Short Communication

Banana puree fermentation by *Lactobacillus acidophilus* immobilized in Ca-alginate

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(Received April 17, 2003; Accepted September 12, 2003)

**Key Words** — banana; calcium alginate; fermentation; fructooligosaccharides; immobilized cell; *Lactobacillus acidophilus*

Lactic acid bacteria (LAB) species are applied in human and animal foodstuffs for preservation, flavor enhancement, and/or probiotic purposes, and therefore become available to the gastrointestinal tract (Carr et al., 2002). Among LAB, *Lactobacillus acidophilus* and related species exhibit probiotic effects and have been utilized widely in food processing. It colonizes the intestinal tracts of man and higher animals and suppresses pathogenic microorganisms (Brennan et al., 1993). In addition, *L. acidophilus* has also been reported to possess functional properties, including antitumor activity, hypocholesterolemic actions, and the ability to synthesize various vitamins (Dash, 1996).

Banana (*Musa* spp.) is one of the major fruits in Taiwan and is an important food crop cultivated extensively in tropical and subtropical areas (Jonas, 1995). Common banana products processed are banana puree, banana pulp, banana flour or powder, banana chips, canned banana slices, banana jam, banana wine, banana juice, and so on. Application of banana for LAB fermentation has also been researched (Aegerter and Dunlap, 1980; de Porres et al., 1985). Banana contains high levels of sugars, mostly sucrose, glucose, and fructose, and is adequate for microbial fermentation (Glatz et al., 1985). The goals of banana processing are to improve the shelf-life of banana products, to enrich their flavors, and to enhance their health effects. However, banana puree, which is often used for LAB fermentation, possesses some components, such as pectin, which are adverse to fermentation, and displays a pH of approximately 4.5 which is not a proper pH for LAB fermentation (Du-paigne, 1974). Accordingly, endeavors such as pH adjustment or cell immobilization are needed to ameliorate the LAB fermentation of banana puree. Meanwhile, banana also contains certain amounts of fructooligosaccharides (FOS) which have been found to exert advantageous health effects by promoting the proliferation of lactic acid bacteria in the human colon (Tomomatsu, 1994). Products combining *L. acidophilus* as a probiotic and certain fruits, such as banana, as a prebiotic that might show beneficial functional properties (as a synbiotic) might be proposed and explored.

Advantages of immobilized cell technology include continuous utilization, enhancement of production and

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excretion of secondary metabolites, and protection against disturbed and unfavorable environments (Nath and Chand, 1996). Besides, cell concentration was effectively raised, and the survival and operating efficiency could be improved by the application of immobilized cells during processing (Champagne et al., 1994; Jen et al., 1996). Therefore, immobilized cell technology has been adopted for various industrial applications. Cell immobilization of yeast was used by del Rosario and Pamatong (1985) to improve the alcohol production of banana pulp fermentation. Immobilization of LAB by Ca-alginate gel entrapment effectively increased resistance to bile acid and gastric juice, and enhanced its viability and activity in human guts (Favaro-Trindade and Grosso, 2000; Lee and Heo, 2000). The purposes of this research were to combine L. acidophilus and banana in a direct fermentation by using banana puree as the medium and to improve the fermentation efficiency by the use of Ca-alginate entrapment cell immobilization, and to develop a novel product with symbiotic effects.

L. acidophilus BCRC 10695 cultures were obtained from the Biosource Collection and Research Center of the Food Industry Research and Development Institute at Hsinchu, Taiwan, and kept on MRS agar (Difco Laboratories, Detroit, MI, USA) and stocked at −20°C. Bananas (Musa sapientum) were purchased at a local market, and were yellow with large black splotches. This fruit was used to make the banana medium. Na-alginate and calcium chloride (CaCl₂) (Sigma, St. Louis, MO, USA) were applied to form the immobilizing matrix for the entrapment of cells. Whole bananas with peels were washed with a solution of 20 ppm chlorine in water and then peeled by hand after Blanching in boiling water for 15 min (Aegerter and Dunlap, 1980; de Porres et al., 1985). Aseptic water was added in a water-to-peeled-fruit ratio of 3:1 (w/w), and the mixture was reduced to a puree by a sterile Waring blender and homogenized by using a homogenizer (Polytron PT3000, Switzerland). Homogenized puree was then poured into flasks for fermentation.

L. acidophilus was cultured statically in 500 ml MRS broth which was adjusted to pH 6.5 with 1 M HCl before autoclaving. A 1% inoculum was added, incubated at 37°C for 12–14 h, collected by centrifugation at 8,000×g for 15 min at 0°C and washed twice with 0.1% peptone water (King and Su, 1993). Cell cultures obtained were then suspended in 0.1% peptone water to make a concentrated cell suspension. The concentrated cell suspension was mixed with 2% (w/v) sterile Na-alginate solution in a 1:1 volume ratio, and the resulting 1% alginate-bacteria mixture was extruded as droplets through a needle by using a peristaltic pump (Eyela MP-3, Japan). The droplets were then dropped into a gently stirred (70 rpm) sterilized 0.1 M calcium chloride solution at room temperature and stored in the solution for 30 min to allow cell-immobilized Ca-alginate gel beads to complete gelation before their use (King and Zail, 1983). Beads of diameters around 2.6 mm were obtained.

Cooled, homogenized puree was inoculated with L. acidophilus and incubated at 37°C for 80 h. The inoculated L. acidophilus was separated into two lots, i.e. free cells and immobilized cells. Two percent (v/v) inoculum was applied in free cell fermentation and 4% (v/v) was employed in immobilized cell fermentation since the cell concentration in the immobilized gel beads had been half diluted during cell immobilization, and therefore equal initial cell concentrations could be obtained. The variation of viable cell number, pH, FOS, and reducing sugar content was monitored periodically during incubation. Free cells were cultured in MRS agar medium after a series of appropriate dilutions, and the number of viable cells was counted by using the standard plate count method at 37°C for 48 h. For immobilized cells, Ca-alginate gel beads containing cells were dissolved in sterile 1% (w/v) sodium citrate solution with gentle shaking for 20 min at room temperature to depolymerize the beads and produce a cell suspension, and cells were then serially diluted and cultured for colony counting (Champagne et al., 1996). Reducing sugars (Toriya et al., 1998) and FOS contents (Campbell et al., 1997) were analyzed by HPLC. The pH was measured directly using a pH meter (HI 9021, Hanna Instruments, Taiwan). All of these determinations were carried out in six replications.

The growth of both free and immobilized L. acidophilus in banana media during fermentation is shown in Fig. 1. The viable cell number of free cells was found to be lower than that of immobilized cells. Since the cell number in the immobilized gel beads increased owing to the growth of cells, some bacteria leaked from the entrapped gel beads and grew in the medium solution which initially had no bacteria ( Jouenne et al., 1992). Bacteria were found to propagate in the medium suspension around 5 h after the start of the fermentation of immobilized cells, and the final cell concentration in the suspension reached the
Growth curves for free and Ca-alginate immobilized *L. acidophilus* in banana media. ▲, immobilized cell (suspension); ■, immobilized cell (gel bead); ●, free cell.

The final viable cell number of the free cell fermentation was found to be around 10^6 CFU/ml. These results indicate that higher viable cell numbers, compared to free cells, are reached during immobilized cell fermentation (Pilkington et al., 1999). This might be due to the protection effect provided by cell immobilization (Champagne et al., 1994) and unfavorable conditions, such as pH as low as 4.7, in the banana media could be overcome, and better results could be obtained.

The variation of reducing sugar content and pH in banana media for free and immobilized *L. acidophilus* fermentation are shown in Figs. 2 and 3, respectively. It was found that the utilization of reducing sugar was higher for immobilized cells compared to free cells during fermentation. The pH value in the free cell media was found to be higher than in immobilized cells. Figures 1, 2, and 3 show possible relationships existed between variation of cell growth, pH, and reducing sugar content observed in this study. Better cell growth might be observed by the better utilization of the media, and higher reducing sugar consumption and higher acid production could be found in immobilized cell fermentation (de Porres et al., 1985). This caused a lower pH and lower residual reducing sugar content in the immobilized cell fermentation compared to that for free cells.

The use of FOS, including 1-kestose (GF_3), nystose (GF_3), and 1′-β-fructofuranosylnystose (GF_4), during the fermentation of banana media by immobilized and free *L. acidophilus* is shown in Fig. 4. It was found that GF_3 and GF_4 were not consumed during fermentation, and the decrease in GF_2 content was higher in free cells compared to immobilized cells. This might be due to the mass transfer resistance of the gel matrix and less GF_2 was obtainable for immobilized cells. On the other hand, because low-molecular-weight carbon-sources, e.g. fructose and glucose, were available and more susceptible to the fermentation, higher-molecular-weight carbon-sources, e.g. GF_3 and GF_4, remained unchanged (Kaplan and Hutkins, 2000). FOS
which remained unfermented could act as prebiotics for *L. acidophilus* growth after human consumption. Based on the symbiotic point of view, Ca-alginate immobilized *L. acidophilus* fermented banana medium was found to be an appropriate product. Its fermentation was more efficient, and *L. acidophilus* and FOS contents were higher than those of free cell fermented banana medium. In conclusion, FOS contents in Ca-alginate immobilized cell fermented banana medium showed no significant change, and this immobilized *L. acidophilus* fermented banana medium, composed of high numbers of immobilized cells as well as FOS contents, could be developed as a potential product with symbiotic benefits.

**References**


