# 行政院國家科學委員會專題研究計畫成果報告
## 利用細胞固定化嗜酸乳酸桿菌發酵香蕉培養基的探討
## Fermentation of Banana Media by Using Cell Immobilized *Lactobacillus acidophilus*

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### 一、中文摘要

本研究使用香蕉作為原料來製備培養基，進行嗜酸乳酸桿菌的發酵，並分別利用褐藻酸鈣及κ-紅藻膠凝膠所製備得之膠球來進行細胞的包埋固定化，所得褐藻膠膠球之直徑約 2.6 mm，κ-紅藻膠膠球直徑約為 3.0 mm，以嘗試提升嗜酸乳酸桿菌在香蕉培養基中的發酵效率。所用香蕉原料包括綠香蕉及成熟香蕉，分別利用固定化及游離態菌體來進行發酵 80 小時。在發酵過程中，不論是固定化或是游離態的嗜酸乳酸桿菌發酵，成熟香蕉培養基中的活菌數均高於綠香蕉培養基。在固定化菌體的發酵生長過程中，部份菌體會自包埋的膠球中脫離進入培養基溶液中生長。在固定化菌體發酵實驗部份，培養基懸浮液中的最終活菌數可達 $10^5$ CFU/ml 的程度，固定化膠球中的活菌體濃度可達 $10^8$ CFU/ml gel 以上；而在游離態發酵組中，最終活菌為 $10^6$ CFU/ml 的程度。固定化菌體能克服綠香蕉培養基中的不利條件，呈現出較佳的生長。在發酵過程中，pH 值與可滴定酸度的變化和菌株的生長情況有明顯的關聯。成熟香蕉培養基經固定化嗜酸乳酸桿菌發酵後，其中果寡醣的含量變化不大，可作為嗜酸乳酸桿菌之益菌物質，具有成為良好 synbiotics 產品之潛力，其中褐藻酸鈣固定化者又優於κ-紅藻膠固定化者。由成本分析的整體結果發現，褐藻酸鈣固定化要優於κ-紅藻膠固定化。另一方面，將嗜酸乳酸桿菌分別以褐藻酸鈣及κ-紅藻膠進行固定化，探討細胞固定化對菌體凍結及冷凍乾燥時存活的保護效果，以及其對冷凍乾燥菌體在 5℃、25℃、45℃、60℃、70℃等不同溫度下之貯藏安定性的影響。實驗所用游離態及固定化菌體的初始濃度均達 $10^{10}$ cells/ml 層次的程度，結果發現褐藻酸鈣與κ-紅藻膠膠球的固定化均能有效地提供保護效果，減少菌株在操作過程中所受到的傷害。在所用的不同貯藏溫度下，不論是游離態或是固定化的菌體，其 Log D 值與貯藏溫度間均具有極高的相關性，而由此二種狀態菌體所得之二條 Log D 與貯藏溫度的迴歸直線所求出之 z 值間亦具顯著差異($p < 0.05$)，顯示細胞固定化能增加冷凍乾燥菌體在貯藏過程中對溫度的耐受性，並且能減低溫度變化對冷凍乾燥菌體貯藏安定性的影響。

**關鍵詞**：香蕉、嗜酸乳酸桿菌、細胞固定化、發酵、益生菌、益菌物質、果寡醣、合生素、冷凍乾燥、貯藏安定性、成本分析

### Abstract

Banana was used as the raw material for the preparation of fermentation media of *Lactobacillus acidophilus*, and cell immobilization was applied to improve the fermentation efficiency of *L. acidophilus* in banana media. Cell immobilization was performed using calcium alginate and κ-carrageenan as the entrapping matrix, and gel beads of diameters around 2.6 mm for the former and 3.0 mm for the latter were obtained. Both green and ripe bananas were used for the preparation of banana media, and both free and immobilized cells were used to conduct the fermentation for 80 hours. The viable cell number in ripe banana media was found to be higher than that in green ones during both free cell and immobilized cell fermentation. During the fermentation of immobilized cell, cells would leak out from the gel beads and grew in the medium solution. In immobilized cell fermentation, the final viable cell number could reach $10^5$ CFU/ml in medium suspension and that in gel beads could become over $10^8$ CFU/ml gel. In free cell fermentation, the final viable cell number was around $10^6$ CFU/ml. Immobilized cell could overcome the unfavorable conditions in green banana media and improved results could be obtained. During the fermentation, the variation of pH and titratable acidity showed obvious relationships with the growth of cells. Variation of fructooligosaccharides contents in ripe banana media was not remarkable in immobilized cell fermentation compared to free cell. Immobilized *L. acidophilus* fermented banana medium was able to be used as a synbiotic product by combining the probiotic effect of *L. acidophilus* and the prebiotic effect of banana. The effect of Ca-alginate immobilization was better than κ-carrageenan. Based on the overall results of cost analysis, Ca-alginate immobilization was a better choice compared to κ-carrageenan immobilization. On the other hand, *L. acidophilus* was immobilized using Ca-alginate and κ-carrageenan, and protection effects of cell immobilization on the viability of the bacteria after freezing and freeze-drying were studied, and its influence on the storage stability of the freeze-dried cells at 5℃, 25℃, 45℃, 60℃, 70℃ was also investigated. Initial concentration of both free and immobilized cells used for experiments all reached the level of $10^{10}$ cells/ml. Results indicated that the immobilization in Ca-alginate gel beads and κ-carrageenan gel beads could provide effective protection to reduce the damage of bacteria under operations. High correlations were obtained between Log D values and storage temperatures for both free and immobilized cells under those various storage temperatures used. the z value which derived from the linear regression equation of Log D and storage temperature for free and immobilized cells were significantly different ($p < 0.05$). Cell immobilization could enhance temperature tolerance of the freeze-dried bacteria during storage and diminish the influence of temperature variation on the storage stability of freeze-dried cells.

Keywords: Banana, *Lactobacillus acidophilus*, Cellimmobilization, Fermentation, Probiotic, Prebiotic, Fructooligosaccharides, Synbiotic, Freeze-drying, Storage stability, Cost analysis.

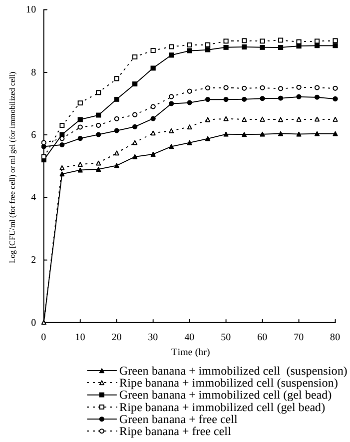
## 二、緣由與目的

嗜酸乳酸桿菌(*Lactobacillus acidophilus*)是一種具有益生效果(probiotic effect)的乳酸菌，對人體具有整腸之療效，常用於保健食品的製造。此菌最初是由幼兒及成人糞便中所分離，其與大多數乳酸桿菌之不同處為對膽汁具有抵抗力，而且可抑制腸內有害菌之生長，因此在一些市售之乳製品及乾燥食品中皆有添加此菌，以助益人體健康。由於市售之乾燥食品中嗜酸乳酸桿菌之存活率很低，因此如何藉由適當的加工方法以提高發酵產品中乳酸的產率、發酵效率，以及液態發酵產品及冷凍乾燥食品中嗜酸乳酸桿菌的存活率，實為一重要的課題。細胞固定化(cell immobilization)為利用聚合物、玻璃、無機鹽類等適當的擔體以結合、架橋或包埋等方式，來將微生物菌體作某種空間限制，以加以有效利用的方法，能提供多項優於傳統發酵程序及固定化酵素程序的優點，因此在各方面的應用日廣，尤其在食品方面的利用更是日益增加。菌體固定化能有效提高菌體對 pH、溫度等外界環境因子的耐受性，增加其貯藏時的安定性，同時並能有效提高菌體濃度及操作處理過程中菌體的存活率，以及增加作用效率。無論是菌株的冷凍乾燥、保存，或是諸如香蕉泥等液態乳酸食品的發酵，在加工與操作過程中，均存在有不利於嗜酸乳酸桿菌生存繁殖的因子。因此藉由嗜酸乳酸桿菌菌體的固定化，可以提高該菌在冷凍、乾燥、發酵等操作過程中菌體的存活，增加最終產品的保存

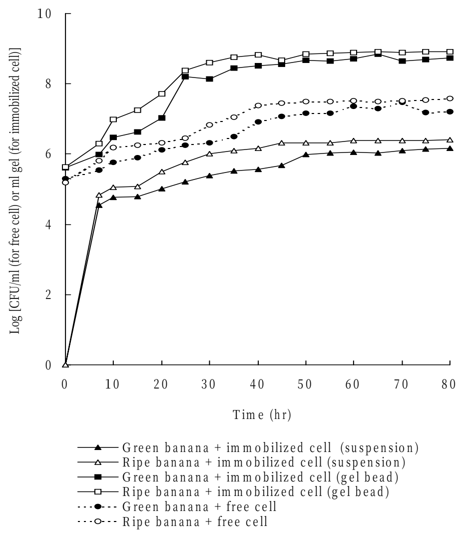
性與菌株的可利用性，並提高產品附加價值以及食用時菌株在人體腸道中的存活率。香蕉為台灣的重要農產作物，營養價值頗高，而且常常會因產銷失調而造成生產過剩，因此開發適當的加工方法以製成風味性質與貯藏性質俱佳的香蕉加工品，確為一值得努力的方向。香蕉含有多量適合乳酸菌發酵所需的醣類，利用香蕉作為培養基以乳酸菌進行發酵來製得乳酸菌發酵香蕉產品的研究，國內外有不少學者做過嘗試，其目的在延長香蕉產品的貯藏性，改善製品的風味，並提升製品的營養保健效果。此外香蕉含有益生菌(probiotics)生長所需之益菌物質(prebiotics)-果寡醣(fructooligosaccharides)，因此在香蕉產品中加入嗜酸乳酸桿菌，以助益乳酸菌在人體腸胃道中之生長發育，是開發乳酸菌營養保健產品時所值得考慮的一個方向。本研究嘗試利用固定化嗜酸乳酸桿菌(immobilized *L. acidophilus*)技術，來探討菌體細胞固定化對嗜酸乳酸桿菌於冷凍乾燥以及隨後之保存過程中菌株存活之影響，並探討嗜酸乳酸桿菌於香蕉培養基中進行發酵時，固定化與游離態菌株二者在發酵時菌體濃度與發酵效率的差異。另一方面，有效結合香蕉的營養價值與乳酸菌的益生特性，配合細胞固定化的技術，以提升香蕉乳酸菌產品的保健效果，亦是本研究所探討的一項課題。

## 三、結果與討論

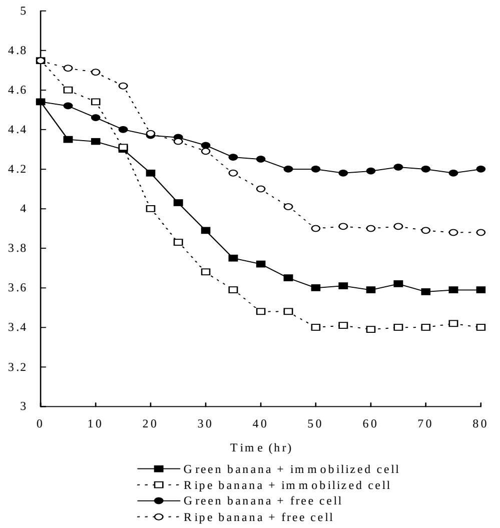
1. 不論是成熟香蕉培養基或是綠香蕉培養基，固定化 *L. acidophilus* 的發酵結果均優於游離態菌體。不論是固定化或是游離態 *L. acidophilus*，在成熟香蕉培養基中的發酵效果均優於在綠香蕉培養基中的結果(圖 一、圖二)。
2. 隨著發酵過程的進行，香蕉培養基中的 pH 值、還原糖量均會隨著菌數的增加而降低，而可滴定酸度則隨著菌數的增加而上升(圖三~圖八)。
3. 不論是褐藻酸鈣或是紅藻膠固定化 *L. acidophilus*，固定化菌體經凍結以及冷凍乾燥後之菌體存活均較游離態菌體為高。固定化 *L. acidophilus* 冷凍乾燥後之貯藏效果要優於游離態菌體，具有較佳之存活率(圖九、圖十、表一~表四)。
4. 成熟香蕉培養基經固定化 *L. acidophilus* 發酵後，發現其中果寡醣的含量變化不大，可作為 *L. acidophilus* 之益菌物質。其中褐藻酸鈣固定化者又優於 κ-紅藻膠固定化者。經固定化 *L. acidophilus* 發酵之成熟香蕉培養基，具有成為良好之 synbiotics 產品之潛力(圖十一)。
5. 成本分析的結果發現，採用固定化菌體來進行香蕉培養基發酵，遠比利用游離態菌體有利，而其中以褐藻酸鈣來進行固定化會比以 κ-紅藻膠來進行固定化具有較佳之投資報酬(表五、表六)。



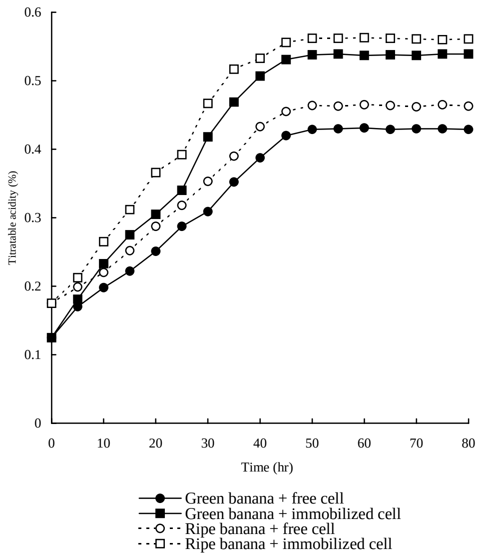
圖一、褐藻酸鈣固定化與游離態嗜酸乳酸桿菌在不同香蕉培養基中之生長曲線



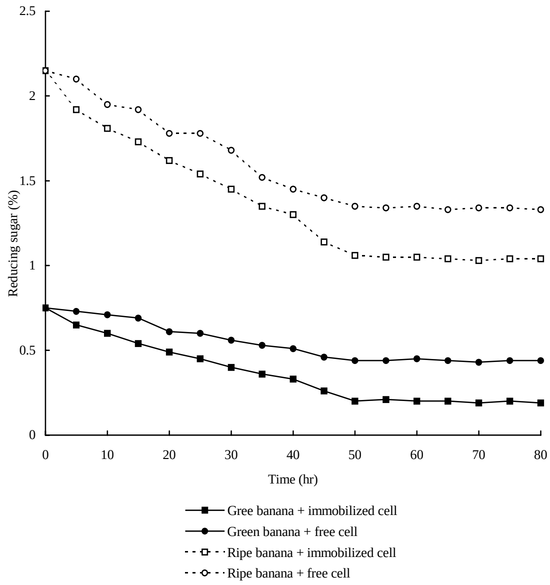
圖二、κ-紅藻膠固定化與游離態嗜酸乳酸桿菌在不同香蕉培養基中之生長曲線



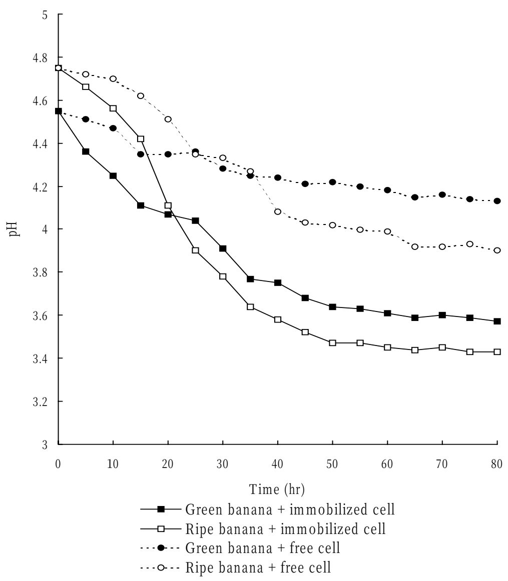

圖三、不同香蕉培養基在褐藻酸鈣固定化與游離態嗜酸
乳酸桿菌發酵過程中 pH 的變化



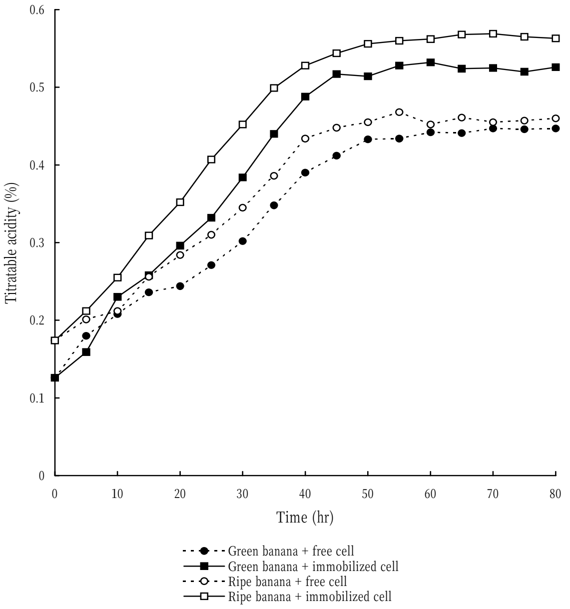

圖四、不同香蕉培養基在褐藻酸鈣固定化與游離態嗜酸
乳酸桿菌發酵過程可滴定酸度的變化



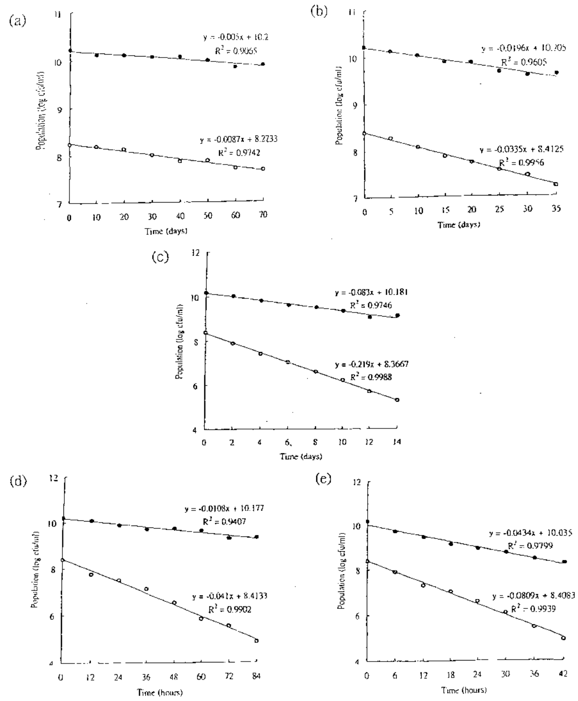

圖五、不同香蕉培養基在褐藻酸鈣固定化與游離態嗜酸
乳酸桿菌發酵過程還原糖量的變化



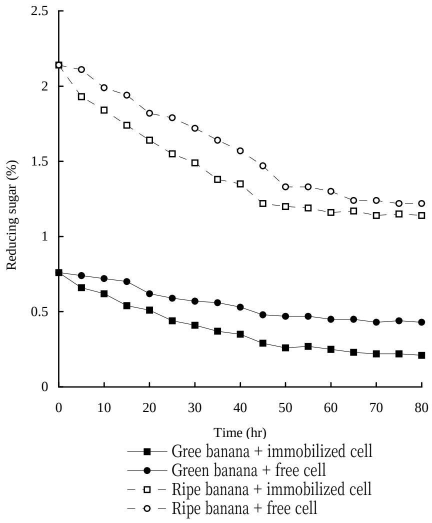

圖六、不同香蕉培養基在 κ -紅藻膠固定化與游離態嗜酸
乳酸桿菌發酵過程中 pH 的變化



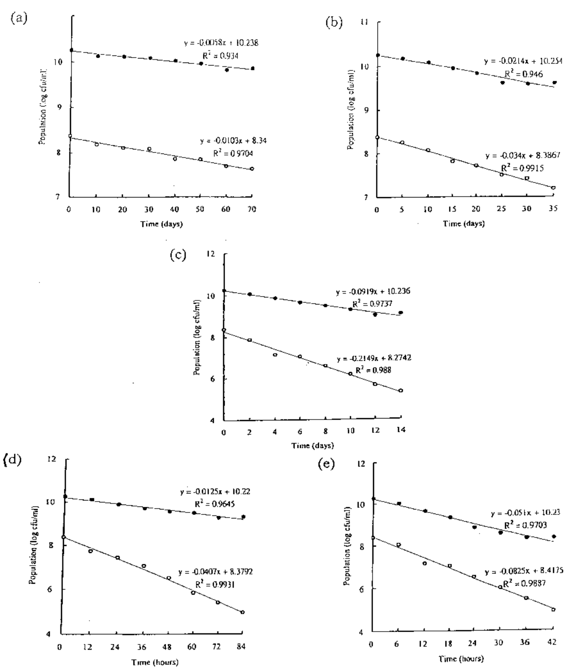

圖七、不同香蕉培養基在 κ -紅藻膠固定化與游離態嗜酸乳酸桿菌發酵過程中可滴定酸度的變化



圖九、冷凍乾燥嗜酸乳酸桿菌在(a)5℃,(b)25℃,(c)45℃,(d)60℃,(e)70℃下貯藏過程中存活率的變化(○：游離態菌體，●：褐藻酸鈣固定化菌體)




圖八、不同香蕉培養基在 κ-紅藻膠固定化與游離態嗜酸乳酸桿菌發酵過程中還原糖量的變化

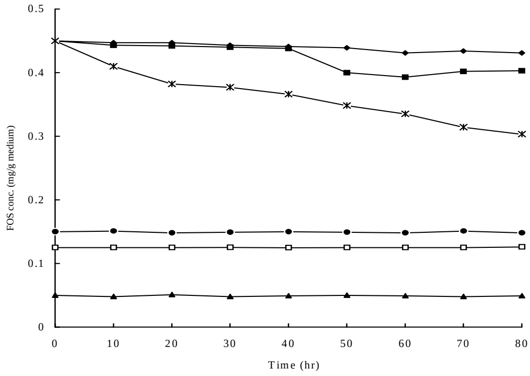


圖十、冷凍乾燥嗜酸乳酸桿菌在(a)5℃,(b)25℃,(c)45℃,(d)60℃，(e)70℃下貯藏過程中存活率的變化(○：游離態菌體，●：κ-紅藻膠固定化菌體)

表一、游離態及褐藻酸鈣固定化嗜酸乳酸桿菌在凍結及冷凍乾燥後之存活菌數(log CFU/ml 及 log CFU/ml gel)

| State | Initial | Freezing | Freeze-drying |
|---|---|---|---|
| Immobilized cells | $10.80^{ax*}$ | $10.49^{bx}$ | $10.22^{cx}$ |
| Free cells | $10.81^{ax}$ | $9.60^{by}$ | $8.45^{cy}$ |

$^{*a\text{-}c}$ Different letters within a row are different at a 5% significant level (n = 6).
$^{x\text{-}y}$ Different letters within a column are different at a 5% significant level (n = 6).




-◆- Ripe $GF_2$ (Ca-alginate immobilization)
-■- Ripe $GF_2$ (κ-carrageenan immobilization)
-✳-Ripe $GF_2$ (Free cell) -●- Ripe $GF_4$
-□- Green $GF_2$ -▲- Ripe $GF_3$

圖十一、固定化及游離態嗜酸乳酸桿菌香蕉培養基發酵過程中果寡醣(FOS)含量的變化

表二、不同貯藏溫度下，褐藻酸鈣固定化與游離態冷凍乾燥嗜酸乳酸桿菌菌體的 D 值與所得之迴歸方程式和 z 值

| Freeze-dried sample | D value 5°C | 25°C | 45°C | 60°C | 70°C | Equations[a] | Correlation coefficient ($R^2$) | Z value[b] (°C) |
|---|---|---|---|---|---|---|---|---|
| Free cells | 2746.6 | 717.6 | 109.6 | 24.4 | 12.4 | Log $D_T$ = 3.69-0.0373T | 0.9951 | $26.8^{x*}$ |
| Immobilized cells | 4838.7 | 1224.5 | 289.2 | 92.6 | 23.0 | Log$D_T$ = 3.92-0.0344T | 0.9857 | $29.1^{y}$ |

[a] $D_T$ is D value (time required to obtain one log variation in population for free cells in solution and immobilized cells in gel) for a given storage temperature T; D values are in hours.
[b] Z value is the temperature span (in ℃) required to obtain a 10-fold variation in D values.
$^{*x\text{-}y}$ Different letters within a column are different at a 5% significant level (n = 6).

表三、游離態及κ-紅藻膠固定化嗜酸乳酸桿菌在凍結及冷凍乾燥後之存活菌數(log CFU/ml 及 log CFU/ml gel)

| State | Initial | Freezing | Freeze-drying |
|---|---|---|---|
| Immobilized cells | $10.80^{ax*}$ | $10.54^{bx}$ | $10.26^{cx}$ |
| Free cells | $10.81^{ax}$ | $9.51^{by}$ | $8.38^{cy}$ |

$^{*a\text{-}c}$ Different letters within a row are different at a 5% significant level (n = 6).
$^{x\text{-}y}$ Different letters within a column are different at a 5% significant level (n = 6).

表四、不同貯藏溫度下，κ-紅藻膠固定化與游離態冷凍乾燥嗜酸乳酸桿菌菌體的 D 值與所得之迴歸方程式和 z 值

| Freeze-dried sample | D value 5°C | 25°C | 45°C | 60°C | 70°C | Equations[a] | Correlation coefficient ($R^2$) | Z value[b] (°C) |
|---|---|---|---|---|---|---|---|---|
| Free cells | 2330.1 | 705.9 | 111.7 | 24.6 | 12.1 | Log $D_T$ = 3.6393-0.0364T | 0.9922 | $27.5^{x*}$ |
| Immobilized cells | 4137.9 | 1121.5 | 261.2 | 80.0 | 19.6 | Log$D_T$ = 3.8723-0.0345T | 0.9836 | $29.0^{y}$ |

[a] $D_T$ is D value (time required to obtain one log variation in population) for a given storage temperature T (℃); D values are in hours.
[b] Z value is the temperature span (in ℃) required to obtain a 10-fold variation in D values.
$^{*x\text{-}y}$ Different letters within a row are different at a 5% significant level (n = 6).

表五、各生產模式所用材料之單位成本

| 材料 | 模式(1) | 模式(2) | 模式(3) | 市價[a] |
|---|---|---|---|---|
| 嗜酸乳酸桿菌 | 2000 元/株 | 2000 元/株 | 2000 元/株 | 1 元/1 億活菌 |
| 果寡醣 | — | — | — | 1 元/公克 |
| 香蕉 | 20 元/公斤 | 20 元/公斤 | 20 元/公斤 | |
| 固定化凝膠 | — | κ-紅藻膠[b]，5000 元/公斤；KCl，900 元/公斤 | 褐藻酸鈉[b]，4500 元/公斤；$CaCl_2$，1000 元/公斤 | |

[a] 為市售台糖公司產品之價格。
[b] 為美國 Sigma 公司產品之價格。

表六、以發酵完成之培養基 1 公升作基準所作之投資報酬的比較

| | 項目 | 模式(1) | 模式(2) | 模式(3) |
|---|---|---|---|---|
| 成本 | 香蕉 | 20 元 X0.25=5 元 | 20 元 X0.25=5 元 | 20 元 X0.25=5 元 |
| | 膠體 | — | κ-carrageenan 4 元<br>KCl (以 500 ml 計) 10 元 | Na-alginate 1.8 元<br>$CaCl_2$ (以 500 ml 計) 11.2 元 |
| | 合計 | 5 元 | 19 元 | 18 元 |
| 收益 | 活菌 | $10^6$ x $10^3$ = $10^9$<br>⇨ 10 元 | gel $10^8$ x 40<br>suspension $10^5$ x $10^3$<br>共 4.1 x $10^9$<br>⇨ 41 元 | gel $10^8$ x 40<br>suspension $10^5$ x $10^3$<br>共 4.1 x $10^9$<br>⇨ 41 元 |
| | FOS | 0.5 g ⇨ 0.5 元 | 0.65 g ⇨ 0.65 元 | 0.65 g ⇨ 0.65 元 |
| | 合計 | 10.5 元 | 41.65 元 | 41.65 元 |
| 總結算 | | + 5.5 元 | + 22.65 元 | + 23.65 元 |

## 四、計畫成果自評

本計畫所得成果已達成原計畫書的目標，內容並切實遵照原計畫書的構想，具產業實際應用及學術上的價值。所得成果已寫成兩篇論文投稿 SCI 學術期刊(J. Gen. Appl. Microbiol., 49: 357-361; Int. J. Food Microbiol., 91: 215-220)。

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