

IDENTIFICATION OF LACTIC ACID BACTERIA ISOLATED FROM KATYK, GOAT'S MILK AND CHEESE

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Summary

Eighteen lactic acid bacterial strains were isolated from home made katyk, goat's milk and cheese. Nine of them belonged to lactic acid cocci and others were referred to genus Lactobacillus. Five strains were identified to species by determination of morphological, cultural, physiological and biochemical characteristics - Pediococcus acidilactici, Pediococcus pentosaceus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus helveticus and Lactobacillus plantarum.

Introduction

Lactic acid bacteria are widely distributed in the nature. In this group are included representatives of the genus *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Leuconostoc*. They could be isolated from soils, waters, plants, silages, waste products, and also from the intestinal tract of animals and humans. The lactic acid fermentation, which these bacteria perform has long been known and applied by the humans for making different foodstuffs. It plays an essential role in the production of all dairy products and is involved in the production of many other foods and drinks – sausages, pickles, boza etc. Since decades by these processes the application of well-studied starter cultures was established. They should possess stable fermentation characteristics and should be resistant to bacteriophages [5]. In our country

at domestic conditions dairy products are prepared from cow, but also from sheepish, goat's and buffalo-cow's milk – such as cheese, curds, katyk (specific national dairy product), yellow cheese, etc. By these products the species composition of lactic acid bacteria is more various and inconstant when compared with those of the trade products. The “wild” strains, in biotechnological aspect are perspective as bacteriocin producers [6, 10] and probiotics [3, 4]. They have a potential use for the establishing of new, the so-called “functional foods” [3, 9].

The aim of the recent study was the isolation and taxonomic determination of lactic acid bacteria from home made katyk and products of goat's milk in order for their including in original Bulgarian collection of lactic acid strains.

Materials and methods

Isolation of lactic acid bacteria. The lactic acid bacteria were isolated from home made kатыk of cow's milk in the Sofia region (2 samples), yogurt of goat's milk (2) and goat's cheese (2) of Tabashko village, Troyan region. The isolation was performed by the routine microbiological procedure and inoculation on a solid medium. Selective media for lactic acid bacteria were used – MRS-agar and M-17 agar. The cultivation was performed without shaking at the appropriate temperature (32 and 40°C) for five days. From kатыk were isolated only thermophilic strains and they were cultivated at temperature 42-45°C. Once single colonies were obtained they were inoculated in MRS broth or M-17 broth. After growing the cultures were diluted and tested for purity on the suitable agar medium.

Identification of the bacterial strains. The determination of the strains was performed

according to their morphological, cultural, physiological and biochemical characteristics by the procedures described in the Bergey's Manual [1]. For performing the biochemical tests a medium with the following composition was used (g/l): bovine extract – 10.0; neopeptone – 10.0; yeast extract – 5.0; Tween 80 – 1 ml; K₂HPO₄ – 2.0; CH₃COONa x 3H₂O – 5.0; diammonium citrate – 2.0; MgSO₄ – 0.2; MnSO₄ – 0.05; bromocresol purple – 0.17. The carbon source was added to the sterile basal medium as filter sterilized solution to a final concentration of 1%. Carbohydrates utilization was assessed at the 24th, 48th hour and on the 7th day of the strain growth at the corresponding temperature.

For identification of the lactic acid bacteria API 50 CH tests (bioMerieux) were also used.

Results and Discussion

From the tested samples eighteen lactic acid bacterial cultures were isolated. After the original characterization nine of them were determined as representatives of the lactic acid cocci, and the rest were referred to genus *Lactobacillus*. The strains K1, K2, Ch1, Ch2 and Ch3 were determined to species.

The strains K1 and K2 isolated from kатыk were cocci – Gram-positive, nonspore-forming, nonmotile, catalase-negative, facultative anaerobes. Their morphological, cultural and physiological characteristics are shown in Table 1, and the biochemical – in Table 2. As a result of the investigations the strain K1 was determined as *Pediococcus acidilactici* and the strain K2 as *Pediococcus pentosaceus*. Both strains curdled the milk very well, which is not typical for the pediococci. This characteristic could be explained with adaptive alterations as a result of growth in the tested substrate. The strains were deposited in the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC) with accession

numbers 3437 and 3545, respectively, and were kept in a freeze-dried state. Representatives of these two species from the genus *Pediococcus* find biotechnological application by the processing and conservation of meat and vegetable foods and play an essential role as components of trade starter cultures by sausage production [8].

The other three lactic acid cultures were referred to genus *Lactobacillus*. They are Gram-positive, nonspore-forming rods, catalase-negative, microaerophilic, with fermentative metabolism. For their identification to species the morphological, cultural, physiological and biochemical characteristics were tested. The data are shown in Table 1 and Table 2.

The strain Ch1 could be determined as a representative of the obligate homofermentative species *Lactobacillus delbrueckii*. The all three subspecies of the species (subsp. *bulgaricus*, *delbrueckii* and *lactis*) are characterized with a narrow fermentation profile. The studied strain was able to ferment glucose,

Table 1. Morphological, cultural and physiological characteristics of the isolated strains.

Characteristics	Strains					
	K1	K2	Ch1	Ch2	Ch3	
Cell morphology	cocci in tetrads	a group of two or four cocci	thin, long rods	medium-long rods, rounded ends	short, rounded rods	
Gram stain reaction	G+	G+	G+	G+	G+	
Spores formation	-	-	-	-	-	
Colony morphology	grey-white, 1-2 mm	grey-white, 1-2 mm	small-sized, slightly convex, R, <1 mm	circular, irregular, "snowflake" type, 1-2 mm	circular, white, glistening, convex, 1 mm	
Catalase activity	-	-	-	-	-	
Glucose fermentation	+	+	+	+	+	
NH ₃ from arginine	+	+	-	-	-	
Growth at temperature (°C)	15	ND	ND	-	-	+
	30	+	+	+	+	+
	45	+	+	+	+	-
	50	+	-	ND	ND	ND
Growth in a medium with NaCl (%)	4	+	+	ND	ND	ND
	6.5	+	+	ND	ND	ND
Gelatin liquefaction	ND	ND	-	-	-	
Milk curdle	+	+	+	+	+	
pH optimum	6.5	6.5	5.8-6.5	5.8-6.5	5.8-6.5	

Legend: positive reaction (+), negative reaction (-), not determined (ND).

fructose, lactose, galactose and maltose. It is the closest to *Lactobacillus delbrueckii* ssp. *bulgaricus* although the dissimilation of the last two carbohydrates is not characteristic for the type strain.

As a result of the taxonomic studies the tested strain Ch2 was determined as *Lactobacillus helveticus*. Its morphological, cultural and biochemical characteristics were identical with those of the type strain. This species belongs to the group of the thermophilic lactobacilli, obligate homofermentative type and is included in starter cultures

by the production of the cheese Gruyere, Gorgonzola, Mozzarella [2].

The phenotype analysis of the strain Ch3 indicated its relativity to *Lactobacillus plantarum*. This strain belongs to the group of the mesophilic lactobacilli, which are commonly met in the later phase of the maturing of the cheese, together with *Lb. casei*, *Lb. brevis* and *Lb. buchneri* [2]. Olarte noted that the presence of *Lb. plantarum* by the production of the cheese Cameros from goat's milk decreased the number of the enterobacteria and fecal coliforms in the final product [7].

Table 2. Biochemical characteristics of the tested strains – acid production by utilization of carbon sources.

Carbon sources	Strains					Carbon sources	Strains				
	K1	K2	Ch1	Ch2	Ch3		K1	K2	Ch1	Ch2	Ch3
Arabinose	-	+	-	-	+	Melibiose	-	+	-	-	+
Cellobiose	+		-	-	+	Raffinose	-	+	-	-	+
Esculin	+	+	-	-	+	Rhamnose	-	-	-	-	-
Fructose	+	+	+	+	+	Ribose	+	+	-	-	+
Galactose	+	+	+	+	+	Salicine	+	+	-	-	+
Glucose	+	+	+	+	+	Sorbitol	-	-	-	-	+
Lactose	-	-	+	+	+	Sorbose	-	-	ND	ND	ND
Maltose	-	+	+	+	+	Sucrose	-	-	±	-	+
Mannitol	-	-	-	-	+	Trehalose	-	+	-	+	+
Mannose	+	+	-	+	+	Xylose	-	-	-	-	-
Melezitose	-	-	-	-	+						

Legend: positive reaction (+), negative reaction (-), not determined (ND).

The two strains from genus *Pediococcus* were isolated from katyk – *Pediococcus acidilactici* and *Pediococcus pentosaceus*. From goat's yogurt was isolated *Lactobacillus delbrueckii* ssp. *bulgaricus* and from home made goat's cheese *Lactobacillus helveticus* and *Lactobacillus plantarum*. These strains showed some non-essential phenotypic

differences from the type strain due to the specificity of the substrates and the growth conditions. The “wild” strains have potential as producers of growth inhibition factors, which play important role in the interaction within the mixed cultures and the investigations in this direction will continue.

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ИДЕНТИФИКАЦИЯ НА МЛЕЧНОКИСЕЛИ БАКТЕРИИ, ИЗОЛИРАНИ ОТ КАТЪК, КОЗЕ КИСЕЛО МЛЯКО И СИРЕНЕ

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Резюме

От домашно приготвени катък, козе кисело мляко и сирене са изолирани 18 щама млечнокисели бактерии. Девет от тях са представители на млечнокиселите коки, а останалите са от род *Lactobacillus*. Пет щама са идентифицирани до вид чрез определяне на морфолого-културалните и физиолого-биохимичните признаци и свойства – *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactobacillus delbrueckii ssp. bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*.