, H. C., and Chou, C.C. ( 2002 ) Survival of *Bifidobacteria* after spray-drying. *Int. J. of Food Microbiology.* 74:79-86. 92.Lilly, D. M. and Stillwell, R. H. ( 1965 ) Probiotics:growth promoting factors produced by microorganisms. *Science.* 147:747-748. 93.Mazur, P. ( 1977 ) The role of intracellular freezing in the death of cells cooled at supraoptimal rates. *Cryobiol.*14:251-272. 94.McMeekin, T. A., Brown, J., Krist, K., Miles, D., Neumeyer, K., Nichols, D. S., Olley, N. J., Presser, K., Retkowsky, D. A., Ross, T., Salter, M., and Soontranon, S. ( 1997 ) Quantitative microbiology:a basis for food safety. *Emerg. Infectious Diseases.* 3:541-549. 95.Moriche, T. ( 1970 ) Nature and action of protection solutes in freeze-drying of bacteria. In: Proceeding of the first Int. Conference on Culture Collcetions. p.121. 96.Nagendra, P. S., Warnakulasuriya, E. V. Lankaputhra, Margaret, Britz, L., and Kyle, S. A. ( 1995 ) Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in commercial yoghurt during refrigerated. *Int. Dairy J.* 5:515-521. 97.Naidu, A. S., Bidlack, W. R., and Clemens, R. A. ( 1999 ) Probiotic spectra of lactic acid bacteria ( LAB ) . *Crit. Rev. Food Sci. Nutr.* 38:13-126. 98.Nikolova, N. ( 1978 ) Freeze-drying of starters for yogurt and of *Lactobacillus bulgaricus* in protective media. XX. *Int. Dairy Congress.* p.584-585. 99.O ' Sullivan, M. G., Thornton, G., O ' Sullivan G. C., and Collins, J.K. ( 1992 ) Probiotic bacteria:myth or reality, *Trends Food Sci. Technol.* 3:309-314. 100.Ockerman, H. W. and Hansen, C. L. ( 1988 ) Animal by-product processing. Ellis Horwood Ltd. Chivhester, England. 101.Panoff, J., Thammavongs, B., Laplace, J., Hartke, A., Boutibonnes, P., and Auffray, Y. ( 1995 ) Cryotolerance and cold adaptation in *Lactococcus lactis* subsp. *lactis* IL1403. *Cryobiol.* 32:516-520. 102.Parker, R. B. ( 1974 ) Probiotics, the other half of the antibiotic story. *Anim. Nutr. Health.* 29:4-8. 103.Paul Conrad, B., Miller, Danforth P., Cielenski, Peter R., and de Pablo, Juan J. ( 2000 ) Stabilization and preservation of *Lactobacillus acidophilus* in saccharide matrices. *Cryobiol.* p.17-24. 104.Playne, M. J. ( 1993 ) Probiotic Food. In Daisy. The food industry conference proceedings. Sydeny convention and exhibition center, Publ. Foodpro-93 Sydey, 12-14 July. p.3-9. 105.Pringle, M. J. and Chapman, D. ( 1981 ) Biomembrane structure and effects of temperature. In " Effects of low temperature on biological systems " ( G. J. Morris and A. Clarke, Eds. ) Academic press, New York. p.21. 106.Robinson, P. K. ( 1987 ) Survival of *Lactobacillus acidophilus* in fermented products. *Suid-Afrikaanse Tydskrif Vir Suiwelkunde* 19:25-7. 107.Sinskey, T. J. and Silverman, G. J. ( 1970 ) Characterization of injury incurred by *Escherichia coli* upon freeze-drying. *J. Bacteriol.* 101:429-437. 108.Smittle, R. B., Gilliland, S. E., Speck, M. L., and Walter, W. M. J. ( 1974 ) Relationship of cellular fatty acid composition to survival of *Lactobacillus bulgaricus* in liquid nitrogen. *Appl Microbiol.* 27:738-743. 109.Speckman, C. A., Sandine, W. E., and Elliker, P. R. ( 1973 ) Lyophilized lactic acid starter culture concentrates: preparation and inoculation of vat milk for cheddar and cottage cheese. *J. Dairy Sci.* 57: 165-170. 110.Spios E. F. and Szuhaj, B. F. ( 1996 ) Lecithins. Hui YH,editor. *Bailey ' s Industrial Oil and Fat Products.* John Wiley & Sons Inc, New York. p.311-395 111. Stadhouders, J., Jansen, L. A., and Hup, G. ( 1969 ) Freezing and freeze-drying of starter bacteria. *Koeltechniek.* 62(2):29-33. 112.Tan, C. S. ( 1997 ) Preservation of fungi. *Cryptogamie Mycol.* 18:157-163. 113.To, B. C. S. and Etzel, M. R. ( 1997 ) Spray drying, freeze drying, or freezing of three different lactic acid bacteria species. *J. Food Sci.* 2 ( 3 ) :576-578. 114.Ward, A. F. and Courts, L. M. ( 1977 ) The science and technology of gelatin. Academoc Press Inc. New York. 115.Wright, C. T. and Klaenhammer, T. R. ( 1981 ) Calcium-in-duced alteration of cellular morphology affecting the resistance of *Lactobacillus acidophilus* to freezing. *Appl. Environ. Microbiol.* 41:807-815. 116.Wright, C. T. and Klaenhammer, T. R. ( 1983 ) Survival of *Lactobacillus bulgaricus* during freezing and freeze-drying after growth in the presence of calcium. *J. Food Sci.* 48:773-777