Evaluation of technological parameters of newly isolated lactobacilli from traditional dairy products

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Evaluation of technological parameters of newly isolated lactobacilli from traditional dairy products: *Lactic acid bacteria (LAB) are industrially important microorganisms which are used all over the world in a variety of food fermentations. Their contribution in these processes primarily consists of the production of different metabolites like lactic acid, carbon dioxide, diacetyl/acetoin, and etc., which define the preserving capacity, flavor, texture and shelf life of fermented foods.*

In the present work, 8 Lactobacillus strains, newly isolated from artisanal samples of white-brined and yellow cheeses were characterized. The aim was to investigate in vitro their coagulation, caseinolytic and antimicrobial activity. Strain-specific features have been observed. All tested lactobacilli can be estimated as slowly curdled strains with low caseinolytic activity. The sensory qualities of obtained fermented milk samples were estimated according to the BDS standard evaluation of organoleptic properties of dairy products. Moreover, fermented milks and supernatants of exponential and stationary phase LAB cultures showed a promising antimicrobial activity against Escherichia coli ATCC 25922.

The obtained results are only an initial step in characterization of the newly isolated strains. For this reason further technological and functional evaluation is needed and is still in progress.

Key words: *Lactobacillus, milk coagulation, caseinolytic and antimicrobial activity*

INTRODUCTION

Nowadays fermented dairy products are still an important part of consumers` daily diet, due to their beneficial health effects. These effects include improving the intestinal microbial balance and reducing the risk of gastro-intestinal diseases, alleviation of lactose intolerance, enhancement of nutrients bioavailability, and etc. [2]. Academic, commercial and regulatory interests are focused on the usage of new processes and products with improved qualities, and low-cost production techniques which have made food fermentations and microbial bioconversion an interesting topic for further investigations [2].

Lactic acid bacteria are one of the most important groups of microorganisms used in food fermentations. They can produce lactic acid, which is a critical parameter on the preservation of fermented foods. Besides lactic acid, LAB also produce carbon dioxide and diacetyl/acetoin which contribute to their flavor, texture and shelf life. There is a growing interest especially on the development of LAB with desired technological features in order to improve the safety and quality of the produced traditional dairy products [3].

The objective of the present study was the evaluation of some important technological properties of lactic acid bacteria, isolated from traditional Bulgarian dairy products and their potential for further usage as food additives.

MATERIALS AND METHODS

Microorganisms, media and culture condition

Eight strains of lactic acid bacteria (*Lactobacillus sp.*) isolated from two traditionally fermented foods in Bulgaria – white-brined (S5, S6, S7, S8, S9, S10 and S12) and yellow cheeses (strain S11) were used in this study. They are a part of recently created laboratory collection of LAB isolates originating from traditional dairy products [4]. The home-made samples and the raw materials for their preparation are presented in Table 1.

The LAB isolates were grown in MRS broth (Himedia, USA) for 24 hours, anaerobically at 37°C in a drying oven with forced convection FD 23 (Binder, Germany).

The technological parameters considered in the study included the ability of LAB to coagulate milk and the determination of organoleptic properties, caseinolytic and antimicrobial activities of the investigated isolates.

Table 1. Samples and isolates from traditional Bulgarian dairy products

Fermented food	Raw material	Number of isolates
White-brined cheese	Sheep milk	2
	Cow milk	4
	Mixed (cow and sheep) milk	1
Yellow cheese	Cow milk	1
Total		8

Coagulation activity

Different methodological approaches have been applied to estimate the coagulation capacity and milk proteolysis of the investigated isolates: (i) the ability to coagulate pasteurized milk (3.5% fat) and 10% w/v skimmed milk (Scharlau, Spain); (ii) a parallel coagulation test with different inoculum by adding 5 and 10% (v/v) exponential cultures to pasteurized milk; (iii) determination of the effect of addition of glucose solution 10% (v/v) and yeast extract (Difco, USA) 0.1% (v/v) to sterile skimmed milk, inoculated with the tested lactobacilli; (iv) preparation of experimental yoghurts in sterile tanks with 5% (v/v) inoculum, consisting of adequate amount (1:1 v/v) of the lactobacilli and industrial starter (LB Bulgaricum^{TM}), for Bulgarian yoghurt according to BDS 12:2010. Control probes (sterile milk without additives) were also applied in the different tests. All of the above samples were incubated at 37°C and pH measurement was also made for each of the obtained fermented milks.

Organoleptic characteristic of fermented yogurts

The Bulgarian standard (BDS 12:2010) was used in assessment of the organoleptic properties of obtained experimental yoghurts. Five non-professional panelists (from the students and staff of the Department of Biotechnology, HTMU in Sofia, Bulgaria) estimated the following parameters of the fermented milks: color and appearance, flavor, body and texture and overall acceptability. Thirty-five point scale, ranging from excellent (score =varying for the different parameters) to very poor (score=0), was used for the evaluation of the fermented milks.

Caseinolytic activity

The agar-well diffusion method using Ca-caseinate agar (Fluka, Switzerland) and milk-agar plates was applied in the determination of the caseinolytic activity. Exponential and stationary cultures in MRS broth (24 h and 72 h) were prepared and tested for each strain. The activity was expressed by the diameter of the obtained clear zones.

Antimicrobial activity

The agar-well diffusion method was applied to determine the antimicrobial activity of cell-free cultures of selected lactobacilli. An overnight culture (20 h) of *Escherichia coli* ATCC 25922 was diluted in sterile saline and inoculated in MacConkey agar (Merck, Germany) plates to a final concentration of 10^6 CFU/ml. Exponential cultures of the investigated *Lactobacillus* strains were prepared in MRS broth. Their cells were removed by centrifugation (6000 g, 5 min, 4°C) and the supernatants were collected and filter-sterilized (0.22 µm, Merck Millipore, USA). The strains were also cultivated for 24 h at 37°C in 10% (w/v) skimmed milk and were tested for antimicrobial activity. The activity was assessed by measuring the diameter of the obtained inhibition zones. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

The autochthonous microflora of artisanal dairy products was estimated as promising source of different strains with beneficial properties (Danova S. et al.,2012) [5]. Therefore, a new laboratory collection of LAB isolates from white-brined and yellow cheeses was created. The 8 strains, characterized as *Lactobacillus s*p. (S5 to S12) were pre-selected for technological characterization.

Coagulation activity

Coagulation activity is an important technological characteristic of LAB. The ability of the strain S5 to coagulate pasteurized milk (3.5% fat) and 10% w/v skimmed milk was firstly investigated. The results showed a coagulation activity only with the sample prepared with pasteurized cow milk (2% fat).

A different time of complete coagulation have to be pointed, when using different LAB inoculums. The best results were obtained with 5% (v/v) of overnight *Lactobacillus* cultures, added as starters in milk. However, due to the long time of fermentation (over 12 h), all of the tested lactobacilli can be estimated as slowly curdled strains. This can also be observed in the results reported by Georgieva et al (2009) [6].

The addition of 10% (v/v) glucose, as well as the addition of 0.1% (v/v) yeast extract to the sterile milks had a positive effect on the growth of the *Lactobacillus* strains. Faster coagulating strains were S6 and S10.

The addition of the strains to the industrial starter helped for the faster coagulation of the yoghurt samples (1 hour earlier) and had a strain-specific influence on the measured pH values, shown in Table 2:

Table 2. Effect of LAB isolates from white-brined and yellow cheeses on acidification and coagulation of milk [a, b]

Home-made product	Strain	[a] Milk coagulation at 37°C
	Control (industrial starter)	+ (3.82)[b*]
White-brained cheeses		
	S5	+ (4.30)[b]
	S6	+ (4.57)[b]
	S7	+ (4.08)[b]
	S8	+ (4.58)[b]
	S9	+ (4.27)[b]
	S10	+ (3.98)[b]
	S12	+ (3.48)[b]
Yellow cheese	S11	+ (3.66)[b]

[a] *The numbers in brackets represent the pH values.*
[b] *Coagulation occurred between 4 and 5 h;* [b*] *coagulation occurred between 5 and 6 h.*

A strain-specific variable coagulation ability and low proteolytic activity were often reported for cheese-originated lactobacilli in comparison with other LAB assayed in the same conditions [7, 8].

The yoghurt samples were stored 24 h at 4°C in a refrigerator and the sensory properties were estimated.

Organoleptic properties

The consumer`s requirements for a good appearance and sensory properties make the organoleptic evaluation one of the most important tests in food industry. For this reason a sensory evaluation of the fermented milks with different LAB culture was made. The results are presented in Table 3.

Soon after the processing, the score of obtained samples differed significantly especially in their appearance and body and texture characteristics. The lowest results about appearance were observed for strains S8, S9 and S10, isolated from sheep and cow cheeses, respectively. According to the parameter “body and texture”, the samples were similar and showed higher score than the control except strain S10, which had the lowest score. The textural differences between the samples can be attributed to the kind of milk used and their composition. According to the other two characteristics – flavor and overall acceptability all the strains showed very good scores. Thus, some strain-specific features should be pointed for this test, too.

Table 3. Sensory properties of fermented milks supplemented with 2.5% industrial inoculum and control sample after coagulation.

TESTED SAMPLE	*PARAMETERS*			
	Color and appearance (max 5 points) *Average ± SD*	Flavor (max 35 points) *Average ± SD*	Body and texture (max 30 points) *Average ± SD*	Overall acceptability (max 5 points) *Average ± SD*
Control	3.4 ± 0.55	34 ± 1.22	24.4 ± 0.89	3.6 ± 0.55
S5	3.6 ± 0.55	33.6 ± 0.55	26 ± 0.71	4.6 ± 0.55
S6	3.2 ± 0.45	34.2 ± 0.84	25.2 ± 0.84	3.6 ± 0.55
S7	3 ± 0.71	33 ± 0.71	29.6 ± 0.55	4.6 ± 0.55
S8	1.4 ± 0.55	32.4 ± 0.89	29.2 ± 0.84	4.2 ± 0.45
S9	2.4 ± 0.55	33 ± 1.00	29.6 ± 0.55	4.8 ± 0.45
S10	1.8 ± 0.45	32.8 ± 0.45	14.8 ± 0.84	3.2 ± 0.84
S11	3.2 ± 0.45	33 ± 0.71	29.8 ± 0.45	4.6 ± 0.55
S12	2.8 ± 0.45	34.2 ± 0.84	29.4 ± 0.55	4.6 ± 0.55

Despite of the detected differences between the experimental fermented milks, several of them showed high similarity with the control. Improvements in the structure of yoghurts with the addition of probiotic have been described for other fermented milks as well [9]. That kind of positive influence on the rheological parameters can be a good alternative to the usage of various additives which may negatively affect texture, taste and aroma of yoghurt [10].

Caseinolytic activity

On milk agar medium all of the *Lactobacillus* strains had very clear zones at the beginning of the cultivation, while on the Ca-caseinate agar medium they didn`t show any activity. The investigation of the total proteolytic activity on milk agar and the caseinolytic activity on Ca-caseinate agar outlined the tested strains as cultures with low proteolytic activity. These results can also be sustained by the investigations of Hebert et al (2000) [11]. It was reported that some of their *Lactobacillus* strains, considered as slow coagulating, also represented poor proteolytic activity.

Table 4. Caseinolytic activity of LAB strains, isolated from white-brined and yellow cheeses.

Strains	*Caseinolytic activity on Ca-caseinate agar of*			
	24 h cultures		72 h cultures	
	Inhibition zone, [mm]	Illustration of the inhibition zones	Inhibition zone, [mm]	Illustration of the inhibition zones
S5	11		14.5	
S6	12		12	
S7	11		12	
S8	13		13.5	
S9	12.5		13	
S10	11		13	
S11	12		12	
S12	12.5		15.5	

The obtained results on Ca-caseinate agar showed that the 72 h cultures possessed better caseinolytic activity than that at exponential phase. From that it can be concluded that the caseinolytic activity increases with time, due to the initial cell lysis at the end of fermentation. The highest activity possessed the strains isolated from white-brined cheeses (S5, S8 and S12). The obtained results are summarised in Table 4.

Antimicrobial activity

The antimicrobial activity of 8 *Lactobacillus* strains against intestinal pathogen was investigated, using the referent strain *Escherichia coli* ATCC 25922. The obtained results are shown in Table 5:

Table 5. Antimicrobial activity of the LAB strains against *E. coli* ATCC 25922.

Strain	*Inhibitory activity against E. coli 25922, [mm sterile zone]*		
	Supernatants from exponential cultures (24 h)	Supernatants from late stationary-phase cultures (72 h)	Coagulated Milks
S5	10	9	9
S6	10	8	8
S7	11.5	8	10
S8	12	9	8
S9	10	10	8
S10	10.5	7.5	8
S11	10.5	10	8
S12	11	11	9

The present results (Table 5) revealed that all the strains slightly diversed in their antimicrobial activity, according to the 3 different assays. The highest activity is achieved with *Lactobacillus* S7, while the strain S6 possessed the lowest antimicrobial activity against *E. coli*. These results complemented the reported about various *Lactobacillus* sp. with broad spectrum of antagonistic activity against different pathogens like *E. coli, Salmonella, Shigella* and etc. [12].

The mechanism of antimicrobial activity is associated mainly with the decreased pH of the medium. The antimicrobial agent is probably the produced lactic acid during the fermentation. The inhibitory effect of lactic acid is provoked by the undissociated molecules prevailing in environment with low pH [13]. The latter affects basic metabolic functions, such as the transport of substrates, oxidative phosphorylation and release of protons which cause a change in the intracellular pH. All this destabilizes the membrane, which affect its permeability, resulting in reduced activity of several enzymes and initiates the action of other antimicrobial substances [1].

Further tests with neutralized and protease treated spent cultures have to be done in order to characterize other produced active metabolites, such as bacteriocins.

CONCLUSION

The results allow the conclusion that our isolated strains can be used successfully as additives in the production of yoghurt and different fermented dairy products. They can improve their properties by minimizing the time for coagulation and increasing the pH values which will make the taste of the products much more pleasant. They can also improve the stability and performance of the coagulum and in combination with their good antimicrobial activity against *E. coli* ATCC 25922 they guarantee the better impact over the humans` health.

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This paper has been reviewed