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Production of Traditional Yoghurt Using Starter Culture Obtained from Koumiss*

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Summary: The objective of this study is to use *Lactobacillus paraplantarum* and *Leuconostoc mesenteroides subs. cremoris* to obtain koumiss and practice them in preparation of yoghurt. Twenty five samples of koumiss bought from different places of Kyrgyzstan were used to obtain *Lactobacillus paraplantarum* and *Leuconostoc mesenteroides subs. cremoris*. Standard microbiological methods were conducted for isolation of starter culture microorganisms. Identification process was performed with characterizing by Matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-TOF MS). The obtained starter culture microorganisms were used in preparation of yoghurt according to the traditional method of yoghurt production. Produced yoghurt samples and control group were exposed to sensorial analysis. Sensorial and physical properties of yoghurt prepared by using the isolated strains from koumiss were not found to be significantly different from commercial yogurt in statistical analyses. In conclusion, it was seen that starter culture obtained from koumiss can be used in production of yoghurt and also received results can be used as a base for investigations on using culture microorganisms obtained from koumiss in production of different types of dairy products.

Key words: Koumiss, *Leuconostoc mesenteroides subs. cremoris*, *Lactobacillus paraplantarum*, *Lactobacillus ssp.,* yoghurt.

Geleneksel Yoğurt Üretiminde Kımızdan Elde Edilen Starter Kültürlerin Kullanımı

Özet: Bu çalışma, kımız'dan elde edilen *Lactobacillus paraplantarum* ve *Leuconostoc mesenteroides subs. cremoris* bakterilerinin yoğurt üretiminde starter kültür olarak kullanımını incelemek için gerçekleştirilmiştir. Kırgızistan'ın farlı yerlerinden temin edilen 23 adet kımız örneğinden *Lactobacillus paraplantarum* ve *Leuconostoc mesenteroides subs. cremoris* standart kültür metotları kullanılarak izole edildi. İdentifikasyon için, matriks ile desteklenmiş lazer desorbsiyon/iyonizasyon kütle spektrometresi (MALDI-TOF MS) yöntemi kullanıldı. Elde edilen kültürler geleneksel yöntemle yoğurt yapımında starter kültür olarak kullanıldı. Üretimi yapılan yoğurt örnekleri ile kontrol olarak kullanılan yoğurt örneği duyusal analiz ile değerlendirildi. İstatistiksel analizde yoğurt örnekleri, kontrol grubu arasında önemli bir fark tespit edilemedi. Sonuç olarak, kımızdan elde edilen starter kültürler ile yoğurt üretilebileceği, aynı zamanda elde edilen kültürlerin farklı süt ürünlerinin üretiminde kullanılmasının mümkün olabileceği ifade edilebilir.

Anahtar kelimeler: Kımız, *Leuconostoc mesenteroides subs. cremoris, Lactobacillus paraplantarum*, *Lactobacillus ssp.*, yoğurt.

Introduction

Traditional yoghurt is produced by using a culture of *Lactobacillus delbrueckii* subsp. bulgaricus and *Streptococcus thermophilus* bacteria to meet the standard of identity for yogurt. In addition, other microorganisms belonged to *Lactobacilli* and *Bifidobacteria* genus, are also sometimes added during or after culturing yoghurt (7,15,18).

Koumiss is fermented milk traditionally made from mare`s milk and is important to the people of the Central Asian steppes, including Mongolia, Kazakhstan, Kyrgyzstan and in some Russian and Chinese regions (5,22). Koumiss is a dairy product similar to kefir, but is produced from a liquid starter culture (usually by backslopping), in contrast to the solid kefir grains. Because mare's milk contains more sugars than the cow's or goat's milk fermented into kefir, koumiss has a higher, though still mild, alcohol content (9,19,22). Even in the areas of the world where koumiss is popular today, mare's milk remains a limited commodity.

Because of the health-protective effects of starter cultures, food produced by probiotic microorganisms is estimated as a functional product. To provide a supply of the high-quality and safe health-protective products, the range of products containing probiotic cultures should be in-

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creased. Probiotics may produce bacteriocins, which are defined as ribosomally synthesized antimicrobial peptides, produced as a defense response and generally active against closely related bacteria (17).

Most probiotics belong to *Lactobacillus* and *Bifidobacteria* genera and, the former is the most abundant member of the group of lactic acid bacteria (LAB). Many lactobacilli are used as starter cultures for manufacturing cheeses, yoghurt, sourdough breads, silage, table olives, sauerkraut, fermented fish and sausages. Lactobacilli play a role as natural biopreservatives in non-fermented vegetables (2,6).

The lactobacilli include more than 25 unique species, and the first level of differentiation is based on end-product composition; some are homofermentative, whereas others are heterofermentative. The former are classified as organisms that produce >85% lactic acid as their end-product from glucose. The latter include organisms that produce approximately 50% lactic acid as an end-product, with considerable amounts of carbon dioxide, acetate, and ethanol (1).

Lactobacillus paraplantarum is a facultative heterofermentative rod shaped Gram-positive bacterium that grows from 15 ^{o}C to 37 ^{o}C, with NaCl concentrations up to 8% and it is closely related to *L. plantarum* and *L. pentosus*. (4,20). *L. paraplantarum* isolated from raw or spontaneously fermented cider, cabbages, capers (8).

The *Leuconostocaceae* family belongs to the order of Lactobacillales that are commonly called LAB like the *Lactobacillaceae* family. Their main trait is the production, exclusively or not, of lactic acid from carbohydrate fermentation. In the past, they formed the *Leuconostoc* genus, which was roughly defined as heterofermentative cocci. To date, this family comprises four genera: *Fructobacillus*, *Leuconostoc*, *Oenococcus*, and *Weissella* (14).

Leuconostoc strains are often present in dairy starter cultures and also in the dairy environment and thus could be considered as non-starter lactic acid bacteria (NSLAB) in the same way as mesophilic lactobacilli. Their role in the formation of aroma and texture of certain dairy products is essential (3).

Strains of the taxa *Leuconostoc mesenteroides ssp. dextranicum* and *Leu. mesenteroides ssp. cremoris* are frequently used, together with *Lactococcus* spp., as mesophilic starter cultures in dairy fermentations. The ability of *Leu. mesenteroides ssp. cremoris* to produce diacetyl and acetoin from citrate has led to its widespread use as a characteristic aroma producer in cultured dairy products, such as cultured buttermilk, creamery butter, cultured sour cream, and some cheeses (11,12,21).

Leuconostocaceae species have a relatively poor acidifying power and mainly are chosen for their capacity to produce typical aromas and flavors and to inhibit some undesirable contaminants. The balance between diacetyl, which is the most aromatic compound, and other products depends on the pH of the medium, temperature, and redox potential, and partially on the strain itself. The sensory quality of fermented milk also includes viscosity resulting from the synthesis of polysaccharides (14).

The aims of this study were, first, to investigate the effects of *L. paraplantarum* and *Leu. mesenteroides subs. cremoris* microorganisms isolated from koumiss in Kyrgyzstan, second, to prepare yoghurt using isolated microorganisms , and third, to compare the sensorial and physical characteristics of the prepared yoghurt with those of commercial yoghurt. This paper reports, for the first time, the successful isolation of *L. paraplantarum* and *Leu. mesenteroides subs. cremoris* from koumiss in Kyrgyzstan, and also shows that the characteristics of the yoghurt prepared by using isolated LAB were better than those of commercial yogurt. Based on the results, we can speculate that koumiss can be used as a source of LAB for preparation yoghurt and other dairy products.

Materials and Methods

Koumiss samples

In the period from June 2015 to March 2016, koumiss samples were collected at Bishkek and Narın regions in Kyrgyzstan. In each of the two regions, samples were taken from several places. Koumiss materials were carefully handled to avoid contamination, and each sample was immediately put in refrigerator and brought to laboratory.

Isolation of culture microorganisms

Keeping aseptic laboratory techniques one ml of each sample of koumiss was taken and added to 9 ml Ringer solution, so 10^{-5}, 10^{-6}, 10^{-7} dilutions was made. One ml of each aliquot was put in Petri dishes and culture media was poured into shallow, covered dishes to harden. Duplicate experiments were done; MRS and M17

broth were used. The plates were incubated anaerobically using the Anaerogen system (Oxoid) at 30 °C for 72 h to obtain Lactic acid bacteria (LAB) colonies.

The LAB (1% v/v) were cultivated in sterile 10 mL aliquots of de Man Rogosa and Sharpe (MRS) broth and incubated for 24 h at 37 °C. The cultures were centrifuged at 5000 g for 15 min to separate bacteria. Biomass washed twice with sterile distilled water. The bacteria were then inoculated into skim milk (12% w/v) and incubated at 37 °C for 24 has a pre-culture to obtain approximately 10^8 colony forming units (CFU)/mL (16).

Characterization and identification of the isolated bacteria

The microorganisms were identified by using a system formed by comparison with a reference spectrum obtained from colonies formed on M17 and MRS agar. Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) (VITEK® MS) (bioMerieux, France) was utilized to identify the protein profiles of cell structures of the microorganisms.

Preparation of yoghurt

For yoghurt production, pasteurized milk of 3.2% fat was purchased from the local market and stored at 6°C until use. Before inoculation milk was heated at 90°C for 10 min in a boiling water bath with continuous stirring to increase the viscosity of the final product. After that milk was immediately cooled to 45°C using tap water (19). Heat treated milk was then inoculated with 1% starter cultures (approximately 10^8 colony forming units (CFU)/mL.) and incubated at 43°C for about 6 hours until a curd formed and pH value of 4.5 was reached. pH values were measured using a glass electrode connected to Fisher Scientific AB 15 plus digital pH meter. After that yoghurt samples were put in refrigerator at 6°C for 12 hours to improve its consistence and sensorial characteristics.

Sensory Analyses

A panel of 23 untrained assessors evaluated the sensory attributes of the yoghurt samples for flavor, appearance and overall acceptance based on the method developed by International Dairy Federation (13). The test was accomplished based on 5-point hedonic scale by panelists and scaled as 1 = dislike extremely, 2 = dislike moderately, 3 = neither like nor dislike, 4 = like moderately and 5 = like extremely.

The samples, each of which was given a three digit code, were served in plastic containers under normal light. The panelists received the samples randomly. They were asked to rinse their mouth with water between each sample testing.

Statistical Analyses

All data were subjected to one-way analysis of variance (ANOVA) using SPSS 20 software (SPSS Inc., Chicago, IL, USA, 2002). Significance of results controlled using by Duncan test (10).

Results

Based on the results presented in this paper, it can be assumed that starter strains for yoghurt have been primarily derived from koumiss, and koumiss produced in Kyrgyzstan is potential source for *Lactobacillus ssp.* strains.

The characteristics of the selected strains isolated from koumiss were compared in relation to yoghurt production with those of the current commercial starter strains. Organoleptic results obtained from 23 panelists were estimated by using Duncan test.

As a result of analysis it is seen that yoghurt prepared with *L. paraplantarum* got 26.63 average mark, yoghurt prepared with *L. paraplantarum* and *Leu. mesenteroides subs. cremoris* got 26.26 average mark and purchased yoghurt got 24.39 average mark. There were no significant differences between groups (P>0.05) (Table 1).

Discussions

It was concluded that the selected LAB strains are similar to the industrial strains in respect to their ability to produce yoghurt. And from the preliminary sensory examinations, the quality of the yoghurt prepared by pure starter and starter combinations, which contained either one or two koumiss-originated strains, was shown to be within the high range compared with those of commercial yoghurt. These results suggest that,

Table 1. Organoleptic Analysis Results of Groups

Yoghurt Samples	Mean±SEM	P
L. paraplantarum	25.63±3.68	0.635
L. paraplantarum and *Leu. mesenteroides subs. cremoris*	26.26±3.36	0.713
Purchased yoghurt	24.39±6.14	0.222

at a minimum, yoghurt with an acceptable quality could be prepared using starter combinations of *L. paraplantarum* and *Leu. mesenteroides subs. cremoris* strains isolated from koumiss produced in Kyrgyzstan.

The results in this study clearly show that yoghurt bacteria exist in koumiss and also koumiss can be used as a source of starter culture for production other diary products. Further microbiological studies as well as genetic studies using the complete genome sequences of strains isolated from koumiss produced in Kyrgyzstan will shed light on these questions in the near future. This is the first report on the isolation of *L. paraplantarum* and *Leu. mesenteroides subs. cremoris* strains from koumiss produced in Kyrgyzstan, as well as on the characterization of the yoghurt prepared using the isolated strains from koumiss.

Findings of this study imply, first, a koumiss produced in Kyrgyzstan is a source of *L. paraplantarum* and *Leu. mesenteroides subs. cremoris*, second, the microbiological and fermentation characteristics of the isolated strains from koumiss are distinguishable from those of the industrial strains currently used for yogurt production. Therefore, it is assumed that starter culture isolated from koumiss can be successfully used in commercial yoghurt production. Such yoghurt can be an alternative product during period of absence of mare's milk. Also this paper reports can be a base for the further studies about using of starter culture obtained from koumiss in production of other dairy products.

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