# Development of spray drying method for the preparation of probiotic bacteria Bifidobacterium longum


## 江捷峰、陳鴻章 ; 李範亞

E-mail: 9008940@mail.dyu.edu.tw



**ABSTRACT**

In order to obtain a better preservation method for commercial purpose, this research investigated the feasibility of preserving Bifidobacterium longum CCRC 11847, a probiotic bacterium in human gastrointestinal tract, with spray-drying process. Initially, the effect of each factor, such as time course, outlet temperature of spray-drying, feed velocity and types and concentration of emulsifiers and antioxidants, on the survival ratio of the bacteriumwere was investigaed by changing one factor at a time. Then the most suitable conditions of each factor were combined for spray-drying process to obtain the best survival ratio of the bacteria. The bacterium was cultured anaerobically at 37 °Cin the MRS broth and spray-dried at different time interval to obtain the best time course for preservation. After 36 hours , the total viable plate count was 3＊108 CFU/mL and the growth of the bacteria was optimum. The effect was the best when the outlet and inlet temperature of the spray dryer were set at 90 °C and 37 °C, respectively. When different kinds of emulsifers such as glycerol monostearate, sorbitan monostearate, and stearic acid were used as the protective agent in the spray-drying process, it was found that glycerol monostearate had the best protective effect on Bifidobacterium longum. The total viable plate count after spray-drying was 9＊105 CFU/g. As for the concentration of the emulsifers, three different concentrations (1%, 2%, 3%) were used and the results indicated that the protective effect increased with increasing concentration of the emulsifers. When different kinds of antioxidants, such as butyl hydroxyanisol (BHA), dibutyl hydoxytoluene (BHT), and ascorbic acid (Vitamin C) were used as the protective agent in the spray-drying process, it was found that BHA had the best protective effect on the bacterium among the three. When the survival ratio test of the bacteria was conducted at antioxidant concentrations of 1000 ppm, 2000 ppm, and 3000 ppm, it was found that higher antioxidant concentrations had better protective effect than lower concentrations. When the optimal conditions of each factor in the spray-drying process were combined, the survival ratios of Bifidobacterium longum right after spray-drying and after 30 days storage at room temperature were higher than those of basal set. With an initial cell concentration of 3＊108 CFU/mL, the survival count right after spray-drying with basal set conditions was 420 CFU/g, and none survived after 30 days of storage. However, the survival count right after spray-drying reached 9＊105 CFU/g when the optimal set of conditions was employed, and the survival count of Bifidobacterium longum after 30 days storage at room temperature was 4.4＊105 CFU/g , representing a survival ratio of 49%.



Keywords : Bifidobacterium longum ; spray-drying ; emulsifiers ; antioxidants


**Table of Contents**

**REFERENCES**

1. Alih, K., and Esam, H. M. (1998) Alginate encapsulated bifidobacteria survival in mayonnaise. J. Food Sci., 63, 3-4. 2. Ariga, H., Vrashima. T., Michihata, E., Ito. M., Morizono, N., Kimura. T., and Takahashi, S. (1992) Extracellular polysaccharide from encapsulated Streptococcus salivarius subsp. thermophilus or 901 isolated from commercial yogurt. J. Food Sci., 57, 625-628. 3. Bateup, J, M., McConnell, M. A., Jenkinson, H. F., and Tannock. G. W. (1995) Comparison of Lactobacillus strains with respect to bile salt hydrolase activity, colonization of the gastrointestinal tract, and growth rate of the murine host. Appl. Environ. Microbiol., 61, 1147-1149. 4. Berrada, N., Lemel, J. F., Laroche, O., Thovenot, P., and Piaia, M. (1991) Bifidobacterium from fermented milks: survival during gastric transit. J. Dairy Sci., 74, 409-413. 5. Cerning, J., Bonillanne, C., Desmazeaud, M. J., and Landon, M. (1986) Isolation and characterization of exocellular polysaccharide produced by Lactobacillus bulgaricus. Biotechnol. Lett., 8, 625-628. 6. Cheah, P. Y. (1990) Hypotheses for the etiology of colorectal cancer-an overview. Nutr. Cancer, 14, 5-6. 7. Clark, P. A. and Martin, J. H. (1994) Selection of bifidobacteria for use as dietary adjuncts in cultured dairy foods. Tolerance to simulated bile concentrations of human small intestines. Cult. Dairy Prod. J., 29(8), 18- 21. 8. Conway, P. L., Gorbach, S. L., and Goldin. B. R. (1987) Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J. Dairy Sci., 70, 1-12. 9. Hughes, D. B. and Hoover, D. G. (1995) Viability and enzymatic activity of bifidobacteria in milk. J. Dairy. Sci., 78, 268-276. 10. Edwin, N. F. (1996) Antioxidants in foods and their impact on food quality. Food Chem., 57(1), 51-55. 11. Faldt, P. and Bergenstahl, B. (1996) Changes in surface composition of spray-dried food powder due to lactose crystallization. Lebensmittel-Wissenschaft and Technologie, 29(5), 438-446. 12. Fernandes, C. F. and Shahani, K. M. (1988) Effect of nutrient media and bile salts on growth and antimicrobial activity of Lactobacillus acidophilus. J. Dairy Sci., 71, 3222-3229. 13. Fuller, R. (1989) Probiotics in man and animals. J. of Appl. Bact., 66, 365-378. 14. Fuller, R. and Cole, C.B. (1986) Theory and application in probiotics. Chalcombe, 1-14. 15. Gibson, G. R. and Roberfroid, M. B. （1994） Dietary modulation of the human colonic microbiota: Introducing the concept of probiotcs. Journal of Nutrition, 125 (6), 1401-1412. 16. Gilliand, S. E. and Walker, D. K. (1990) Factors to consider when selecting a culture of Lactobacillus acidophilus as a dietary adjunct to produce a hypocholesterolemia effect in humans. J. Dairy Sci., 73, 903-911. 17. Goldin, B. R., Gorbach, S. L., and Salminen. S. (1992) Survival of Lactobacillus strain GG species in human gastrointestinal tract. Dig. Dis. Sci., 37, 121-128. 18. Abu-Taraboush, H. M. Al., Dagal, M. M., and Al-Royli, M. A. (1998) Growth, viability, and proteolytic activity of bifidobacteria in whole camel milk. J. Dairy Sci., 81, 354-361. 19. Hull, R. R., Conway, P. L. and Evans, A. J. (1992) Probiotic food a new opportunity, Food Australia, 44 (3), 112-113. 20. John, W. and Sons, I. (1970) Engineering factors in single-cell protein production. Biotechnology and Bioengineering, 12(1), 135-140. 21. Jos, H. J., Huis, I. V. and Havenaar, R. (1992) Probiotcis and health in man and animal. European Journal of Chemical Nutrition, 1, 29-31. 22. Judie, D. D. (1986) Antioxidant. Food Technol., 40, 94-103. 23. Kanawjia, S. K., Pathania. V., and Singh, S. (1992) Micro-encapsulation of enzymes. Microorganism and flavours and their applications in foods. Indian Dairyman, 44, 280-287. 24. Kitazawa, H., Itoh, T., and Yamaguchi, J. (1991) Induction of macrophage cytotoxicity by slime products produced by encapsulated Lactococcus lactis subsp. Cremoris. Anim. Sci. Technol., 62, 861-865. 25. Kim, I. S., Kamara. B. J., Good, I. C., and Enders. G. (1988) Method for the preparation of stabile microencapsutated lactic acid bacteria. J. Ind. Microbiol., 3, 253-257. 26. Klaenhammer, T. R. (1993) FEMS Microbiol. Rev., 12, 39-85. 27. Klaver, F. A. M. and vander Meer, R. (1993) The assumed assimilation of choleslerol by Lactobacilli and Bifidobacterum bifidum is due to their bile salt deconjugating activity. Appl. Environ. Microbiol, 59, 1120-1124. 1. Lee, Y. K. and Salminen, S. （1995） The coming of age of probiotics. Trends in Food Science and Technology, (67), 241-245. 2. Lin, M. Y. and Yen, C. L. (1999) Antioxidative ability of lactic acid bacteria. J. Agric. Food Chem., 47, 1460-1466. 3. Lilly, D. M. and Stillwell, R. H. (1965) Probiotics: growth promoting factors produced by microorganisms. Science, 147, 747-748. 4. Ludbrook, K. A., Russel, C. M., and Greig, R. I. (1997) Exopolysaccharide production from lactic acid bacteria isolated from fermented foods. J. Food Sci., 62(3), 597-604. 5. Martcau, P., Pochart. P., Flourie. B., Pellier, P., Santos, L., Desjeux. J. F., and Rambaud, J.

C. (1990) Effect of chronic ingestion of a fermented dairy product containing Lactobacillus acidophilus and Bifidobacterium bifidum on metabolic activities of the colonic flora in humans. Am. J. Clin. Nutr., 52, 685-688. 6. Marteau, P. (1992) Effect of probiotics on intestinal metabolism. European Journal of Clinical Nutrition, 3, 35-37. 7. McKay, L. L. and Baldwin, K. A. (1990) FEMS Microbiol. Rev., 87, 3-14. 8. Mitsuoka, T. (1984) Taxonomy and ecology of bifidobacteria. Bifidobacteria Microbiol., 3, 11-28. 9. Moura, T. F., Jacob, M. Pauvert, B., Gaudy, Terol, A., and Chauvet A. (1996) Vitamin C spray drying: Study of the thermal constraint. Drug Development and Industrial Pharmacy, 25(3), 393-400. 10. Nil (1994) Establishing a scientific basis for probiotic R&D. Trends in Biotechnology, 12(1), 6-8. 11. O''Sullivan, M. G., Thornton, G., O''Sullivan, G. C., and Collins, J. K. (1992) Probiotic bacteria: myth or reality, Trends in Food Science & Technology, 3, 309-314. 12. Rasic, J. L. and Kurmann, J. A. (1983) Bifidobacteria and Their Role. Birkhaus Verlag, Boston. MA ,USA. 13. Roberts. C., M., Fett, W. F., Osman, S. F., Wijcy, C., O''Connor, J. V., and Hoover, D. G. (1995). Exopolysaccharide production by Bifidobacterium longum BB-79. J. Appl. Bacteriol., 78, 463 -468. 14. Robinson, R. K. (1983) Starter culter for milk and meat processing. Biotechnolog., 3, 191-202. 15. Scardovi, V. (1986) Genus Bifidobacterium in Bergey's manual of Systematic Bacteriol. MD, USA., Vol 2, 1418-1434, 16. Tannock. G. W., Tangerman, A., Schaik, A., V., and McConnel, M, A. (1994) Deconjugation of bile acids by Lactobacilli in the mouse samll bowel. Appl. Environ. Microbiol., 60, 3419-3420. 17. 王有忠 (1993) 食品添加物。華香園出版社第二版，台北市。 18. 朱文深 (1997) 雙歧桿菌屬之分類與應用。菌種保存及研究簡訊， 10（4)， 1-8。 19. 佐佐木隆 (1998) 乳酸菌生物技術之進展。生物產業，9（4）, 227-233. 20. 李國鏞，游若荻 (1992) 微生物學。華香園出版社第四版，台北市。 21. 金安兒，蘇照堂，黃守潔 (1993) 香蕉泥的添加對嗜酸桿菌乳中 Lactobacillus acidophilus 噴霧乾燥後存活率之影響。中國農業化學會誌，31 (1), 68-77。 22. 林慧生 （1994） 脂肪氧化與抗氧化劑。雜糧與畜產，249 （1）, 14 - 19。 23. 吳淳美 (1979) 食品中之氧化-還原系統及食品抗氧化劑。 食品工業，11(5)，42-49. 24. 施宗雄 (1998) 台灣乳酸菌類研究之介紹及在動物添加劑上之應用。生物產業，9 (4), 239-245。 25. 拱玉郎 (1997) 天然抗氧化劑發展近況。食品工業，29 (3)， 29-37。 26. 務台方彥 (1978) 使用發酵乳雙歧桿菌之特性。New Food Industry , 20(8), 17-23. 27. 財團法人食品工業發展研究所 (1997) 雙歧桿菌屬之分類與應用。菌種保存及研究簡訊，10 (4)，1-8。 28. 許文浩 (1994) 菌元介紹。食品工業，26 (12)，39-45。 29. 許夏芬 (1996) 天然抗氧化劑之添加對花生油安定性之探討。食品科學，23 (6)，788-800。 30. 陳茂元，吳俊忠 (1999) 食用優酪乳可增加腸道比菲德菌並壓抑腸桿菌。乳酸菌與人體健康研討會，5月1日台北市，統一企業主辦。 31. 陳厚基 (1990) 併用生菌劑及 Isomalt oligo 糖於子豬之效果試驗。飼料營養雜誌，2, 3-7。 32. 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), 234-238 。 33. 郭明潔 (1993) 以反應曲面法探討添加維生素 C、單棕櫚酸山梨糖酯和氯化鈉對濕酵母冷藏期產氣活性之影響。東海大學食品科學研究所碩士論文。 34. 楊基礎，劉佳 (1996) 雙歧桿菌固定化的研究。 微生物學通報，23 (1)， 35-36 (大陸)。 35. 趙承琛，陳純純，吳忠能 (1983) 乳化劑之化學構造和用量對乳態的影響。界面科學，6 (4), 2-15. 36. 戴乃倫 (1981) 酪多精對仔豬飼養效果之研究。台灣糖業公司畜牧研究所 69-70 年期研究試驗報告 (1981年12月)。 37. 蔡英傑 (1998a) 乳酸菌與益生菌。生物產業，9 (2)，98-104. 38. 蔡英傑 (1998b) 乳酸菌應用綜論。生物產業，9 (4)，258-264. 39. 廖啟成 (1998) 台灣雙歧桿菌研究介紹及食品產業上之應用。 食品工業研究所乳酸菌研究及應用研討會，3月29日新竹市。 40. 楊媛絢 (1998a) 原生保健性菌種與益菌助生質之應用。食品工業，30 (2)，11-22。 41. 楊媛絢 (1998b) 雙歧桿菌環境耐性菌株之相關研究及專利。食品工業，30 (2)，32-47。 42. 劉英俊，汪金追 (1982) 微生物應用工業。中央圖書出版社，台北市。 43. 賴滋漢，金安兒，柯文慶 (1993) 食品加工學(方法篇)。精華出版社，台中市。 44. 謝旻峰 (1993) 包覆抗氧化劑及乳化劑對即溶活性乾酵母儲藏安定性之影響。東海大學食品科學研究所碩士論文。