Research of fermentation processes of protein substrates by consortiums of lactic acid bacteria

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Abstract. The paper presents data on the assessment of the biotechnological activity of new consortiums of lactic acid bacteria Lactobacillus with the proteolytic activity. A comparative assessment of the biotechnological use of previously isolated strains of lactic acid bacteria and their combinations using methods that are highly effective in the technology of fermented food products is carried out. Biotechnological properties were studied on various food objects and substrates of animal and plant origin with a high protein content. As a result of the studies, consortiums of lactic acid bacteria, they can be recommended for inclusion in the composition of starter cultures for the production of functional products.

1. Introduction

In the modern world, environmental protection and rational use of natural resources are becoming very important. During the processing of raw materials, a significant amount of low-grade raw materials and waste with various compositions and properties is formed. The main direction of rational use of agricultural resources is the maximum degree of processing of raw materials and waste reduction. One of the promising ways of rational use of low-grade food raw materials is its biotransformation, which includes processing with living cells of microorganisms, primarily with starter cultures of lactic acid bacteria (LAB).

LAB are a group of Gram-positive non-sporulating anaerobic or facultative aerobic cocci and bacilli that produce lactic acid as the main product [1]. Originally, four genera: *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* had recognized as LAB. This group has now expanded to include more than 10 genera [2, 3]. LAB take part in the process of food fermentation and are used as starter cultures. The use of LAB improves the sensory characteristics of the product, such as taste, aroma and texture, affects the formation of color and reduces the ripening time. Products made with LAB are better digested and safer because pathogenic bacteria are inhibited [4].

Some strains of LAB are probiotics and have a beneficial effect on human intestinal microflora [5]. Biochemical processes occurring during the life activity of LAB include a number of numerous enzymatic reactions leading to a decrease in pH, inhibition of competing microflora due to the formation of metabolites such as lactic acid, diacetyl, acetaldehyde and peptides [4]. LAB fermentation produces products of fat and protein breakdown that contribute to flavor characteristics through the release of lipolytic and proteolytic enzymes [6]. The proteolytic potential of LAB is of the greatest interest for our study.

Proteolysis is the hydrolysis of peptide bonds of proteins with the formation of peptides and free amino acids, which take part in decarboxylation, deamination, and transamination reactions and have a

significant impact on the formation of organoleptic characteristics in such products as cheese and fermented sausages [7, 8]. In dairy products, proteolysis of milk protein improves the digestibility of products, which finds its application in the production of dietary food. Proteolysis also reduces the antigenicity of whey protein, which can induce an immune response for some people, and the resulting peptides reduce microbial infections [9]. In addition, hydrolyzed peptides are useful for the gut microbiota because they exhibit antimicrobial activity and inhibit pathogens such as E. coli, L. monocytogenes, and S. aureus, thereby promoting the growth of LAB [10]. Many LAB, including Lactococcus, Lactobacillus and Streptococcus, are strains with proteolytic activity [11] Lactobacillus genus, particularly L. delbrueckii and L. helveticus, produce proteases capable of hydrolyzing α- and ß-casein [8]. Representatives of the Pediococcus genus have a high level of proteolysis and are widely used in the production of cheese and fermented sausages [12].

LAB have a complex proteinase and peptidase system consisting of several components: proteases associated with the cell membrane, peptide transporters, and intracellular peptidases that break down peptides into shorter and free amino acids [13].

The relationship between the location of these enzymes and the type of their activity allows us to understand their role during fermentation. There is evidence that proteolytic enzymes are released into the extracellular space and will affect food matrices during food production, hydrolyzing proteins and influencing the development of flavor and biochemistry of dairy products [14]. In addition, the extracellular proteolytic system LAB plays a key role in the degradation of casein to oligopeptides in milk, which can use in metabolic processes [7]. There is also evidence that some strains of LAB effectively inhibit the activity of S. aureus. This is due to the formation of an endopeptidase, which cleaves the peptide bonds between two amino acids in the cell membranes of the bacteria [15].

Starters with proteolytic activity can be used in the production of fermented meat products and in cheese technology. Therefore, studying the ability of microbial strains to affect protein substrates of animal origin is necessary to realize the potential of LAB for food production. These bacteria are capable of enhancing the safety and palatability of dairy products by exhibiting dual activity, contributing to the metabolism of lipids and proteins contained in milk. The ability of different LAB to coexist in the same food suggests the potential of using a selected consortium of bacteria with high values of enzymatic activity in the development of foods to promote consumer health [16].

Selecting promising LAB strains by their level of proteolytic activity and to developing a consortium of microorganisms containing functionally active strains of Lactobacillus is the aim of this research.

2. Materials and methods

The objects of the study were new starters containing strains of Lactobacillus: *L.fermentum, L.plantarum, L.casei, L.curvatus*, as well as consortia obtained on their basis in the ratio 1:1.

Characteristics of the starters are presented in Table 1.

All selected starters perform mainly homo-fermentative lactic fermentation, except Lactobacillus fermentum. The temperature optimum of the strains lies in the range of 30 - 37 °C, the strains are well reproduced in a wide temperature range from 15 to 45 °C, the optimal pH is 5,5 to 6,2, the strains are resistant to alkaline reaction of the environment (pH = 9,2), they can grow in acidic environments (pH = 3).

These strains are characterized by high proteolytic activity [17], exhibit antioxidant [18], gene-protecting and antimutagenic properties [18], and actively inhibit the growth of sanitary-positive food microflora [19], which confirms their prospects for inclusion into the consortium intended for functional food products.

The activity of LAB was studied by registering absorption spectra in the wavelength range of 220 - 280 nm according to the method of Warburg and Christian [20], as well as according to the method [21], the principle of which is to measure the amount of products of bacterial proteolysis not precipitated by trichloroacetic acid. Skimmed milk as well as sterile extracts of myofibrillar and sarcoplasmic proteins were used as model substrates.

Table 1. Characteristics of LAB

Stains	Shape and size of		Growth temperature °C		Type of fermentation
	cells	colonies	optimal	limits	
Lactobacillus plantarum 21	Rods 4-5 × 0,6-1 µm	Average, dome-shaped, whitish	30-37	15-40	Homo-fermentative LAB
Lactobacillus curvatus 8	Rods 1-1,2 × 4,5 µm	Small, rounded, white	30-37	15-40	Homo-fermentative LAB
Lactobacillus casei 32	Rods 2-4 × 0,7-1 µm	Small, curved, whitish sparkly	30-37	15-45	Homo-fermentative LAB
Lactobacillus fermentum 12	Rods 0,5 - 0,9 µm	Small, curved, sparkly	35-40	15-45	Hetero-fermentative LAB

3. Results and discussion

Microbial proteases play an important role in the breakdown of proteins into small peptides and in their subsequent cleavage into amino acids [22]. We previously found that the microorganisms under study are effective on casein [19]. The sarcoplasmic protein fraction characterizing muscle tissue proteins was selected for further studies [23]. The results of the studies are presented in Figure 1.

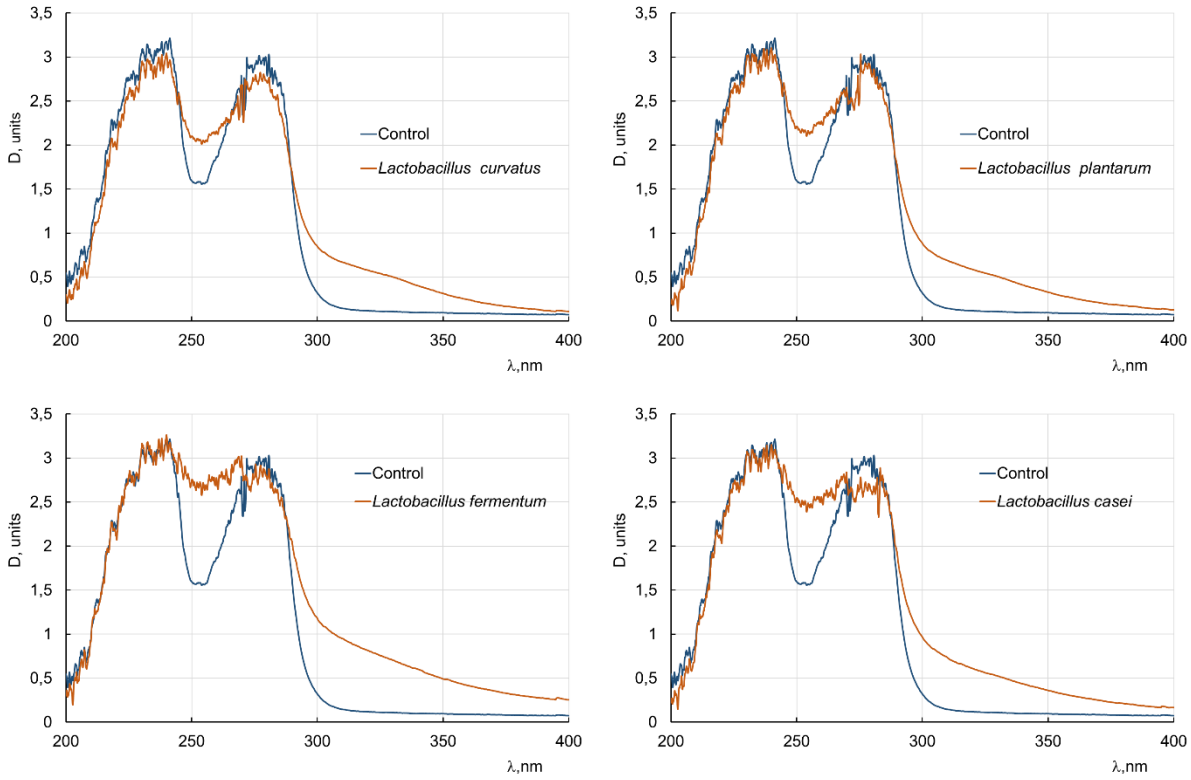

Figure 1. Spectrogram of hydrolysates of sarcoplasmic proteins model systems fermented by LAB

A decrease in total protein background and accumulation of free amino acids in the extracts in the 240-280 nm wavelength range was found for all strains studied, with *Lactobacillus casei* and *Lactobacillus fermentum* strains showing the greatest activity. The degree of hydrolysis of sarcoplasmic proteins was 65-70 % for these strains. For *Lactobacillus casei* the degree of hydrolysis was about 35 %, and for *Lactobacillus curvatus* - not more than 20 %. Earlier studies on protein substrates of animal origin [17] showed that the proteolytic potential of *Lactobacillus curvatus* is insufficient for effective action on protein complexes of meat and dairy raw materials and further study of this strain in the work is inexpedient, so it had excluded from the study.

In order to determine the possibility of creating multispecies compositions of selected LAB, we studied the ability of their co-growth on sterilized skim milk. Four combinations of cultures mixed in equal ratios (1:1) and representing the basis of a multistrain starter were made:

Sample 1: *L.plantarum, L.casei;*

Sample 2: *L.casei, L.fermentum;*

Sample 3: *L.plantarum, L.fermentum*;

Sample 4: *L.plantarum, L.casei, L.fermentum.*

The results of combinability studies are presented in Table 2. Samples 1 and 2 yielded the highest number of cells. The fermentation time in these samples was 28 h with a pH shift to 4,89. The acidity was best in samples 3 and 4 and ranged from 67 to 87 °T with a pH of 4,46 to 4,48.

Sensory evaluation of multistrain starters showed that the leading position was taken by sample 4, which had a dense consistency, a very pleasant aroma and clean fermented milk taste.

Table 2. Fermentation of milk with different consortiums

Consortium	Lg LAB	Fermentation time, h	pH	Acidity, °T	Sensory characteristics		
					consistency	taste	aroma
Sample 1	8,58	28	5,09±0,15	63,94±1,92	homogeneous	pure	fine
Sample 2	8,63	28	5,20±0,16	59,61±1,79	homogeneous	pure	fine
Sample 3	8,21	24	4,46±0,13	87,50±2,63	dense	pure	faint
Sample 4	8,26	17	4,48±0,13	66,89±2,01	dense	pure	very nice

After that, we studied the proteolytic activity of the obtained consortiums on protein substrates of animal origin (sterilized skim milk, sterile extracts of myofibrillar and sarcoplasmic proteins). The results of the study, presented as the degree of protein hydrolysis, are shown in Figure 2.

The combination of *Lactobacillus plantarum, Lactobacillus casei* and *Lactobacillus fermentum* (sample 4) show the greatest proteolytic activity; the degree of hydrolysis of milk proteins was 82 %, myofibrillar and sarcoplasmic proteins - 68 % and 75 % respectively. The results of the study indicate the manifestation of a symbiotic effect when LAB is used together, which agrees with the researches [16]. The obtained effect exceeds the biotechnological potential of individual strains presented in consortium.

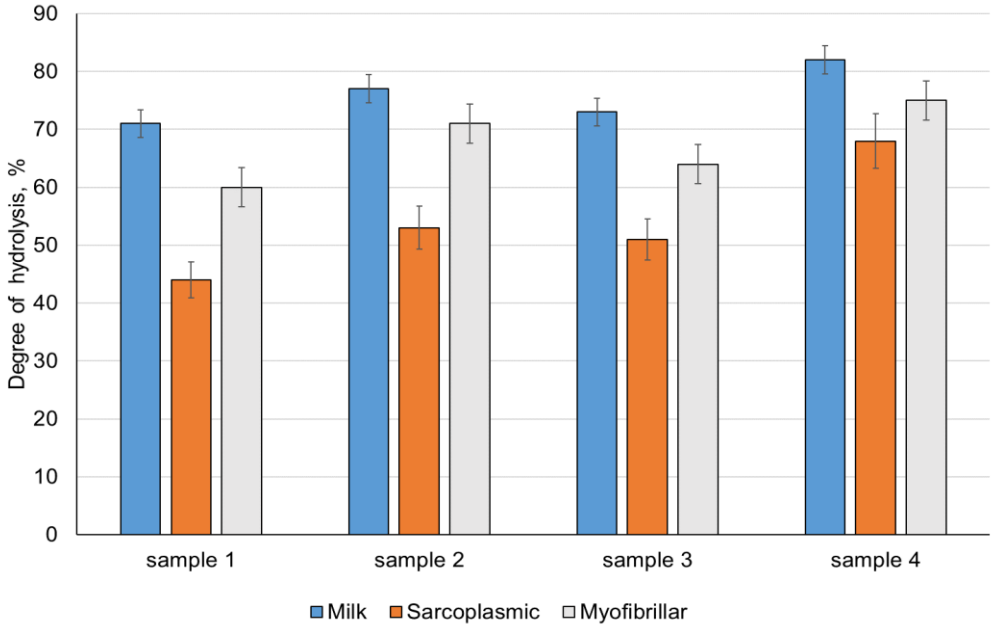

Figure 2. Degree of protein hydrolysis by consortiums

4. Conclusion

Proteolysis is an important biochemical process in the technology of many fermented foods, therefore, when selecting starter cultures, special attention should be paid to the research of the proteolytic activity of individual strains and their combinations. The strains of LAB presented in this research are producers of extracellular proteases and exhibit proteolytic activity on various food substrates.

The selected LAB strains when cultured together will provide biochemical transformations of proteins due to their high proteolytic potential have a positive effect on the functional and technological characteristics of the raw materials and improve the quality of the food products. Possible applications of the studied strains and their combinations are the technology of fermented food.

In addition, the developed consortium can be used for the disposal of agricultural waste and complex processing of low-grade protein raw materials for the creation of feed and food protein additives.

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