Microorganisms and Characteristics of Laban

A. A. G. BAROUDI and E. B. COLLINS
Department of Food Science and Technology
University of California
Davis 95616

ABSTRACT

Laban had a titratable acidity of about 1.0%, a pH of 4.25, an ethanol content of 1.25%, and contained 4.2 μg acetaldehyde and 34 μg acetoin/ml. There was no diacetyl. Five microorganisms, classified as *Streptococcus thermophilus, Lactobacillus acidophilus, Leuconostoc lactis, Kluyveromyces fragilis*, and *Saccharomyces cerevisiae*, were responsible for the fermentation. *Streptococcus thermophilus* and *L. acidophilus* were responsible for acid production with *S. thermophilus* producing acid more rapidly. Most of the acetaldehyde was produced by *K. fragilis*, little ethanol was found in absence of *S. cerevisiae*, and the acetoin was produced by *S. thermophilus*.

INTRODUCTION

Fermented milks, with different names and ripened by microorganisms under similar conditions, are available in most countries (3, 5, 6, 10). Laban, the popular fermented milk in Lebanon, and some others (6, 11) are both acidic and alcoholic. Laban is made from the milk of cows, sheep, goats, or camels and is eaten plain or with sugar, used in the preparation of cakes, soups, soft drinks, or other foods, or drained to make a type of cream cheese.

A laban starter was brought by the senior author from Lebanon in an insulated container packed with ice, used to make laban, and subsequently studied. This is a report of the identity of the microorganisms in the starter, their characteristics, and the characteristics of laban made from homogenized milk.

MATERIALS AND METHODS

Taxonomic Procedures

The bacteria and yeasts isolated from the laban starter were identified according to Bergey's Manual (2) and the taxonomic study of Lodder (9), respectively. Standard microbiological procedures described in *Manual of Microbiological Methods* (1) were used. Subsequent to their isolation, the microorganisms were maintained by transferring them every 3 wk on slants of Standard Methods agar (bacteria) or malt agar (yeasts). The normal incubation prior to refrigeration was 36 h at 30 C.

Chemical Methods

Ethanol was determined by the alcohol Stat-Pak method (Calbiochemical Co., Los Angeles, CA). Acetaldehyde was determined colorimetrically by the method of Lindsay and Day (8). Acetoin and diacetyl were determined by the Westerfeld method (13), as the sum of both or separately, after separating them with salting-out column chromatography (12). A Klett-Summerson colorimeter, model 800-3, with filter No. 54, was used for Westerfeld analysis. Effluent during chromatographic separations was collected and distributed into test tubes by a Redi-Rak fraction collector (Stockholm, Sweden). Acidity was determined by titration with .1 N sodium hydroxide, and pH was determined with a Coleman pH meter.

RESULTS

Characteristics of Laban

Laban was made by the procedure normally used in Lebanese homes, and the temperatures and times were determined. The procedure was to boil milk for 1 min, permit it to cool to 50 C (45 C, after inoculation), inoculate the milk with 2.5 to 3.0% starter saved from a previous batch and not refrigerated more than 3 wk, permit the temperature to drop to room temperature (26 C) during incubation for about 9 h, and continue the incubation 3 to 5 h at room temperature prior to refrigeration. Subsequent to the experiments on composition reported below, laban was made routinely in the laboratory by cooling 300 ml heated milk to 50 C, inoculating it with 2.5% starter, incubating it at

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30 C for about 9 h (i.e., until the titratable acidity was 1.0 to 1.1%), and holding it at room temperature for 3 to 5 h prior to refrigeration.

Laban made from commercially homogenized milk had an average titratable acidity of 1.05% (range .9 to 1.2%), a pH of about 4.25, an ethanol content of 1.25%, and contained 4.2 μg acetaldehyde and 34 μg acetoin plus diacetyl/ml. Organoleptically, it was tart, slightly yeasty, and had no detectable diacetyl odor.

Identity of Microorganisms

Microscopic observations revealed that the laban culture contained a mixture of bacteria and yeast, about 50% streptococci. Subsequent plating on Standard Methods agar (bacteria) and malt agar (yeasts) resulted in the isolation of five microorganisms that are considered responsible for the fermentation. Three are bacteria; two are yeasts. Specific taxonomic characteristics and identifications follow.

Each of the three bacteria was a Gram positive, catalase negative, nonmotile nonspore-forming organism that produced acid from lactose, glucose, galactose, or sucrose and did not liquefy gelatin. None grew at 22 or 55 C. Two had spherical cells that occurred in pairs and short chains, and the other had rod-shaped cells with rounded ends, that occurred singly, in pairs, and usually as long filamentous chains. One of the cocci produced no gas from glucose and thus was homofermentative. It produced acid, reduction, and coagulation of litmus milk, did not produce ammonia from arginine or acid from maltose, grew at 50 C, had an optimal growth temperature of 40 to 45 C, and thus was identified as *Streptococcus thermophilus*. The other organism with spherical cells produced acid and gas from glucose showing that it was heterofermentative. It did not acidify litmus milk, grew at 40 C, had an optimal growth temperature of 37 C, and was classified as *Leuconostoc lactis*. The bacterium with rod-shaped cells acidified litmus milk, produced no gas from glucose, and produced acid from maltose. It grew optimally at 35 to 38 C and was judged to be *Lactobacillus acidophilus*.

Each of the yeasts produced 1 to 4 spores per ascus, was nonmotile, did not liquefy gelatin, grew optimally at 30 C, well at 22, and slowly at 37 C. Each produced acid and gas from glucose, galactose, or sucrose. One, classified as *Kluyveromyces fragilis*, produced kidney-shaped spores, oval to elongated oval cells, colonies on malt agar that were fringed by pseudomycelia, slight acid without gas from maltose, and acid and gas from lactose. The other was *Saccharomyces cerevisiae*. It produced spherical to slightly oval spores and cells, no mycelia, acid and gas from maltose or raffinose, and slight acid and no gas from lactose or melibiose. It produced a strongly alcoholic odor in 24 h at 22 C on malt agar.

Contribution of Each Isolate to Laban

Laban made by inoculating heated milk with a mixture of the five organisms grown in sterile milk was similar to that made with the original laban culture. Subsequently, laban was made with different combinations of the five. With *S. thermophilus* and *L. acidophilus* excluded, coagulation did not occur; and with only *S. thermophilus* absent, coagulation was slower and little acetoin plus diacetyl were produced. *Leuconostoc lactis* is considered unessential, though, in its absence, there seemed greater tendency for whey separation if incubation at 30 C was continued beyond the normal time. Results showed that *S. thermophilus* produced some acetaldehyde, but most was attributable to *K. fragilis*. There was little ethanol in absence of *S. cerevisiae*.

Diacetyl Not Produced

Though the odor of diacetyl was not detected in laban made with the original culture or with the mixture of organisms, analysis had shown that each contained acetoin plus diacetyl, produced by the culture that had been classified as *S. thermophilus*. To determine whether diacetyl was present, one quantity of milk was inoculated with laban culture, another was inoculated with *S. thermophilus*, and both were treated according to the procedure for making laban. Subsequently, each was tested for diacetyl and acetoin after separation by column chromatography (12). Neither contained diacetyl. There were 36 μg acetoin/ml of the laban and 34 μg/ml of the milk soured by *S. thermophilus*.

DISCUSSION

The laban culture we studied was developed by natural selection and has been used in Lebanon many years for making laban. Finding

it contained five different microorganisms apparently living in association was surprising, though the procedure for making laban undoubtedly contributed to the lack of species domination. Inoculating milk at 50 C with 2.5 to 3.0% starter results in cooling it immediately to about 45 C. *Streptococcus thermophilus* should start growing immediately, followed in order, as the temperature is permitted to decrease slowly, by *L. acidophilus, L. lactis*, and finally by the yeasts, which grew optimally near room temperature. Also, it is possible that some of the species produce growth factors that encourage growth of others.

Each of the microorganisms we isolated from the laban culture, except possibly *L. lactis*, made an important contribution to the characteristics of the final product. *Streptococcus thermophilus* produced acid most rapidly, *K. fragilis* was primarily responsible for the production of acetaldehyde, and *S. cerevisiae* contributed ethanol. *Lactobacillus acidophilus* contributed acidity and possibly enhanced the therapeutic value of laban.

Fermented milks made in the home are important in the diet in several countries including Lebanon. The gain of any health benefits from the microorganisms (except possibly *L. acidophilus*) is controversial (4, 7), but there are many claims indicating that fermented milks are beneficial for the relief of human ailments, especially intestinal disorders (3, 6, 10). In any event, fermented milks are nutritious, and fermentation serves as an excellent means of preserving milk for later consumption.

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