ABSTRACT

Over the last few years the traditional dairy products have been gaining much more popularity among scientists and consumers. They are a part of the nutritional diet of people since ancient times and are a proven source of lactic acid bacteria (LAB) with confirmed and beneficial effects on human health. LAB are the main cause of lactic acid fermentation and determine the specific properties of the resulting products. Home-made fermented dairy products are a part of the daily diet and tradition is saved in several rural regions of Bulgaria. However, the beneficial microbiota of some of them is poorly characterized. Therefore, any research aimed at the isolation and identification of LAB from traditional foods helps to define the autochthonous microbiota, which is typical for the area/product. With these aims 26 artisanal samples of 4 types Bulgarian dairy products: yoghurt, white-brined cheese, yellow cheese and curd, were collected. Their lactic microflora was studied and 74 pure cultures were isolated. They were identified as Lactobacillus spp. using classical approaches for identification. For this reason further characterization of the created collection of lactobacilli is considered perspective.

Keywords: Lactobacillus spp., yoghurt, white-brined cheese, yellow cheese, curd, antagonistic activity.
the flavor, odor and texture of products and serve as bio-protectors which extend naturally their shelf life [3]. This is due to the rich enzyme system, by which they perform their metabolic processes on one hand, while on the other they synthesize organic acids, hydrogen peroxide, diacetyl, bacteriocins and other biologically active metabolites.

According to the Food and Agriculture Organization and the World Health Organization guidelines (FAO and WHO) the probiotic effects are usually strain-specific. This requires a thorough study and characterization of the strains for subsequent use as probiotics [4, 5]. It is essential that ecological and natural sources are used for their isolation. There are regions in Bulgaria, where the traditions in producing various dairy products, such as yoghurt and white-brined cheese, have been well-preserved for centuries. A diversity of well-known fermented milk products occurred [6] as a result of the traditions conserved and consequently applied in small farms only. The variety of dairy products of specific physicochemical and organoleptic qualities is due not only to the technology used and the type of milk, but also to the type of the surrounding microflora of the habitats. Therefore, a renewed interest of microbiologists towards specific dairy products of the Balkans rural regions is rapidly growing during the last few years [7 - 9].

Considering all the above facts, this study aims to the isolation and identification of lactobacilli from traditional Bulgarian products - yoghurt, curd, white-brined and yellow cheese. This is the initial step in search of strains of probiotic properties and technological relevance, promising for the development of new functional foods. Therefore only home-made dairy products prepared by traditional technology in absence of any industrial starter cultures were the object of the study.

EXPERIMENTAL

Materials

Sample collection of traditional Bulgarian dairy products

All dairy products providing samples for this study were home-made in correspondence with the traditional recipes used up to day in the different regions of Bulgaria (Fig. 1). They all contained no industrial starter cultures. A major part of the cheeses were produced from raw milk without pasteurization. All samples were collected in sterile containers, transported to the laboratory and stored at 4ºC until the beginning of the experiments (no longer than 5 days).

Enumeration and isolation of LAB

An optimized protocol for quantitative microbiological analysis of vital lactic microflora according to Danova et al. was used [8]. An amount of 10 g of the inside of all collected samples was homogenized in 90 ml of 0.9 % (w/v) sterile saline (for 10 min on a magnetic stirrer MSH 300N, Boeco, Germany) and used for serial decimal dilutions. In case of cheese samples, an additional step of enrichment of the initial cultures was applied as followed: 1 ml was initially transferred to 9 ml MRS broth and was incubated over-night at 37ºC. Then the mixture was serially diluted and cultured in agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h. The number of single colonies grown on agar plates under anaerobic conditions (using anaerobic jar GasPak 100 Anaerobic system, BD Bioscience, USA) at 37ºC for 48 h.

Phenotypic characteristics of LAB isolates from traditional milk products

At least three colonies were picked from each morphotype found on the countable plate with MRS or Rogosa agar. A binocular loupe (Kruss Optron, Germany) was used to determine the colonies’ morphology.

The strains were identified to a genus level based on the classical cultural, morphological and biochemical
characteristics in accordance with the Bergey’s Manual of Systematic Bacteriology, vol. 2 [10]. The initial selection among the isolates was based on the classical microbiological tests for Gram staining, catalase reaction and cell morphology. The cell morphology was identified by microscopic observation (using light microscope Olympus BX53, Japan). Isolates of a gram-positive status, which showed a negative reaction to the catalase test (as determined by transferring fresh colonies from an agar medium to a grease-free glass slide and adding 1 ml of 3 % (v/v) H₂O₂), were non-motile and did not form spores were selected.

The conducted biochemical test referred to formation of a gas from glucose for determination of the homo or hetero-fermentative type of metabolism of each isolate. It was performed in a modified MRS broth (meat extract omitted) containing glucose (20 g l⁻¹) as a sole source of carbon placed in Durham’s mini-tubes. The ability of gas evolving was determined at the 48th and the 168th h of cultivation at 37°C under anaerobic conditions.

RESULTS AND DISCUSSION

Collection of artisanal samples of Bulgarian dairy products from different rural regions

Bulgaria is the motherland of the first probiotic – the yoghurt and one of the well-known countries with preserved traditions of production of fermented dairy products. The increased consumers’ attention to a healthy lifestyle is the major driving force for intensive investigation of the biodiversity of LAB microbiota responsible for the unique qualities of these products.

This study focused on the microflora of 4 dairy products traditional for the Balkans - yoghurt, white-brined cheese, yellow cheese and curd. When selecting the samples, we headed to those produced at home or at small farms from raw milk as well as to products based on pasteurized milk. A total of 33 samples from different geographic regions of Bulgaria were collected in an effort to mainstream a sufficient number of representative samples from each of the products (Fig. 1a). The dairy products were prepared in accordance with the traditions of the geographical area and did not contain industrial starters as found on the ground of surveys carried out at the domestic farms. Yoghurt, white-brined and yellow cheese are the most widely consumed products in Bulgaria. Therefore, samples of these three products were collected and studied extensively (Fig. 1b).

Eleven home-made yoghurts were collected from the mountainous areas above the towns of Harmanli, Lovech, Troyan, Tvarditsa and Tran. They were prepared from pasteurized buffalo, cow, goat and sheep milk. At the same time the samples of white-brined cheese (from regions close to Sofia and Pernik – Lulin moun-
tain, Veliko Turnovo, Lovech, Vratsa, Stara Zagora and Kyustendil) were mainly made from raw buffalo, goat, cow and sheep milk (Table 1).

**Isolation of lactic acid bacteria and strains characterisation**

Classical microbiological methods using selective agar media for different LAB (MRS), lactobacilli (Ro-gosa), streptococci (M17) and enterococci (Slanetz-Bartley (SB)) were applied for initial characterization of LAB microbiota’s biodiversity. A high vitality (equal or greater than $10^6$ CFU g$^{-1}$ product) of the autochthonous lactic acid microbtiota was observed in almost all of the samples tested (Figs. 2, 3 and 4). This indicates that most of the samples possess characteristics desirable for a functional food, i.e. they provide a high number of living cells in the course of consumption.

However, a significant difference between the samples collected from the 4 products is hardly visible (Fig. 4a). The highest total count of LAB ($\sim 3.7 \times 10^6$ CFU g$^{-1}$)
is reported for samples of white-brined cheese, designated by “P” (Fig. 3b) made from pasteurized buffalo milk in a family farm near Dryanovo (Table 1). The additional step of enrichment applied to the cheese analyses contributed probably to this result, but it was required to provide identical conditions for multiplication of the minor-represented LAB group. The total bacterial counts in Nostrano-cheese samples are also in the range of $10^8$ – $10^9$ CFU g$^{-1}$ [13].

The microbiological analyses show that curd and yoghurt samples possess lower vitality in the range of $10^6$ CFU g$^{-1}$ – $10^7$ CFU g$^{-1}$ (Fig. 2a) while the vital microbiota in the cheese samples is in the range of $10^7$ CFU g$^{-1}$ – $10^{10}$ CFU g$^{-1}$ (Fig. 3a).

The most probable cause for the variety and high vitality of the LAB in some samples refers to the application of raw milk for the dairy food production. The main advantage of the raw milk in respect to the pasteurized one is the content of complex and unstudied diverse microflora from different genera of LAB like *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Weisella* and *Pediococcus*. In addition, strains of the genera *Propionibacterium*, *Staphylococcus*, *Corynebacterium*, *Brevibacterium*, various yeasts, fungus etc. are also reported [11]. A study [12] of the chemical and microbiological quality of buffalo raw milk reports the presence of $2.95 \pm 0.21 \times 10^{10}$ CFU mL$^{-1}$ of LAB in 120 commercially available milk samples in Pakistan. The analyses also show a high contamination of all collected raw milk samples collected with undesirable coliforms (*Escherichia coli*, *Staphylococcus aureus*), yeasts and molds. In contrast, we did not find *E. coli* and moulds in our samples of artisanal milk products from raw milk (strains from S5 up to S12).

Non-starter microflora, which is introduced to the milk by animals, farms or dairies, defines the specific flavor and odor of the manufactured products, which is
Enterococci are detected mainly in the tested cheese samples in their early stage of ripening (samples G, H, P, Q, N, O), while representatives of the genus *Lactobacillus*, reported on Rogosa agar medium dominate subsequently (Fig. 3b).

Our results are confirmed by Franciosi et al. [13] who report that LAB microflora is mainly observed in cheeses from raw cow milk, while species like *Lactobacillus paracasei*, *Streptococcus thermophilus* and *Leuconostoc mesenteroides* predominate at all stages of the cheese making process.

Another research group studies [14] the diversity of species in fresh and ripened cheeses made from unpasteurized goat milk. It reports the dominance of starter LAB species (such as *Lactococcus lactis* and *Leuconostoc mesenteroides*) in samples from young cheeses (with period of ripening of 1 and 3 months) and their absence in mature cheeses. Meanwhile, the dominance of non-starter LAB microflora including the species *Enterococcus faecalis*, *E. faecium*, *L. sakei* and *Staphylococcus epidermis* is observed in mature cheeses.

319 LAB strains were isolated [15] from various samples of traditional Chinese yak milk products. It was found that the group of rod-shaped lactobacilli predominates (51.41 %) over coci LAB (48.59 %). This data lead to classification of six genera (*Lactobacillus*,

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**Fig. 3a.** Quantitative microbiological analyses of the vital microbiota of collected samples of white-brined and yellow cheese from different rural regions.

**Fig. 3b.** Petri dishes with cultured serial dilutions (in the range from $10^{-4}$ to $10^{-7}$) on the ground of white-brined cheese N (made from mixed buffalo-cow milk), cultivated in MRS, M17, Slanetz-Bartley (SB) and Rogosa (R) agar media for 48 h under anaerobic conditions.

*(Dairy products: white-brined cheese: from cow milk – G, H, P; from buffalo milk – Q; from mixed: buffalo and cow milk – N; from sheep milk – O; yellow cheeses from goat milk: I, L).*
Fig. 4a. Comparative microbiological analysis of the vital LAB microbiota of samples of yoghurt, white-brined cheese, yellow cheese and curd cultivated (after serial dilutions in the range from $10^{-4}$ to $10^{-7}$) carried out in parallel in MRS agar.

Fig. 4b. Petri dishes with cultured serial dilutions (in the range from $10^{-4}$ to $10^{-7}$) of samples from goat curd J, buffalo white-brined cheese Q and cow yoghurt F in MRS agar, M17, Slanetz-Bartley (SB) and Rogosa (R) agar media. (Dairy products: yoghurt: from goat milk - A; from cow milk - B, D, E, M; from sheep milk - F, FF, Ro1, Ro2, Ro3; from buffalo milk – V; white-brined cheese: from cow milk: C, G, H, F; from buffalo milk - Q, R; from mixed: buffalo and cow milk – N; from sheep milk - O; goat yellow cheese: I, L; goat curd: J).
**Lactococcus, Leuconostoc, Streptococcus, Enterococcus** and *Weissella* and 21 species. We also find predominant lactobacilli (Fig. 2a, Fig. 3a and Fig. 4a) in samples of artisanal white-brined and yellow cheese and yoghurt. They are often in combination with enterococci and streptococci growing on Slanetz-Bartley and M17, respectively (Fig. 3b and 4b). Despite the risk of a negative impact on the flavor and safety of cheese inherent to some species of the genus *Enterococcus*, they have a great potential as supplements and probiotic cultures and are widely used in the production of dairy products such as Mozzarella, Cheddar, Pecorino Sardo, etc. [16].

The presence of lactic acid bacteria of technologically important properties, biological activity and probiotic potential has been a long-term task in our research [8]. Selective MRS medium is used to isolate *Lactobacillus* strains, which are of interest in our work. The results are summarized in Fig. 4. The few LAB counts as well as the high number of yeasts detected in some of the samples hamper the development of pure bacterial cultures on MRS agar medium. That is why all samples are cultured in parallel in a selective Rogosa medium supporting the growth of *Lactobacillus* strains.

The macroscopic analysis on countable plates of colonies from different samples (Figs. 2b and 3b) shows a greater morphological diversity in their shape and size when using MRS and Rogosa agar, while a limited one in M17 and Slanetz-Bartley agar media. As expected different morphotypes of colonies are present in the 5 types of yoghurt studied (Fig. 2b) compared to those found in curd and cheeses (Fig. 4b).

Seventy-four pure cultures are isolated (Table 1) from the single colonies grown on countable agar plates (Fig. 2b, 3b and 4b). All isolates have rod-shaped and polymorphic cells. They are gram-positive, catalase-negative, and anaerobic or aerotolerant. They grow well in MRS broth (pH 5.4 - 6.5) at 37°C and 42°C acidifying significantly the medium. Thus, these 74 pure cultures possess the LAB characteristics required.

A greater number of the LAB isolates are strains from white-brined cheese (43 strains from cow, sheep, goat, buffalo and mixed cow and buffalo milk). They are followed by the group of strains isolated from yoghurt (19 LAB from cow, sheep and buffalo milk), 2 strains from curd (goat milk) and 10 strains from yellow cheese (goat and cow milk). They are primarily selected on the basis of their colonies characteristics (colour, shape, profile, margin, surface and gloss).

A special attention is paid to the isolates from the cheeses and yoghurts made from buffalo milk. Aiming these 10 strains are isolated and characterized. They are a big part of the group of 41 *Lactobacillus* strains out of the total LAB isolates (74 strains), which are selected for further characterization. Presently, a very limited data exists on LAB microbiota of dairy products from buffalo milk and especially of those home-made. Silva et al. [17] were the first to elucidate the LAB diversity involved in Brazilian water buffalo mozzarella cheese using classical microbiological tests (Gram staining, catalase test, capacity to assimilate citrate, and production of CO₂ from glucose) and RAPD PCR analysis. They isolated successfully and identified 20 LAB belonging to the genera *Lactobacillus*, *Leuconostoc*, *Enterococcus* and *Streptococcus* (*Str. thermophilus* species only). Two samples of the tested artisanal cheeses from different regions of Bulgaria show also the presence of different LAB such as lactobacilli, streptococci, enterococci (Fig. 3b). However, only the strains belonging to genus *Lactobacillus* are the subject of further characterization.

In addition, 5 strains from dairy products (white-brined cheese, yellow cheese and curd) made from goat milk (Table 1) are included in the group of 41 LAB pre-selected for further characterization. The interest shown is determined by the fact that dairy products from goat milk are increasingly valued because of their nutritive and therapeutic properties. They are considered as one of the milk products of greatest marketing potential. Moreover, fermented goat milk incorporating live probiotic cells is very prospective with regard to human health [18].

Representatives of genus *Lactobacillus* can be found in home-made cheeses from different types of milk. For example, their representatives were mainly observed in the study [19] of microbial diversity of the Spanish cheese alberquilla obtained by fermentation of a mixture of goat and sheep milk. Other studies [20] reported the isolation of strains from genus *Lactobacillus* like *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus casei*...
Veronica Nemska, Nevena Lazarova, Nelly Georgieva, Svetla Danova

Studies [23, 24] focused on the diversity of species in traditional home-made Indian yoghurt dahi derived from cow or buffalo milk reported contents of some probiotic strains like Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus rhamnosus, Lactobacillus lactis, Lactobacillus plantarum and Lactobacillus fermentum.

The 74 isolates described above are subjected to an additional microscopic control of their purity and a subse-

<table>
<thead>
<tr>
<th>Isolate(s)</th>
<th>Gram staining</th>
<th>Colonial morphology</th>
<th>Cell morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>S5, S6, S7, S8, S9, S10, S12, 1V, 2V, 3V, 7V, 8V, 10V, OC1, BS32, Kz1, Kz2, Kz3, Ko1, Ko3, Ko2</td>
<td>G+</td>
<td>Pin point, circular, white, convex, with entire margin</td>
<td>Short rods, with rounded ends, single, in pairs or short chains</td>
</tr>
<tr>
<td>Ro33</td>
<td>G+</td>
<td>Circular, white, glossy, convex, with undulate margin</td>
<td>Medium length, straight rods, arranged in long chains</td>
</tr>
<tr>
<td>9V, G7D</td>
<td>G+</td>
<td>Pin point, circular, greyish white, convex, with entire margin</td>
<td>Short single rods, with rounded ends</td>
</tr>
<tr>
<td>Ro34, Ro304, Ro32, J6B</td>
<td>G+</td>
<td>Creamy greyish, with circular or irregular shape Short, circular or scaphoid, with cream color, glossy</td>
<td>Long straight rods with rounded ends, single and shorter rods in pairs Short or long rods with rounded ends, single, in pairs or rarely in short chains</td>
</tr>
<tr>
<td>6V</td>
<td>G+ G+</td>
<td>Pin point, circular, white, convex, with entire margin</td>
<td>Medium long rods, slightly curved, with rounded ends, single or in short chains</td>
</tr>
<tr>
<td>S11</td>
<td>G+</td>
<td>Short, circular, creamy-yellow, convex, smooth, with entire margin</td>
<td>Medium long rods, slightly curved, with rounded ends, single or in short chains</td>
</tr>
<tr>
<td>BS42, KC1, KC2, OC2</td>
<td>G+</td>
<td>White, circular, convex, with entire margin</td>
<td>Short rods, single, rarely in pairs</td>
</tr>
<tr>
<td>4V</td>
<td>G+</td>
<td>Pin point, circular, white, convex, with entire margin</td>
<td>Medium long rods with rounded ends, single, in pairs or in short chains</td>
</tr>
<tr>
<td>5V</td>
<td>G+</td>
<td>Pin point, circular, white, convex, with entire margin</td>
<td>Short rods with rounded ends, single, in pairs, rarely in short chains</td>
</tr>
</tbody>
</table>

Legend: G+ – Gram Positive strain with Gram (+) type cell wall.
quent phenotypic characterization with the application of classical tests appropriate for LAB heterogeneous group [10]. Forty one of the isolates are tested for genus Lactobacillus. Their cells are detected as single one, or arranged in chains. Some are found to form aggregates (Table 2).

During the fermentation processes LAB have the ability to utilize carbohydrates in order to produce energy. Organic acids, accompanied by small amounts of ethanol and gases such as hydrogen and carbon dioxide, are the end metabolic products. Some of the latter play a key role in determination of the products sensory qualities. All newly-isolated lactobacilli are subjected to the glucose fermentation test (Table 3), which has an important taxonomic significance. A predominating presence of gas-forming strains during the experimental growth in modified MRS medium (with glucose as a sole carbon source and meat extract omitted) is found. Lactobacilli of hetero-fermentative type of metabolism were reported [25] as non-starter microbiota in the production of some types of cheeses. It was found that they had a significant contribution to the products flavor and taste. Only two strains (Ro34 and KC2) isolated from yoghurt and cheese are found in our study to produce no gas. They are identified as members of the homo-fermentative lactobacilli group (Table 3).

Our results confirm that artisanal dairy products prepared in accordance with the traditional technologies in the country are a promising source of strains of beneficial properties.

CONCLUSIONS

The isolated lactic acid bacteria are a part of autochthonous microflora of the home-made fermented dairy products prepared in accordance with the traditional technologies in Bulgaria.

The present study shows that yoghurt, white-brined and yellow cheese samples collected from different rural regions of Bulgaria contain rich lactic acid microbiota, which is vital in the course of consumption. The predominant content of lactobacilli is proved in samples of white-brined cheeses, even in the samples produced from raw milk in absence of starters. 74 LAB cultures are successfully isolated and 41 strains are identified as Lactobacillus spp.

The presence of vital LAB of antagonistic activity in the traditional dairy products analysed provides opportunities for their implementation as bio-protective and probiotic starters in modern food technology.

A new collection of original LAB strains is obtained. Their identification to species level and subsequent characterization as beneficial bacteria is in progress.

Table 3. Biochemical characteristic of newly isolated lactobacilli from dairy products.

<table>
<thead>
<tr>
<th>Dairy product</th>
<th>Isolate(s)</th>
<th>Catalase reaction</th>
<th>Gas from glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoghurt</td>
<td>Ro34</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1V, 2V, 3V, 7V, 8V, 10V, Ro33</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>9V</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>White-brined cheese</td>
<td>S5, S6, S7, S8, S9, S10, S12, OC1, OC2, BS32, BS41, Ko1, Ko3</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>KC2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>9V, G7D</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Yellow cheese</td>
<td>S11, Kz1, Kz2, Kz3</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Legend: Catalase test: (+) – positive; (-) – negative; and Glucose fermentation test: (+) - gas detected in Durham’s tubes at 48 h, (-) no gas detected;
REFERENCES