

# THE ISOLATION AND DIFFERENTIATION OF PROBIOTIC STRAINS OF LACTIC ACID BACTERIA FROM NATIONAL FERMENTED MILK PRODUC



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**Introduction:** Lactic acid bacteria (LAB) have been used since time immemorial by people, in particular in some ancient communities, over time, each nation has its own national lactic acid products used not only as food products, but also as therapeutic, preventive and curative agents for the treatment of the cardiovascular system, respiratory organs, gastrointestinal infections, etc. The LAB isolated from the national lactic acid products draw a special interest among the probiotic correctors of normal intestinal microbiota plays an important role in human ecology. LAB are cultures of strategic importance to the nation's health. The basic property of LAB, by which they are combined into a separate broad group of microorganisms, is the ability to form lactic acid as the main product of fermentation. Lactic fermentation is carried out by bacterial organisms heterogeneous in morphology, physiological and biochemical properties. A sharp increase in the man-caused and anthropogenic pressures on the environment, conditions and habitats contribute to the variability of the morphological and cultural properties of these bacteria, which is the cause of greater difficulty in their differentiation and identification. The advantage of the method of natural selection is that nature itself tests the properties of microorganisms. Sometimes even in nature stand out the most adapted form from existing cultures. The basis of LAB group are the genus of *Lactococcus*, *Lactobacillus* and yeast.

Fig.1).

**The aim of this work:** the isolation and differentiation of probiotic strains lactic acid bacteria from this national fermented milk products.

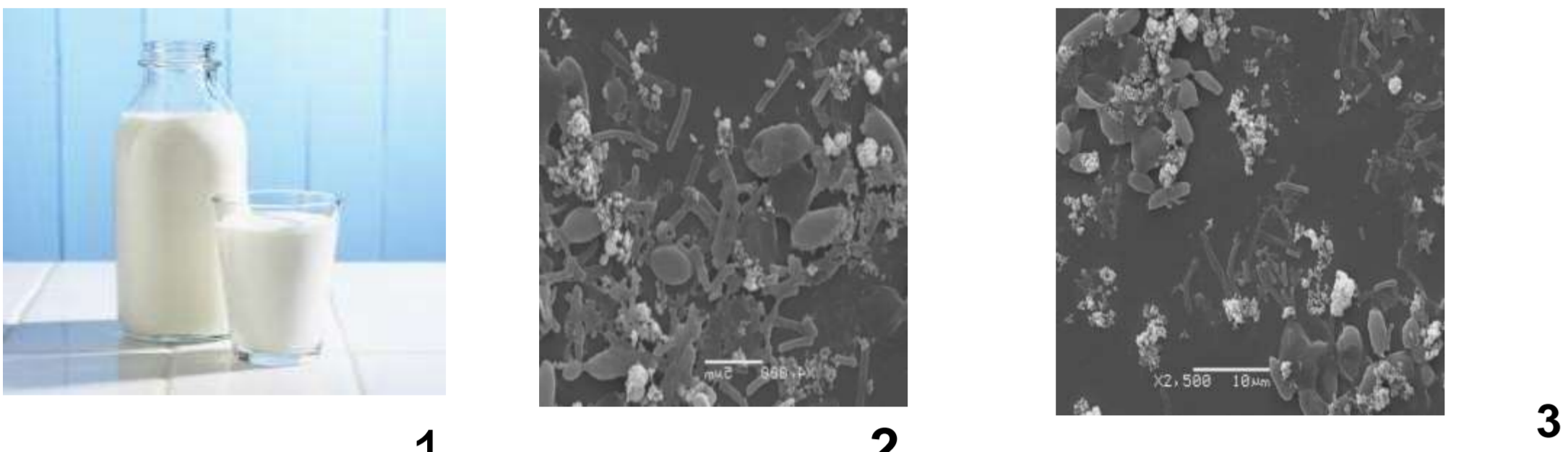


Fig.1. Beverage Kurunga and microbiota of Kurunga (2), "Doogh"(3) (Electron scanning microscope "Can Scan", Gresham (1 x 4000))

**Method:** We used: lebanese milk products (Beirut) of lactic acid fermentation: Laban - cow's milk + starter culture, then squeezing the product (thus, a pure protein product without whey); Leben - cow's milk + starter culture, without pressing; Kurunga and Doogh are a fermented beverages of mixed lactic acid and alcoholic fermentation, which are produced from cow milk and widely used by people of ((Buryatia Republic and Iran).

The best environment for the cultivation of LAB is the skim milk. In test tubes, the product under study was introduced for self-fermentation at different temperatures (30°, 37°, 42°C) under steady-state conditions. Then, 112 the original test tubes were selected, in which a different density of the milk clot formed, which is characteristic of lactococci or lactobacilli. This strains were lyophilized and stored in a household refrigerator. The homoenzymatic lactococci and *Lactobacillus acidophilus* strains were resuscitated and cultivated in fat-free milk. There upon, the inoculum (5%, v/v) was added to the medium of the following composition (g / l): KH<sub>2</sub>PO<sub>4</sub> - 20.0; glucose -10.0; NaCl- 1.0; MgSO<sub>4</sub> -0.2, and yeast autolysate (35 mg% of ammonium nitrogen), pH 6.8-7.0. The antibiotic activities of strains was determined by measurement of growth inhibition zone of test-cultures. The probiotic properties were determined as levels of resistance to conditions of the gastrointestinal tract (GIT).

**Results.** We have isolated some strains of lactococci and lactobacilli with high antagonistic activity against pathogens (Tabl.2).

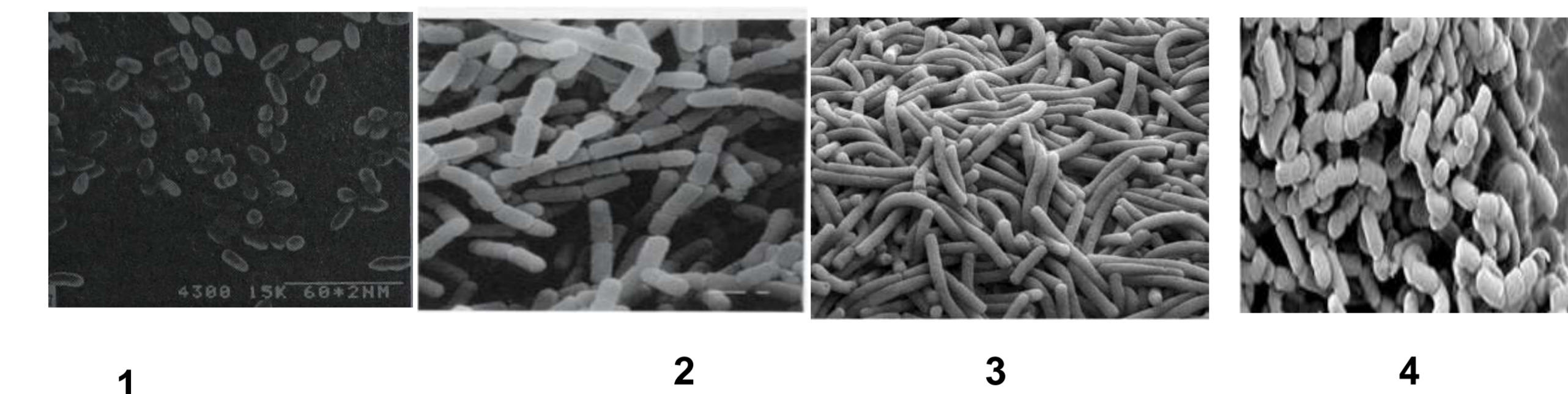


Fig.2. *Lactococcus lactis* ssp. *lactis* K – 205 (1), *Lactobacillus diolivoran* (2), *L.delbrueckii* ssp. *bulgaricus* 2.5 M GU (from Laban,3), *L. paracasei* 9MGU (4), from Lébén.)

Electron scanning microscope "Can Scan", Gresham (1 x 4000)

Table 1. Differentiating characteristics of *Lactococcus lactis* subsp. *lactis* and *Lactobacillus* spp. isolated from kurunga

Characteristics	<i>L. lactis</i> subsp. <i>lactis</i>	<i>Lactobacillus</i> spp.
Predominant position of the cells	Chains of up to eight cocci	Long thin rods, single or forming short chains
Motility	-	-
Growth at 10°C	+	-
Growth at 45°C	-	+
pH 9.6	-	-
pH 5.5	+	+
Growth in the presence of 4% NaCl	+	+
Growth in the presence of 4% NaCl	-	-
Gas formation during glucose fermentation	-	+

Note: The sign "+" denotes the presence of a characteristic; the sign "-" denotes the absence of a characteristic.

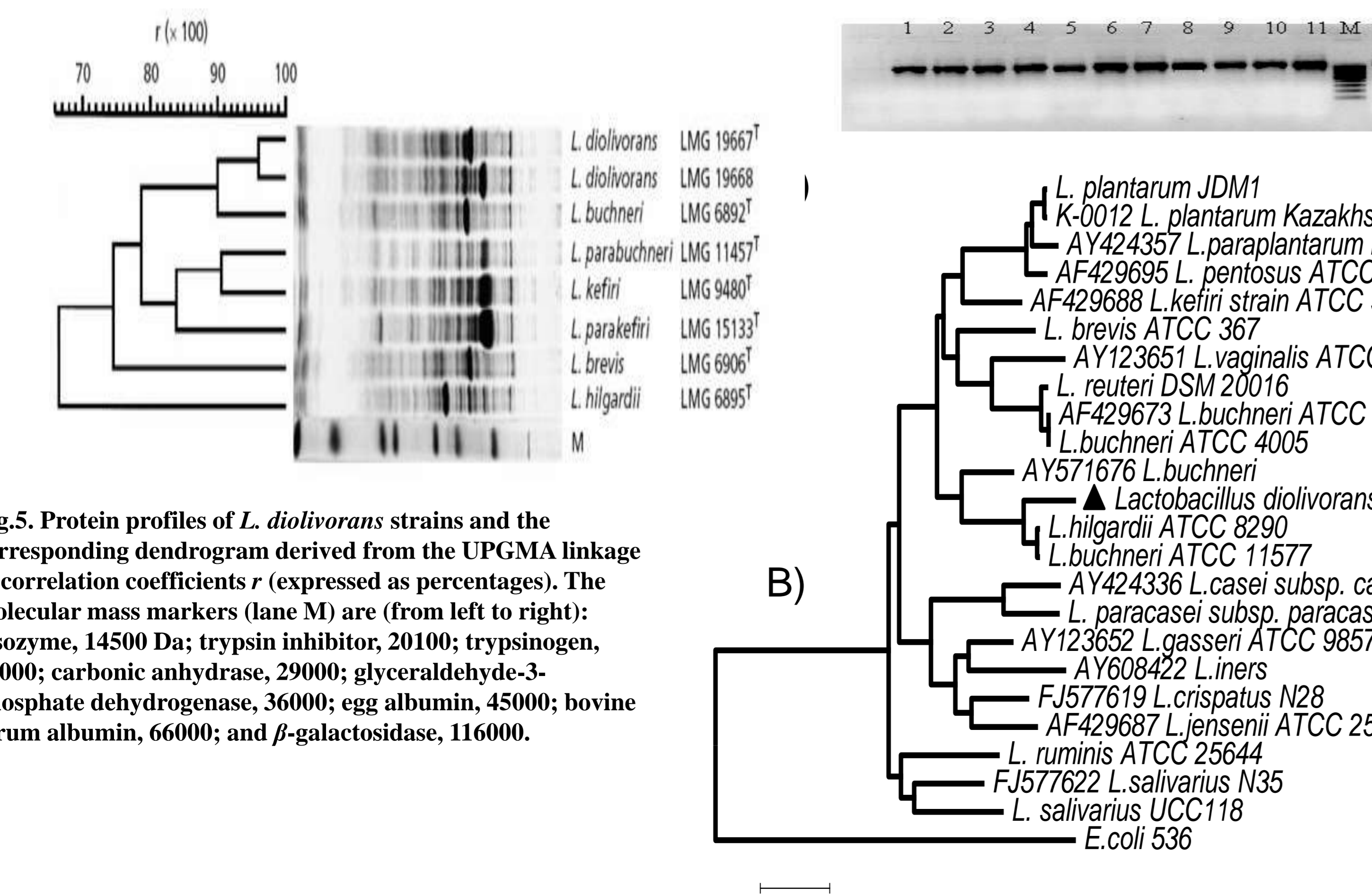


Fig.5. Protein profiles of *L. diolivorans* strains and the corresponding dendrogram derived from the UPGMA linkage of correlation coefficients *r* (expressed as percentages). The molecular mass markers (lane M) are (from left to right): lysozyme, 14500 Da; trypsin inhibitor, 20100; trypsinogen, 24000; carbonic anhydrase, 29000; glyceraldehyde-3-phosphate dehydrogenase, 36000; egg albumin, 45000; bovine serum albumin, 66000; and  $\beta$ -galactosidase, 116000.

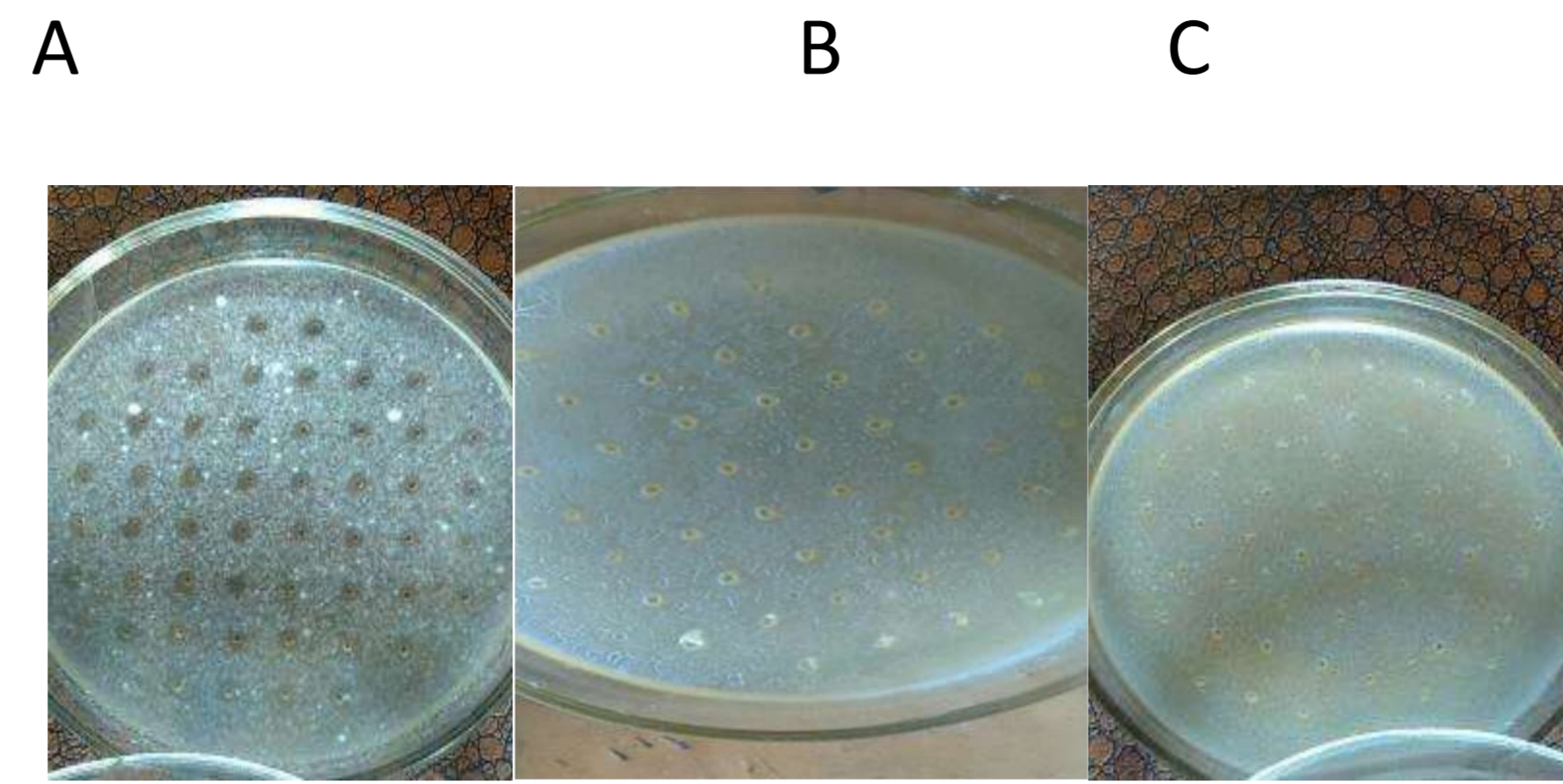


Fig 3. Isolation of the most active colonies with high inhibitory activity ("replica" method)

Test microorganisms: A - *Candida albicans*; B - *Micrococcus flavus*; C - *Proteus vulgaris*

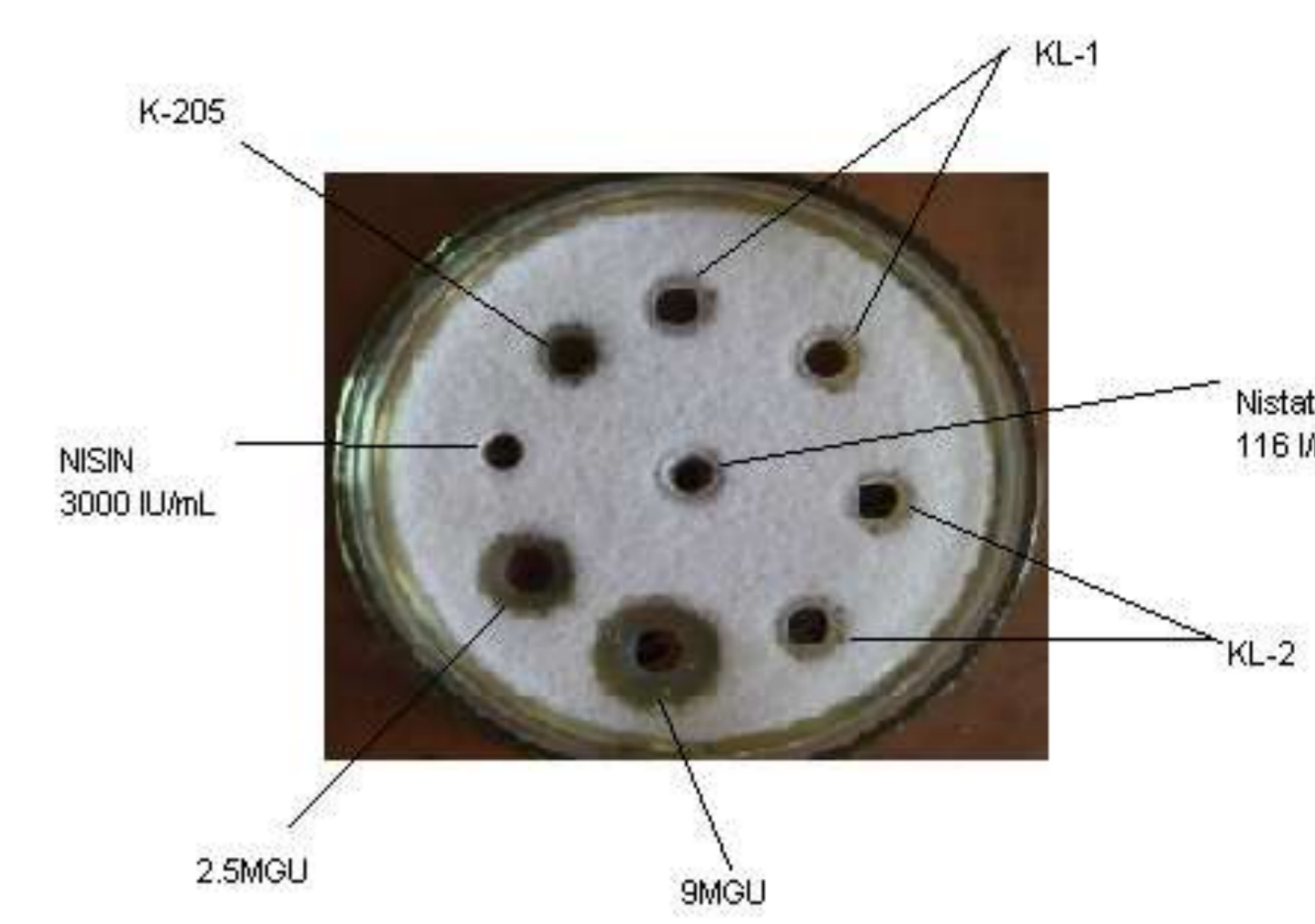


Fig 4. Fungicidal activity of strains of *L.lactis* ssp. *lactis* K-205 and *Lactobacillus* strains.

Up to 50% of colonies of lactic acid bacteria isolated from the microbiota of the Lebanese dairy products Laban and Leben, inhibiting the growth of gram-positive and gram-negative bacteria and up to 30%, possessing fungicidal activity.

According to the phenotypic and genotypic of gene 16S rRNA the most effective lactococcal strain from kurunga was identified as *Lactococcus lactis* ssp. *lactis* K-205 (GenBank EF 114305), two of lactobacilli strains from Kurunga were *Lactobacillus diolivorans* KL-1 and KL-2 (GenBank JF 520627, JF520433), and *L. delbrueckii* ssp. *bulgaricus* 2.5 M GU (from Laban), *L. paracasei* 9MGU (from Lébén).

In our experiments the isolated lactococci and lactobacilli were studied as probiotic cultures. It was conducted in model experiments of the effects of adverse conditions of the gastrointestinal tract by the action of high concentrations of bile acids (0-50%) and hydrochloric acid (0 to 1.0 %), added to fermentative medium, on the viability of strains during the incubation for 1 – 3 h.

Table 2. Antimicrobial action of novel strains different origin

Test- microorganisms	Strains					Nisaplin
	K-205	KL-1	KL-2	2.5 MGU	9 MGU	3000 IU/ml
	Diameter of growth inhibition zone, mm					
<i>Bacillus coagulans</i>	18	0	18	14	22	18
<i>Staphylococcus aureus</i>	23	10	15	12	20	19
<i>Micrococcus luteus</i>	18	12	14	16	18	20
<i>Escherichia coli</i>	14	16	0	18	22	0
<i>Salmonella gallinarum</i>	16	10	12	12	18	0
<i>Klebsiella pneumoniae</i>	16	14	12	13	15	0
<i>Aspergillus niger</i>	18	10	10	11	24	0
<i>Penicillium chrysogenum</i>	14	10	9	12	16	0
<i>Candida albicans</i>	12	12	10	14	18	0

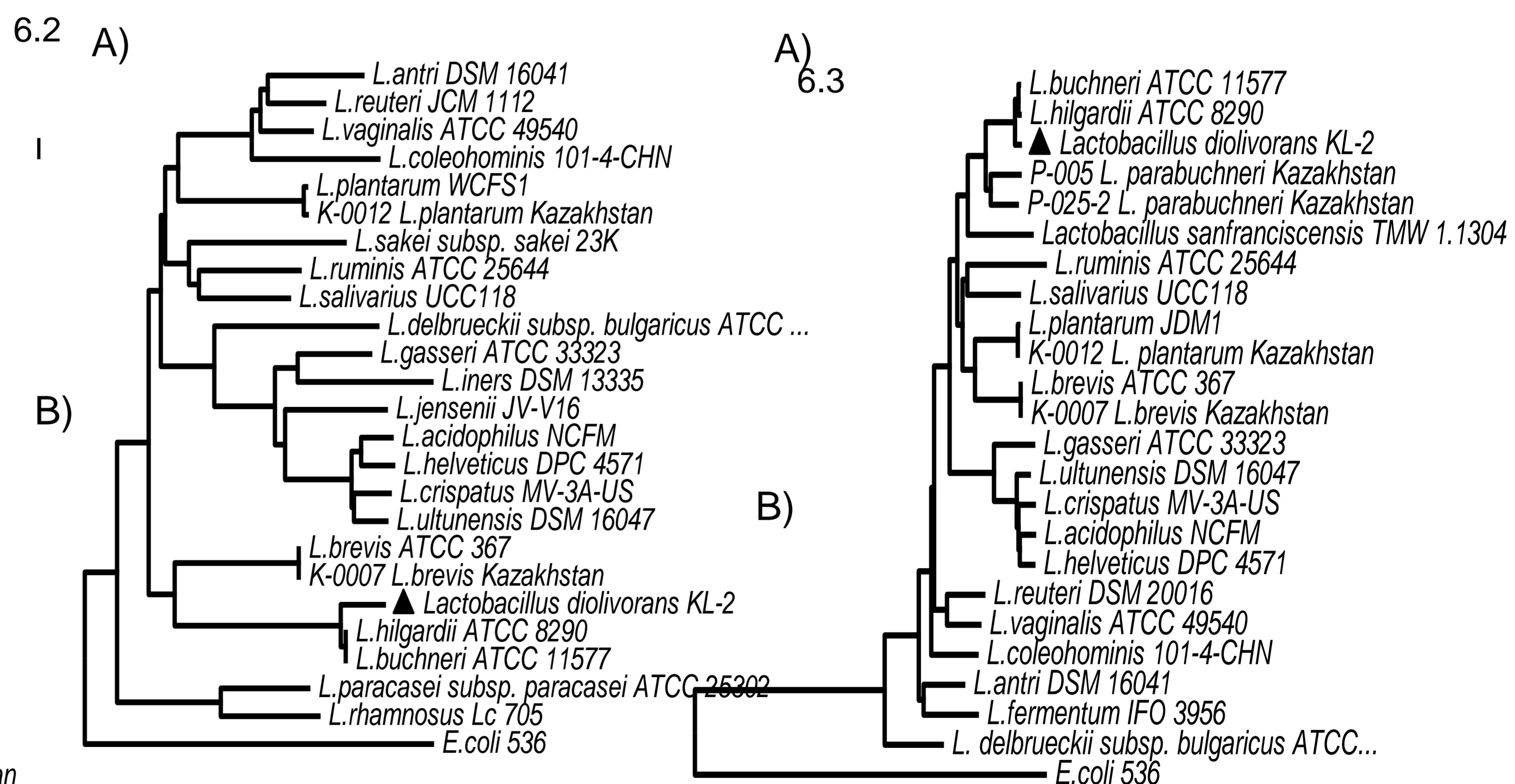
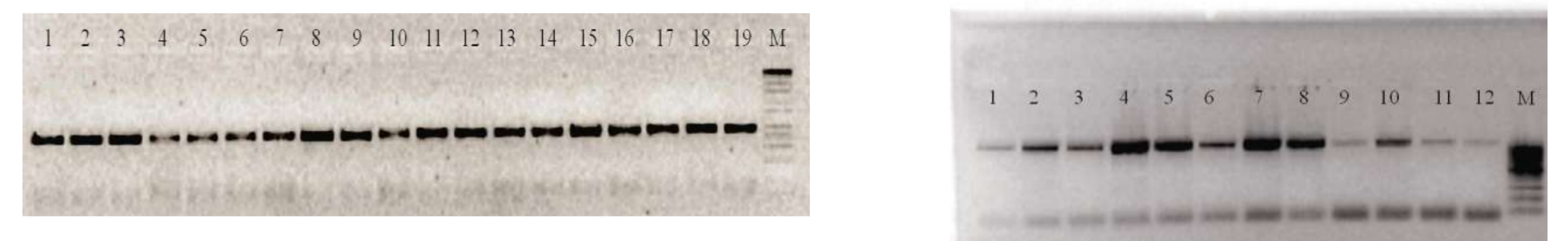


Fig. 6. The identification of *Lactobacilli* based on analysis of the *groEL* (1), *rplB* (2), *groEL* (3) genes

A - Electrophoregramm of PCR amplification products of the *groEL* gene ( 1–12 samples).  
B- The phylogenetic tree of LAB based on nucleotide sequence

**Conclusions.** The present study is the first report of LAB isolated from national lactic acid products of functional nourishments. In our experiments the isolated lactococci and lactobacilli were studied as probiotic cultures. The gene nucleotide sequences of all the genotyped strains were deposited in the GenBank database. All strains have status "GRAS" (absolutely harmless for human health and animals). It should be noted that the new strains of LAB have replenished the collection of cultures and are of scientific and practical interest in terms of expanding biological diversity, their possible use as probiotics, as well as bacterial starter cultures, for the production of lactic acid products





