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Preservation of Lactic Acid Bacteria on Anhydrous Silica Gel for Three Years

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ABSTRACT

This study, which covers three years of storage, analyzes the application of silica gel preservation methods to lactic acid bacteria widely used in yogurt and cheese fermentation. Strains of *Streptococcus lactis*, *Streptococcus lactis* subsp. *diacetylactis*, *Streptococcus cremoris*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus helveticus* and a yogurt culture were adsorbed on anhydrous silica gel in screw-cap tubes or in ordinary test tubes which were subsequently flame-sealed under vacuum. During 3 years, the bacteria were tested for viability by incubation in sterile milk. All of the bacteria retained their acidifying activity, with the exception of the yogurt culture. Extending preservation for more than 2 years had a negative effect on the activity of the yogurt culture. Results obtained support the use of screw-cap tubes which, in general, were suitable to preserve suspensions of lactic acid bacteria adsorbed on anhydrous silica gel.

Storage and maintenance of lactic acid bacteria are important aspects in the manufacture of consistently good starter cultures (*4*). Preliminary studies by Tessi et al. (*9*), using the method of Trollope (*10*) to assess the preservation of lactic acid bacteria on anhydrous silica gel, have shown that these cultures can be stored at about 5°C for 4 years.

This report is a continuation of the above mentioned studies; it shows a 3 year evaluation of the acidification power of mesophilic and thermophilic lactic acid bacteria, which are frequently used to manufacture many dairy products. Comparatively, we employed Sordelli's modified method (*9*).

MATERIALS AND METHODS

Origin and maintenance of cultures

Lyophilized strains were obtained from commercial sources. The following cultures were used: *Streptococcus lactis*, *Streptococcus diacetylactis* (*S. lactis* subsp. *diacetylactis*; *1*), *Streptococcus cremoris*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus helveticus* and yogurt starter. Cultures were maintained by weekly transfer of a 2% inoculum into 13% (wt/vol) constituted non-fat-dry milk. After incubating mesophilic lactic acid bacteria for 24 h at 30°C and thermophilic bacteria for 3 to 4 h at 43°C, the cultures were stored at 4°C. Reserve stock was maintained by Sordelli's modified methods (*9*).

Each culture of mesophilic and thermophilic lactic acid bacteria was streaked for identification onto duplicate plates of MRS agar (Oxoid) (*3*) and incubated anaerobically in a GasPak system (BBL) at 30 and 37°C. Four to eight colonies were picked from each plate of a convenient dilution for microscopic examination and subsequent identification. Purified strains of lactic acid bacteria stored in stab cultures of MRS agar (*3*) were identified according to the criteria of *Bergey's Manual of Determinative Bacteriology* (*1*) using the methods of Sharpe et al. (*7*) for streptococci and Rogosa and Sharpe (*5*) and Rogosa (*6*) for lactobacilli.

Preservation on anhydrous silica gel

The method described by Trollope (*10*) was used for the preservation of lactic acid bacteria. Precooled cell suspensions were adsorbed on anhydrous silica gel in screw-cap tubes (SCT) or in ordinary test tubes that were subsequently flame-sealed under vacuum (VT) in the manner described by Tessi et al. (*9*). Sordelli's modified method (*9*) was used for comparison purposes. In this procedure, cells were suspended in 0.05 ml of sterile bovine serum in Pyrex glass tubes (50 × 8 mm) with cotton-wool plugs. Each tube was then placed inside a test tube filled with $CaCl_2$ and connected to a vacuum pump until complete dehydration was achieved. The tubes were then sealed under vacuum.

Acidification activity

The acidifying activity of each strain adsorbed on silica gel was determined periodically by placing the granules in sterile skim milk. After three consecutive subcultures, the pH and titratable acidity (°D) were determined as described previously (*9*).

Changes of titratable activity (TA) and pH values through time were the basis for assessment of acidification activity. This was obtained by subtracting the value of titratable acidity at zero time from the final acidity. The same procedure was used for pH values. The acidifying activity calculated for mesophilic bacteria was $\Delta TA_{16}(TA_{16h} - TA_{0h})$ and ΔpH_{16} $(pH_{16h} - pH_{0h})$; for thermophilic bacteria was $\Delta TA_9(TA_{9h} - TA_{0h})$ and $\Delta pH_9(pH_{9h} - pH_{0h})$.

For the strains preserved by Sordelli's modified method, activation in sterile milk was used and calculations were done as above.

Statistical analyses

Data were analyzed using analysis of variance. When significant effects ($P<0.05$) were observed, mean separation was accomplished by Student's t test (*8*). The coefficient of linear correlation was calculated using statistical methods for digital computers (*2*).

RESULTS AND DISCUSSION

Mesophilic lactic acid bacteria

Figure 1 illustrates changes in acidifying activity (ΔTA_{16} and ΔpH_{16}) in skim milk during fermentations by

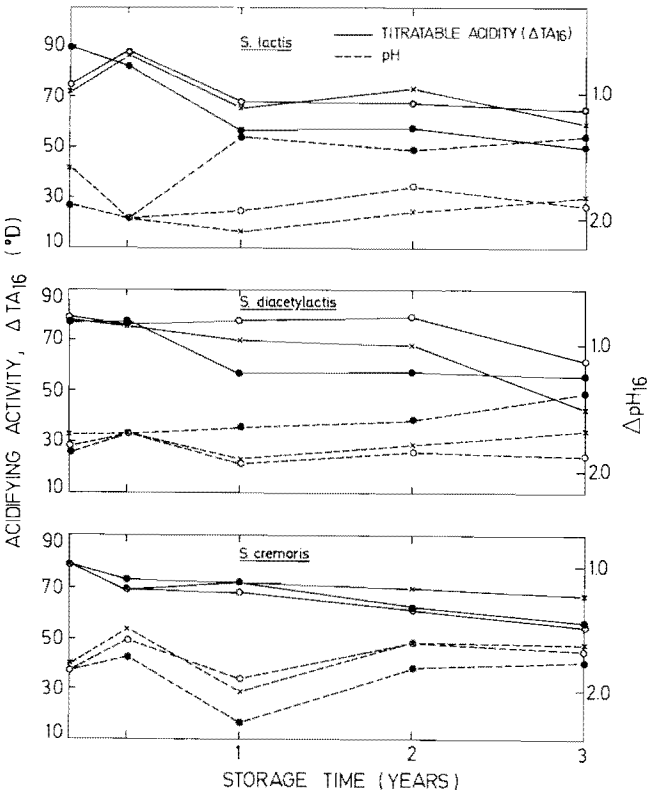

Figure 1. *Effect of storage time on the acidifying activity, ΔTA_{16} (TA_{16h} - TA_{0h}) (°D) and ΔpH_{16}(pH_{16h} - pH_{0h}) of mesophilic lactic acid bacteria, preserved on silica gel in screw-cap tubes (○), in test tubes sealed under vacuum (●), and by Sordelli's modified method (×).*

TABLE 1. *Relationship between acidifying activity of mesophilic and thermophilic lactic acid bacteria (ΔTA_{16} and ΔTA_9)[a] preserved over silica gel and by Sordelli's modified method (SCT, VT and SM)[b].*

Microorganisms	Acidifying activity (SCT, VT and SM)[b]		
	ΔTA(°D)[a]	Regression coefficient	Determination coefficient
S. lactis	16	0.93[c]	0.86
S. diacetylactis	16	0.99[c]	0.98
S. cremoris	16	0.10[c]	0.98
L. bulgaricus	9	0.69[c]	0.47
L. helveticus	9	0.97[c]	0.94
S. thermophilus	9	0.98[c]	0.95
Yogurt starter	9	0.99[c]	0.99

[a]TA, Titratable acidity (°Dornic). $\Delta TA_{16} = (TA_{16h} - TA_{0h})$; $\Delta TA_9 = (TA_{9h} - TA_{0h})$.
[b]SCT, screw-cap tubes; VT, tubes sealed under vacuum; SM, Sordelli's modified method.
[c]No significant difference (P>0.05).

mesophilic lactic acid bacteria that were preserved on anhydrous silica gel (SCT and VT) and by Sordelli's modified method (SM) during a 3-year period. Each plotted point is the average of duplicate determinations from the separate samples. Strains of *S. lactis*, *S. diacetylactis* and *S. cremoris* preserved over silica gel in screw-cap tubes retained their activity during this time.

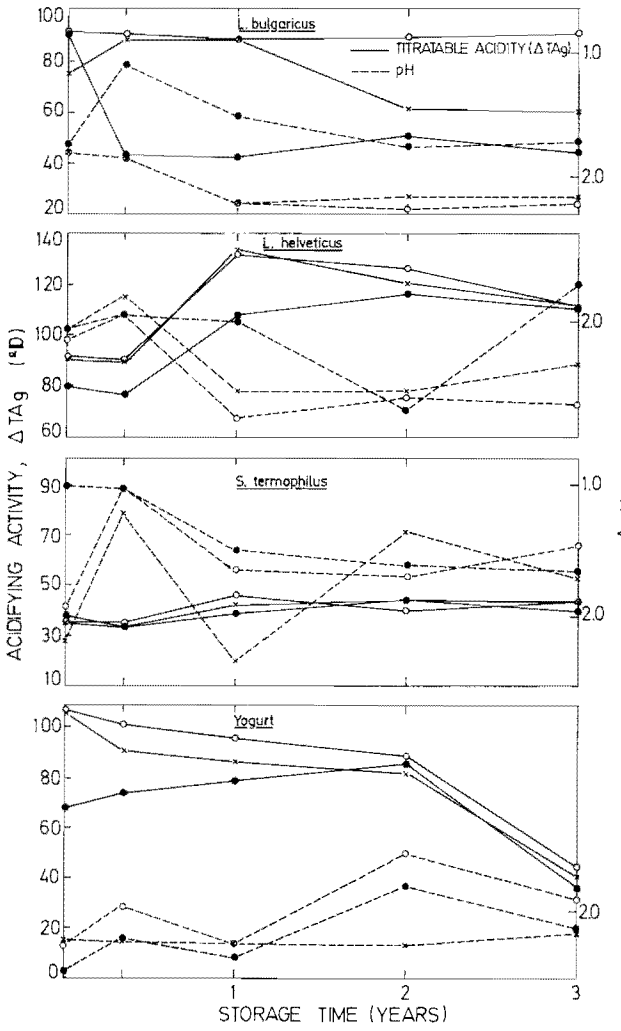

Figure 2. *Effect of storage time on the acidifying activity, ΔTA_9 (TA_{9h} - TA_{0h}) (°D) and ΔpH_9(pH_{9h} - pH_{0h}) of thermophilic lactic acid bacteria, preserved on silica gel in screw-cap tubes (○), in test tubes sealed under vacuum (●), and by Sordelli's modified method (×).*

The data presented in Fig. 1 show a high multiple correlation (P>0.05) between the activities ΔTA_{16} of *S. lactis* and *S. diacetylactis* preserved by the three methods (SCT, VT and SM) (Table 1), but not with respect to *S. cremoris*. Analysis of the data (Fig. 1) by linear regression analysis revealed no correlation (P<0.05) between the values of titratable acidity (°D) and pH.

Thermophilic lactic acid bacteria

Figure 2 presents changes in the acidifying activities (ΔTA_9 and ΔpH_9) in skim milk during fermentations by thermophilic lactic acid bacteria that were preserved by the three methods (SCT, VT and SM) during a 3-year period.

Cultures of *L. helveticus* and *S. thermophilus* preserved on silica gel in screw-cap tubes and by Sordelli's modified method retained their activity. Cultures of *L. bulgaricus* remained stable only when adsorbed on silica gel (SCT). Ex-

tending storage for more than 2 years had a negative effect on the yogurt starter (SCT, VT and SM).

Data shown in Fig. 2 reveals no significant ($P>0.05$) difference between ΔTA_9 for the *L. helveticus, S. thermophilus* and yogurt cultures. The multiple correlation of data presented in Fig. 2 is recorded in Table 1. The regression coefficient (r) showed a highly significant correlation among the acidifying activities (ΔTA_9) of *L. helveticus, S. thermophilus* and the yogurt starter (SCT, VT and SM). Linear regression analysis between the data presented in Fig. 2 revealed no correlation ($P<0.05$) between titratable acidity (°D) and pH values.

The preservation of lactic acid bacteria by either the adsorption method on silica gel in screw-cap tubes or by Sordelli's modified method was very satisfactory. The two first methods (SCT and SM) showed a significant correlation ($P>0.05$) from the statistical point of view. It is important to note that the use of screw-cap tubes to preserve these bacteria has the additional advantage of being easy to accomplish.

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