Ribotyping of *Lactobacillus helveticus* from the Koumiss.

Ivo Sedlacek, Altanzaya Yansanjav, Dana Novakova, Pavel Svec

Czech Collection of Microorganisms, Masaryk University Brno, Czech Republic

Introduction

The genus *Lactobacillus*, belonging to the Lactic Acid Bacteria (LAB) group, represents a large and phylogenetically heterogeneous group of species. Lactobacilli are widespread in nature and they are involved in fermentation processes during production of different kinds of food including dairy products.

Koumiss is a traditional frothy and refreshing fermented dairy product originating from the Central Asian steppes. It is mostly produced from mares' milk by spontaneous fermentation due to natural LAB and yeast microflora. *Lactobacillus helveticus* strains are part of thermophilic starters used for production of high-temperature cook cheeses. This species can also be found in traditional fermented milk products such as yoghurt or sour milk.

The aim of this work was to characterize the biochemical and genetic variability of *L. helveticus* strains isolated from the Koumiss.

Results of biotyping

Biochemical and physiological characteristics obtained by API 50 CH kit and conventional tube tests allowed us to assign analysed strains as members of the genus *Lactobacillus*, but it was not possible to identify them to the species level. However, further characterization by the whole-cell protein fingerprinting (performed at the BCCM/LMG Bacterial Collection, Gent University, Belgium) assigned all strains as members of *L. helveticus* species (data not shown).

Phenotypic heterogeneity among *L. helveticus* isolates was observed, mainly due to acid formation from sugars (Table 1). No clear relationship between the biotype profiles and the origin of the strains was found. All *L. helveticus* strains from Koumiss showed positive fermentation of glucose and mannose and with exception of strain CCM 7061 were positive for galactose and lactose in full agreement with the species description.

Test results for fermentation of glucose and mannose and with exception of strain CCM 7061 were positive for N-acetylglucosamine, fructose, maltose and trehalose were variable among strains. Only strains CCM 7064 and CCM 7066 had identical biochemical properties. The strain CCM 7061 had a very low biochemical activity in general. Strain CCM 7062 showed atypical acid formation from sucrose. The phenotypic variability revealed among strains of *L. helveticus* implies that strains from Koumiss represent different biotypes.

<table>
<thead>
<tr>
<th>Strain number</th>
<th>Source</th>
<th>GAL</th>
<th>LAC</th>
<th>NAG</th>
<th>FRU</th>
<th>MIL</th>
<th>TRE</th>
<th>SUC</th>
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<tbody>
<tr>
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<td>Arkhangai</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>CCM 7066</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
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</tbody>
</table>

Positive reactions: growth at 37°C, acid from glucose and mannose (on API 50 CH)
Negative reactions: growth at 10°C and 45°C, growth on nutrient agar, NHI, from arginine, catalase, gas from glucose, hydrolysis of esculin, acid from GLY, ERY, D-ARA, L-ARA, RIB, D-XYL, L-XYL, ADO, BMX, SOE, MAN, RHA, DUL, INO, SOR, AMM, AMG, AMY, ART, SAL, CEL, INU, MLI, ML2, MBF, RAF, AMI, TUR, TAG, GLC, XLT, BGE, LYY, D-FUC, L-FUC, D-ARL, L-ARL, GGN, 2KG, 5KG (on API 50 CH)

Material and Methods

A group of six *L. helveticus* strains was isolated from natural Koumiss made from mares' milk sampled in Arkhangai and Khotouk in Mongolia. Isolation of LAB strains was performed on MRS agar or in MRS broth (Merck) supplemented with 0.05% cystein and cultivated for 48 h at 36 °C in a 5% CO₂ atmosphere. Reference cultures *Lactobacillus helveticus* CCM 7193 and *L. helveticus* CCM 4280 were obtained from the Czech Collection of Microorganisms (http://www.sci.muni.cz/ccm).

Biochemical characterization was done by commercial kit API 50 CH (bioMérieux) and by additional conventional tube and plate tests. Obtained results were evaluated by APILAB Plus software (bioMérieux).

Ribotyping was performed with EcoRI restriction enzyme and a digoxigenin-labeled DNA probe made complementary to *E. coli* 16S and 23S rRNA. Obtained hybridization profiles were processed by GelCompar II software (Applied-Maths).

Results of ribotyping

Ribotyping with EcoRI and a DNA probe made complementary to 16S and 23S rRNA yielded hybridization profiles containing from eight to ten bands. Obtained results clearly separated analysed group from the type strain CCM 7193 and strain CCM 4280 and allowed us to differentiate individual strains (Figure 1).

Despite the low number of strains examined, the comparison of the obtained ribotypes showed high heterogeneity among analysed and reference strains. This trait seemed not to be only strain-dependent, but it was also related to the source of isolation as all Koumiss-originating strains formed a single cluster clearly separated from the cheese-originating strains CCM 7193 and CCM 4280. Moreover, ribotyping revealed high genetic variability among *L. helveticus* isolates obtained from Koumiss. These results confirmed ribotyping as a suitable and useful taxonomic tool for characterization of lactobacilli.

Conclusion

*L. helveticus* strains are mainly known as the dominant organisms in the natural whey starter cultures used for the production of cheeses. However our study proved this species surprisingly as the prevailing spontaneous flora in traditional production of Koumiss. The presence of different *L. helveticus* biotypes and ribotypes seems to be related to the specific source of isolation. We concluded that analysed isolates from Koumiss represent a new *L. helveticus* ecovar as clearly proved by ribotyping.