

PRESERVATION OF *STREPTOCOCCUS THERMOPHILUS* STRAINS AFTER LONG-TERM STORAGE IN LYOPHILIZED STATE

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Summary

The stability of 75 strains Streptococcus thermophilus, preserved during 10 years in lyophilized state, was studied. It was found out, that all the investigated strains were viable. No change in their morphological, biochemical and technological characteristics was registered.

Introduction

The production of original Bulgarian yoghurt, as well as, the yoghurt produced elsewhere in the world are a result of milk fermentation by selected strains of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus*. According to Krueger et al [4] the milk acidification by lactic acid bacteria to pH values around 4.8 is due predominantly to thermophilic cocci.

Recently, a great attention has been paid to investigations of metabolic properties of *S. thermophilus* concerning the production process. *S. thermophilus* strains are thermostable and ferment only few sugars - lactose, sucrose and glucose, and sometimes also galactose [3].

The metabolic activity of the strains from *S. thermophilus* species when inoculated in milk is related to such important strain technological properties like acidification, biosynthesis of aroma components, consistence and viscosity of the obtained fermented milk.

The acidification ability of *S. thermophilus* strains is a function of their carbohydrate metabolism [8]. It is difficult to evaluate objectively the acidification ability of the strains due to the high buffering capacity of milk. According to Accolas et al [1] mistakes in this analysis will be made until new methods for objective evaluation of the strains acidification ability are created. These methods will substitute

the official procedure based on measurement of the titrable milk acidity.

Spünlér and Corrieu [7] used pH meter permanently recording the pH changes during bacterial growth in milk. The same authors compared the acidification ability of the strains on the basis of the obtained pH values for one and the same cultivation period.

The unchangeable strain metabolic activity during long-term preservation in lyophilized state is of special importance for yoghurt production standardization. Rumian et al. have performed investigations on the viability of lactic acid microorganisms [5, 6].

LBB Collection of LB Bulgaricum - Research, Development and Production Center (RDPC) keeps more than 80 strains *S. Thermophilus* isolated from Bulgarian natural sources as a result of long-term investigations. The chosen strains have proved to possess desired organoleptic and technological properties and have been identified according to Bergey's Manual [3].

The aim of the present work was to check the viability and to determine possible changes in the morphological, biochemical and technological properties of strains of *S. thermophilus* species, preserved lyophilized at $6 \pm 1^{\circ}\text{C}$ for 10 years.

Materials and Methods

Microorganisms. Investigations had been performed with 75 strains *S. thermophilus*, property of LBB Collection of LB Bulgaricum - RDPC, registered in the National Bank for Industrial Microorganisms and Cell Cultures and kept lyophilized in glass ampoules at $6 \pm 1^{\circ}\text{C}$ for 10 years.

The preparation and identification of the strains according to morphological and biochemical characteristics was performed using Bergey's Manual [3].

The strains were also studied for several additional characteristics.

Survival determination (bringing out of anabiosis). Ten ml sterile milk was inoculated with 1% lyophilized culture and kept at 42°C for 17 h. The degree of milk coagulation was recorded by potentiometric determination of pH.

Acidification determination. Sterile skim milk was inoculated with 1% liquid starter culture (from the recovered lyophilized

inoculum) and kept at 42°C for 14 h (sample I) and 14 days (sample II). Then the samples were cooled to room temperature and the acidity was measured by different methods such as:

- i) potentiometrical determination of pH;
- ii) determination of titrable acidity in $^{\circ}\text{T}$ (Bulgarian State Standard 1111-80) and its recalculation as percentage lactic acid (according to the accepted formula $\% \text{ lactic acid} = 0.009 \times ^{\circ}\text{T}$). The obtained results showed the acidifying ability of the tested strains;
- iii) enzyme determination of the lactic acid existence using Boehringer tests [2];
- iv) enzyme determination of the glucose and galactose as products of lactose hydrolysis using Boehringer tests [2];
- v) organoleptic evaluation of the fermented by these strains milk.

Results and Discussion

It was found out that all 75 *S. thermophilus* strains, being kept in lyophilized state at 6°C were viable.

The carried out experiments which use traditional biochemical methods (according to Bergey's Manual) confirm the belonging of the strains to *Streptococcus salivarius subsp. thermophilus* species.

No morphological changes of the cell were observed, when they were included in the collection, i.e. the data of the strains corresponded completely to the initial ones. Diplococci, short chains and single longer chains with typical for *S. thermophilus* dimensions were observed microscopically.

The carried out organoleptic tests showed that the consistence of the coagulated with these strains milk is different for the various strains: from hard smooth to slightly granular. The milk that fermented with them had a specific taste and smell of milk acid. No undesirable deviations in the organoleptic properties were registered when the *S. thermophilus* strains were recovered after being kept in a lyophilized state for 10 years.

The studies on the acidifying properties of the strains showed that they can coagulate the milk when kept for 14 h at 42°C . The pH

values measured are between 4.35 and 4.60. The titrable acidity was between 84 and 94°T , i.e. 0.74 - 0.85 % lactic acid. between 0.4 and 0.6 % optically active lactic acid was accumulated in the milk, only L (+) isomer. The strains were found to hydrolyze between 14 and 28 % of the initial lactose (Fig. 1 and 2). After 14 days at 42°C , pH values of 4.0 - 4.2

were found in the milk inoculated with these strains, as well as a titrable acidity of 105 - 130°T (or estimated as 0.94 - 1.18 % lactic acid). The strains biosynthesized 0.76 to 0.94 % optically active lactic acid, only L (+) isomer, and metabolized 25 - 32 % of the initial lactose (Fig.1 and 2). The experiments carried out showed that the strains did not decompose the galactose obtained as a result of lactose hydrolysis, i.e. it accumulates in the medium in amounts of 0.6 - 0.95 %. Most of the glucose obtained during lactose hydrolysis was assimilated, i.e. it hydrolyzed to lactic acid. These results coincide fully with the findings reported by other authors working with *S. thermophilus* strains from microbial collections abroad.

Comparing our results with the data of the strains, originally deposited it can be

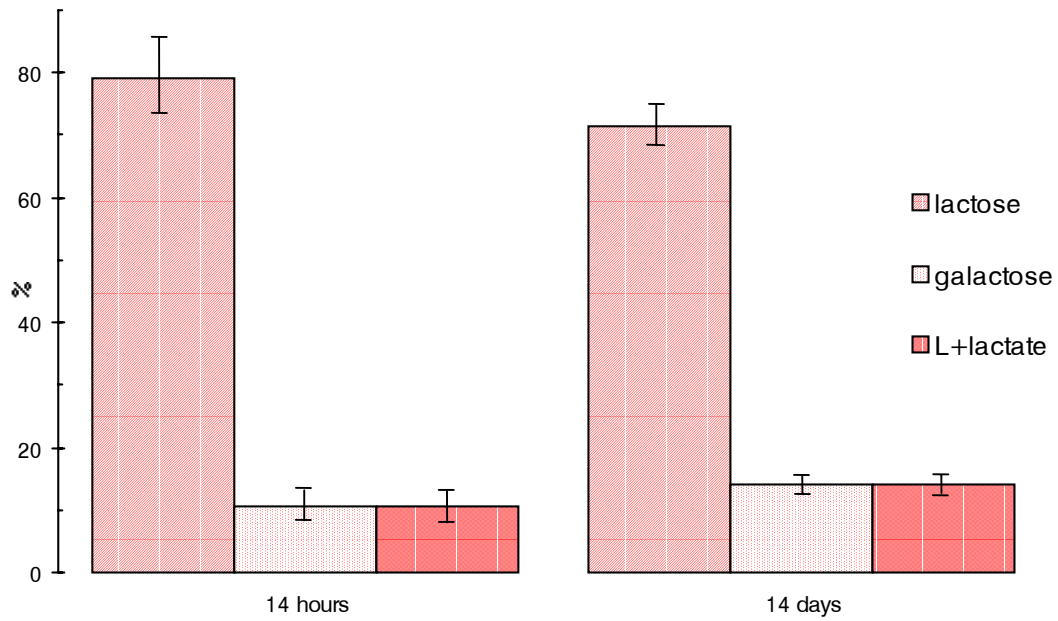


Fig. 1. Characteristics of the acidifying properties of the studied 75 *S. thermophilus* strains. The average data and standard error are presented.

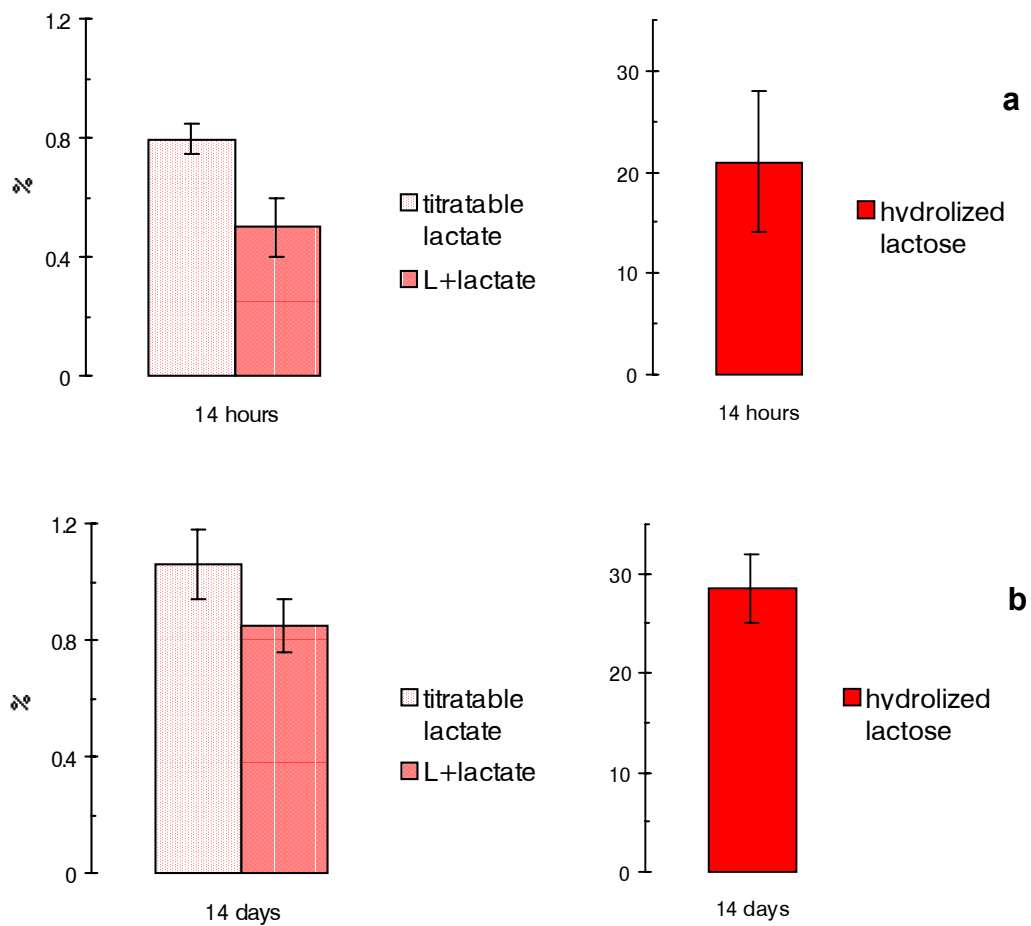


Fig. 2. Carbohydrate content in fermented milk under the action of the studied 75 *S. thermophilus* strains (in rel. %). The average data and standard error are presented. 14 hours (a) and 14 days (b).

summarized that the tested *S. thermophilus* strains, kept lyophilized for 10 years, did not change their initial acidification ability. This fact allowed us to speculate that those strains which express weak acidification can be used for production of yoghurt with low degree of post acidification when stored at 10 - 12°C for a long time.

As a conclusion it can be emphasized

that The LB Collection keeps *S. thermophilus* strains with weak postacidification, which are suitable for production of yoghurt and similar fermented milk products. Also, the method used in the collection for preparation of strains for lyophilization, as well as the lyophilization regime chosen and the storage conditions, guarantee survival and viability of the *S. thermophilus* strains for 10 years.

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