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GENERATION OF BIOACTIVE PEPTIDES IN MEAT RAW MATERIALS EXPOSED TO LYSATES OF BACTERIAL STARTER CULTURES

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Abstract

Nowadays, preparations based on bacterial lysates are mainly applied in medicine. In food industry, bacterial lysates are still not widely used, in particular for manufacturing meat functional foodstuff. Though their potential for functional foodstuff production is predictable, the efficiency and specificity of action which depend on the characteristics of the strain and the method of cell disintegration require study. A set of peptidases identified in starter cultures, in particular endo-peptidases, aminopeptidases, dipeptidases, tripeptidases, and proline-specific peptidases stimulate interest in the lysates of these microorganisms for food biotechnology. In this work, we have shown that lysates of *Pediococcus pentosaceus* 28, *Staphylococcus carnosus* 108, *Lactobacillus curvatus* 1, *P. acidilactici* 38, *L. sakei* 103, *L. sakei* 105, *L. curvatus* 2, *L. acidophilus* AT-41 that we obtained by physical destruction of bacterial cells have the widest spectrum of enzymes and biologically active substances. Our goal was to determine the biochemical composition and enzymatic activity of the lysates of starting bacterial cultures and their role in the formation of biologically active peptides in raw meat. The bacterial suspensions were exposed either to lysozyme treatment followed by separation of the extract from the cell debris by centrifugation, or to ultrasonic treatment to compare two methods of cell destruction. The physical method was proved to be the most effective. For biochemical characterization, the proteolytic, lipolytic and collagenase activities of the lysates, and the concentration of organic acids, proteins, and free amino acids were measured. Enzymatic activities of the lysates were determined using API®ZYM tests. The *Lactobacillus curvatus* 2, *Lactobacillus acidophilus* AT-41, *Pediococcus acidilactici* 38 and *Staphylococcus carnosus* 108 lysates showed the widest range of intracellular enzymes, including leucine and valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and β-galactosidase. The proteolytic activity was the highest in *Staphylococcus carnosus* 108 (115.94 proteolytic capability PC units per mg protein), *Lactobacillus acidophilus* AT-41 (66.7 PC units per mg protein), *Lactobacillus curvatus* 1 (91.03 PC units per mg protein), and *Lactobacillus curvatus* 2 (72.20 PC units per mg protein) as compared to other strains. The level of malic, lactic and succinic acids in the lysates varied in the range of 0.002-0.02, 0.02-0.06, and 0.2-0.9 mg/100 g, respectively. The highest enrichment in free amino acids with 13 AA detected out of 17 AA studied was characteristic of *P. acidilactici* 38 lysate while only 7 AA were detected in the *L. sakei* 105 lysate. A comparison of 2D electrophoregrams of fermented raw meat showed both general effects on reducing total proteins and the lysate-specific effects toward various proteins, e.g., formation of protein conjugates and cleavage of target proteins, in particular actin skeletal muscle. Therefore, lysates of the studied starter cultures can serve as a source of various enzymes for practical use in the food industry, for example to improve the functional, technological and biocorrective characteristics of meat products.

Keywords: lysates, starting cultures, enzymatic lysis, biologically active peptides, two-dimensional electrophoresis, IEF-PAGE, MALDI-TOF, mass spectrometry

The modern human diet should include biologically active ingredients with known physicochemical characteristics, for which properties useful for maintaining and improving health have been identified and scientifically substantiated, and the daily physiological need has been established. Daily consumption of functional foods is seen as a way to reduce the risk of disease [1]. Numerous studies have focused on the production of bioactive peptides as nutraceuticals and functional food ingredients for their health benefits. These short peptides, exhibiting antihypertensive, antioxidant, mineral binding, immunomodulatory, and antimicrobial activities, are latent in the primary sequences of food proteins and are released during enzymatic proteolysis [2].

During microbial fermentation, bacteria synthesize vitamins and mineral compounds, with the participation of proteinases and peptidases, they form biologically active peptides and remove some non-nutritive substances. Microbial fermentation is considered as one of the main and economically most suitable processes for the production of biologically active peptides [3]. Lactic acid bacteria with complex proteolytic systems are successfully used as starter cultures in the production of a variety of fermented meat products. A deeper understanding of the functionality of the proteolytic system of starter cultures opens up future opportunities for obtaining new food compounds with potential health benefits [4]. Fermented foods combine a range of health benefits through antioxidant, antimicrobial, antimycotic, anti-inflammatory, antidiabetic, and antiatherosclerotic activities [5].

Recently, to obtain functional preparations and products, interest has been growing in lysates of cells of microorganisms subjected to mechanical, chemical, or enzymatic destruction. Cells are made up of water, inorganic ions, and carbon-containing (organic) molecules. Water is the most abundant molecule in cells, accounting for 70% of the total mass of cells. Inorganic ions of the cell, including sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), phosphate (HPO_4^{2-}), chloride (Cl^-) and bicarbonate (HCO_3^-), make up to 1% of the cell mass. These ions are involved in cellular metabolism and play an important role in the functioning of cells. However, the uniqueness of a living cell is determined by organic molecules, most of which belong to one of four classes of compounds, i.e., carbohydrates, lipids, proteins, and nucleic acids. Proteins, nucleic acids, and most carbohydrates (polysaccharides) are macromolecules formed as a result of polymerization of low molecular weight precursors — amino acids, nucleotides, or simple sugars. Such macromolecules make up 80-90% of the dry weight of most cells. Lipids are also among the main components of the cell. The rest of the cell mass consists of many small organic molecules, including macromolecular precursors [6]. The cell wall of gram-positive bacteria is a complex structure formed by glycopolymers and proteins. It consists of layers of peptidoglycan (murein sac) surrounding the cytoplasmic membrane, stitched in the transverse direction with teichoic acids, and contains polysaccharides and proteins [7].

Bacteria serve as a source of various intra- and extracellular enzymes. Starter cultures synthesize proteases and peptidases, glycosidases, polysaccharide enzymes, malolactic enzymes, esterases, ureases, phenol oxidases, and lipases [8]. The proteolytic system of lactic acid bacteria used as starter cultures consists of proteinases (break down proteins into peptides), peptidases (break down the resulting peptides into smaller peptides and amino acids), and transport systems that are involved in the cellular uptake of small peptides and amino acids. A wide range of peptidases has been identified in lactic acid bacteria, in particular, endopeptidases, aminopeptidases, dipeptidases, tripeptidases, and proline-specific peptidases [9, 10]. The proteolytic systems of lactococci and lactobacilli are surprisingly similar

in components and mode of action; their proteolytic system consists of extracellular serine proteinase, transport systems specific for di-tripeptides and oligopeptides (> 3 residues), and many intracellular peptidases [11]. In addition, the importance of proteolytic and peptidolytic enzymes of lactic acid bacteria is that a number of strains, for example, *Lactobacillus helveticus* CP790, *L. rhamnosus* GG, *L. bulgaricus* SS1, and *L. lactis* subsp. *cremoris* FT4 are involved in the release of bioactive peptides [12].

Bacteria can produce both intermediate and final products of bacterial metabolism, e.g., lactic acid, hydrogen peroxide, and bacteriocins, as well as metabolites of small molecules (histamine, vitamins, short-chain fatty acids, polyunsaturated fatty acids, serpins, lactocepins, secreted proteins). Moreover, the metabolic potential of microbes varies greatly between species and even among strains of the same species [13].

Thus, from a chemical point of view, cell lysates are a mixture of short-chain peptides, free amino acids, organic acids, polysaccharides, vitamins of groups B, C, PP, folic acid, volatile fatty acids, peptidoglycan of cell walls, as well as various enzymes.

The study of the intracellular enzyme systems of bacteria requires cell disintegration [14, 15]. A comparison of some conventional methods (ultrasonic treatment, mechanical grinding with glass beads, freeze-thaw, chemical lysis with toluene solutions in acetone or ethanol) showed that ultrasonic treatment and grinding of *Bacillus subtilis*, *Pseudomonas putrefaciens*, and *Streptococcus durans* cells made it possible to obtain more protein in cell-free extracts than other methods [16].

Currently, preparations based on bacterial lysates are used mainly in medicine. Clinical studies have shown that oral bacterial lysates reduce the need for antibiotics and the risk of recurrent respiratory infections in children and adults [12]. The use of these drugs for the treatment of bronchial asthma has been described [17, 18]. It was reported about the regenerative effect of bacterial lysates of resident non-pathogenic microflora of spring water Comano (Comano Terme, Trento, Italy) in the culture of human skin fibroblasts in vitro. Bacterial strains isolated from this water were characterized by genomic sequencing. The collection included 182 isolates; bacterial lysates were obtained by autoclaving (121 °C, 20 min) [19]. Re-epithelialization of damaged tissues using a soluble fraction from the lysate of seven different probiotic strains belonging to the genera *Streptococcus*, *Lactobacillus,* and *Bifidobacterium* was shown using the HaCaT human keratinocyte line model *in vitro* [20]. An *in vitro* study revealed an increase in the content of hyaluronic acid in HaCaT cells treated with *L. plantarum* K8 lysates [21]. The use of microfluidized *Lactobacillus rhamnosus* lysates in the reconstructed Keraskin™ human epidermis improved the barrier function of the skin [22]. Lysates of *E. coli* and other pathogens are successfully used in the treatment of urinary tract infections in cases where there is a high resistance of bacteria to antimicrobial drugs [23]. Interventional studies using probiotics, prebiotics, and their hydrolyzed forms and bacterial lysates have shown a decrease in food sensitization and a positive effect in allergic diseases, including atopic dermatitis [24, 25]. Lysates of lactic acid bacteria are used to treat diseases of the gastrointestinal tract [26].

Cell-free gene expression systems are becoming an important platform for solving a wide range of problems in synthetic biology and biotechnology, including the production of reliable biosensors [27].

In food production, in particular, in the meat industry, bacterial lysates are still not widely used, although their prospects are predictable, which stimulates interest in this kind of research. For example, it was shown that under the influence

of the culture of the *L. plantarum* CRL 681 strain (originally isolated from meat products) in combination with its cell lysate, proteolysis of both sarcoplasmic and myofibrillar proteins occurred with the formation of various peptides of a hydrophobic nature [28]. With increased fermentation, the substrate is enriched with biologically active compounds that are produced by bacteria responsible for fermentation (conjugated linoleic acids lower blood pressure, exopolysaccharides exhibit prebiotic properties, bacteriocins exhibit antimicrobial effects, sphingolipids exhibit anticarcinogenic and antimicrobial properties) [29], in addition to bioactive peptides, exhibiting antioxidant, antimicrobial, opioid antagonistic, antiallergenic, and blood pressure lowering effects [30].

Earlier, the authors examined the effect of starter cultures on the formation of bioactive peptides in meat and meat products [31, 32]. In the development of these studies, in this work, the authors have shown that lysates of the studied starter cultures have a set of enzymatic activities, including high general and specific proteolytic activity (the presence of target proteins, the formation of protein conjugates) with the formation of spectra of low molecular weight peptides and can find a practical application for improving the functional, technological and biocorrective characteristics of meat products.

The aim of the work was to determine the biochemical composition and enzymatic activity of lysates of starting bacterial cultures and their role in the formation of biologically active peptides in raw meat.

Methods. The authors used the strains *Pediococcus pentosaceus* 28, *Staphylococcus carnosus* 108, *Lactobacillus curvatus* 1, *P. acidilactici* 38, *L. sakei* 103, *L. sakei* 105, *L. curvatus* 2, *L. acidophilus* AT-41(collection of the the Moscow State University of Food Productions).

To obtain the bacterial biomass of each strain, a cell suspension (10^9 CFU/ml) was introduced into a de Man, Rogosa, and Sharpe liquid nutrient medium (MRS) at the rate of 1 ml of suspension per 10 ml of medium. It was cultivated for 24 h at 37 °C [33]. The culture fluid was placed in two 40 ml centrifuge tubes. The cells were precipitated (4000 rpm, 4 °C, 15 min), the supernatant was decanted. The pellet was resuspended in 10 ml of 100 mM phosphate buffer (pH 7), the samples were combined, centrifuged (4000 rpm, 4 °C, 15 min), resuspended in 10 ml of 100 mM phosphate buffer (pH 7), and additionally centrifuged at the same conditions.

Lysates were obtained in two ways — by treating cells with lysozyme and by ultrasonic disintegration.

When using lysozyme, after removing the supernatant (second centrifugation), 100 mM phosphate buffer (pH = 7) was added to the biomass with the addition of lysozyme (2.5 mg/ml) and sucrose (20 mg/ml) to a cell suspension density of 10^9 CFU/ml (according to the McFarland turbidity standard). The biomass was carefully resuspended. In a glass test tube with a volume of 40 ml, 20 ml of the lysed suspension was taken and placed in a thermostat on a shaker (for uniform treatment with lysozyme) for 1 h at 30 °C. Then the samples were centrifuged (4000 rpm, 4 °C, 15 min) to separate cell debris from protoplasts. The supernatant was decanted, the pellet was resuspended in 10 ml of 100 mM phosphate buffer (pH 7) with sucrose (5 mg/ml) to create a hypotonic environment, leading to physical rupture of protoplasts. The samples were thoroughly mixed for 5 min, centrifuged (15,000 rpm, 8 min). The precipitate was separated from the supernatant (lysate).

For ultrasonic disintegration of cells after removing the supernatant (second centrifugation), 100 mM phosphate buffer (pH 7) was added to the biomass until the cell concentration in the suspension was 10^9 CFU/ml (according to McFarland turbidity standard). In a centrifuge tube with a volume of 40 ml,

20 ml of the lysing suspension was taken. The tube was placed in an ice bath and then processed on an ultrasonic disintegrator Soniprep 150 (MSE, UK) in the following mode: processing for 30 s, cooling for 30 s (six cycles with stirring every two cycles for uniform disintegration; operating wavelength 9 μm). Upon completion of ultrasonic disintegration, the mixture was thoroughly mixed for 5 min, centrifuged (15,000 rpm, 8 min), and the precipitate was separated from the supernatant (lysate).

The completeness of lysis was monitored by transmission electron microscopy (TEM) (JEM-1400, Jeol, Japan; operating voltage 80 kV, wavelength 500 nm). For this, the pellet obtained after centrifuging the lysing suspension and separating the supernatant (lysate) was resuspended in 0.5 ml of 100 mM phosphate buffer (pH 7), and preparations for TEM were prepared from aliquots of each suspension diluted 100 times with 100 mM phosphate buffer. The samples were applied to a support copper mesh coated with a formvar film and reinforced with carbon, dried for 15 min in air, viewed, and photographed; negatives were scanned and processed in a graphic editor.

The protein concentration in the lysate was measured by the Bradford method on a BioSpectrometer basic spectrophotometer (Eppendorf, Austria) based on the reaction with Coomassie Brilliant Blue R-250 (λ = 595 nm). Bovine serum albumin was used as a standard for constructing the calibration curve [34].

The proteolytic activity of the lysates was determined using the modified Anson method according to GOST 20264.2-88 "Enzyme preparations. Methods for determining proteolytic activity (with Amendment No. 1)" by the amount of tyrosine ptoduced during the hydrolysis of the substrate sodium caseinate (λ = 670 nm). When recalculating optical density (OD) values, a calibration curve was used for a series of standard solutions with a known tyrosine concentration. The values of proteolytic activity were expressed in units of proteolytic capacity in 1 mg of protein (PC units/mg protein).

The lipolytic activity of the lysates was measured by the modified method of Oto and Yamada [35] using an alkali to titrate fatty acids formed by lipase and olive oil as a substrate. The values of lipolytic activity were expressed in units of lipolytic capacity in 1 mg of protein (LC units/mg protein).

The collagenase activity of lysates [36] was assessed by a method based on determining the content of hydroxyproline in a mixture of native collagen formed as a result of hydrolysis, with the construction of a calibration curve for the relationship between the concentration of hydroxyproline in a buffer solution (in the range of 2-20 mmol/ml; OD_{555}). Collagenase activity values were expressed as a percentage of collagen dissolved (% cd).

To measure the enzymatic activities of the lysates, the API®ZYM test systems (BioMйrieux, France) were used, which allowed the determination of alkaline phosphatase, esterase, esterase lipase, lipase, leucine, valine and cysteine arylamidases, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucose-aminidase, α-mannosidase and α-fucosidase.

The amino acid composition of the lysates was investigated by a method based on the precipitation of proteins with trichloroacetic acid (TCA) followed by the extraction of free (unbound) amino acids. The isolated amino acids were derivatized with o-phthalic aldehyde (OPA) and 9-fluoromethyl chloroformate (FMOC). OPA was used to determine primary amino acids, FMOC − secondary, followed by high-performance liquid chromatography − diode-array detector (HPLC-DM) analysis and detection at wavelengths 338 and 262 nm, respectively (C18 PA column, 3.5μm×150 mm). The following reagents were used: deionized water obtained using a Milli-Q water purification system (Merck Millipore, USA),

acetonitrile for HPLC (≥ 99.9%) (Panreac, France), methanol for HPLC (Merck, USA), hydrochloric acid (≥ 37%), TCA (≥ 99.0%), FMOC (9-fluorenylmethylchloroformate, 10 mg/ml) (Sigma-Aldrich, USA), OPA (ortho-phthalic aldehyde, 10 mg/ml) (Sigma-Aldrich, USA). A mixture of D, L-amino acids (Merck, USA) was used as standards. Composition of solutions for gradient elution: eluent A — acetonitrile:methanol:water (45:45:10), eluent B — borate buffer (10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, pH 8.2). Amino acids were analyzed according to standard protocols on an Agilent 1260 Infinity LC liquid chromatograph with a diode array detector (Agilent Technologies, United States), column temperature in a thermostat 40 °C, operating pressure 1.6 MPa, eluent flow rate 1.5 ml/min, analysis time 25 min.

Organic acids in lysates were determined by a method based on the extraction of organic acids with an aqueous solution of TCA. Proteins from the extracts interfering with the determination of organic acids were precipitated by centrifugation; the samples were analyzed by HPLC on an Agilent 1260 Infinity LC liquid chromatograph with a UV detector (Agilent Technologies, United States) according to standard protocols. An anion-exchange HPLC column with a length of 50-150 mm and a diameter of 2.1-4.6 mm, a particle size of 1.8-5.0 μm, was used. The standards were solutions of organic acids with a basic substance content of at least 99.0% (Merck, USA). The acids were identified by the absolute retention time, the mass fraction was determined from the area of the chromatographic peak of the analyzed sample, comparing it with the peak of the reference sample with a known concentration. The reagents used for the determination and the conditions of the analysis are similar to the reagents and conditions established for the study of the amino acid composition.

The protein profiles of raw meat after processing were determined in the *longissimus dorsi* muscle of *Bos taurus*. Lysate was injected into a muscle tissue sample (5 ml per 50 g of raw material, ratio 1:10). The samples were kept in vacuum containers for 48 h at a temperature of +4±1 °C and, prior to analysis, were stored at –30 °C for 5 days. For the proteomic study of the processed meat raw material, 100 mg of the crushed sample was homogenized in 2 ml in a Teflon-glass system in a lysis solution (9 M urea, 5% mercaptoethanol, 2% Triton X-100, 2% ampholines, pH 3.5-10). The resulting homogenate was clarified by centrifugation at 800 rpm for 5 min, the supernatant fraction containing solubilized proteins (extract) was used for fractionation.

For proteomic analysis, proteins were separated by O'Farrell two-dimensional (2D) electrophoresis with isoelectric focusing in ampholine polyacrylamide gel (IEF-PAGE), as described previously [37, 38]. For visualization, proteins on 2D electropherograms were sequentially stained with Coomassie Brilliant Blue R-250 and silver nitrate [39]. The molecular weights of the protein fractions were determined using a set of highly purified recombinant proteins with molecular weights of 10-170 kDa PageRuler™ Prestained Protein Ladder (#SM0671 — 10 proteins, Fermentas, USA).

Computed densitometry used two-dimensional electropherograms in a wet state. Their complete digital images and/or images of individual fragments were obtained by scanning (Expression 1680, Epson, USA) [40] (resolution 300 dpi, 48 bit Color, saving the results in *.tiff format). The obtained digital images were processed in a graphics editor and the protein content was calculated using the ImageMaster 2D Platinum version 7 software package (GE Healthcare, Switzerland). When determining the amount of protein, at least three electropherograms with equal application were used. The deviation in optical density values was no more than ±1.5%.

To identify proteins, the cut fragments of 2D gel were homogenized and trypsinolized as previously described [41]. Sets of peptides were studied by MALDI-TOF MS and MS/MS mass spectrometry on an Ultraflex MALDI time-of-flight mass spectrometer (Bruker, Germany) with a UV laser (λ = 336 nm) in the positive ion mode in the mass range 500-8000 Da calibrated against known peaks of trypsin autolysis. Traditional bioinformatics technologies were used to decode mass spectra (peptide fingerprints). The mass spectra of tryptic peptides were analyzed using the Mascot program, the Peptide Fingerprint option (Matrix Science, USA) (0.01% accuracy of determining the MH+ mass) using the Protein NCBI database (https://www.ncbi.nlm.nih.gov/protein/). In a comparative analysis of the proteomic profiles of the presented samples, the authors used the information modules Proteins of bovine skeletal muscle (*Bos taurus*) from the Proteomics of Muscular Organs database (http://mp.inbi.ras.ru).

The quantitative data were statistically processed using the STATISTICA 14.0 software package (StatSoft, Inc., USA). All measurements were performed in 3 replicates. The results are presented as weighted arithmetic mean (*WAM*) with standard deviation ($\pm$SD). Statistical significance was calculated using the non-parametric Mann-Whitney U-test and the Kruskal-Wallis H-test. The critical level of significance of the null statistical hypothesis (p) was taken equal to 0.05.

Results. Peptides produced by starter cultures include ribosomally synthesized bacteriocins and protein hydrolysis products — bioactive peptides that can act as natural preservatives and nutraceuticals, respectively. Bioactive peptides are formed from substrate proteins under the action of intramembrane proteases and are extracellular protein residues that are not used by the proteolytic system of starter cultures for nitrogen assimilation and are released from the cell [11]. In some cases, cell lysis and the release of enzymes involved in proteolysis and generation of bioactive peptides are required [42].

The authors lysed the cell biomass of the starter cultures in two ways – enzymatic (treatment with lysozyme) and physical (ultrasonic disintegration of cells in suspension). In both cases, the cell debris was separated by centrifugation. The resulting supernatant was a lysis product of the cells of the starter cultures.

Electron microscopy. Microscopy showed that cell lysis by lysozyme was less effective than ultrasonic disintegration (Fig. 1). In this case, the cell wall is only slightly subject to destruction: both in the *Pediococcus pentosaceus* 28 strain and in the *Lactobacillus sakei* 103 strain, one can observe a violation of the integrity of the surface layers of the cell wall (the appearance of roughness), as well as an increase in its porosity. The treatment of cells with ultrasound gave a similar result, however, it is noticeable that the destruction occurs evenly over the cell surface, while when the cells are treated with lysozyme, it occurs locally.

Ultrasonic treatment of samples for TEM was first carried out in four cycles at an operating wavelength of 4 μm. Based on the results of microscopy, it was decided to increase the number of processing cycles to six at a wavelength of 9 microns. The obtained data are consistent with the results of the study by Tabatabaie and Mortazavi [43], where ultrasonic disintegration of cells of probiotic bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactococcus lactis* subsp. *cremoris*, and *Lactococus lactis* subsp. *lactis*) was carried out. Their TEM results confirmed the destruction of the bacterial cell wall after treatment. The authors noted that the nature of the damage depends on the duration of treatment. With the minimum time of exposure, microcracks and microvoids are formed on the cell surface, with a longer time – ruptures of the cell wall, its porosity increases. A further increase in the duration of disintegration leads to cell rupture and the release of intracellular contents into the working environment [43].

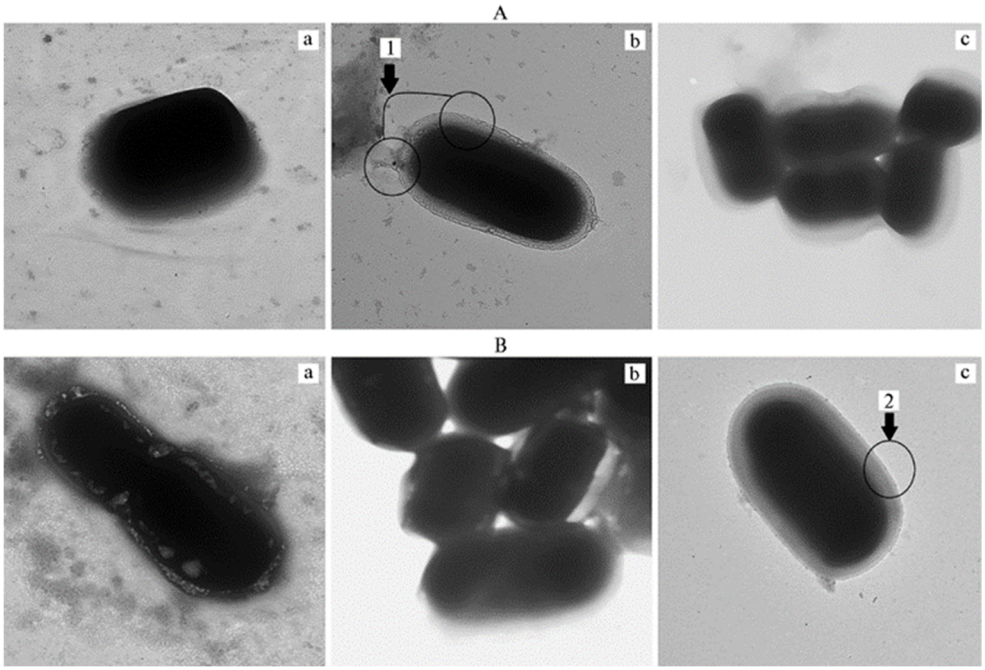


Fig. 1. Control of the completeness of lysis of *Pediococcus pentosaceus* 28 (A) **and *Lactobacillus sakei* 103 cells with different methods of obtaining lysates** (×30,000)**:** a — control sample (*P. pentosaceus* 28 and *L. sakei* 103 cells, no treatment); b — experimental sample (treatment of *P. pentosaceus* 28 and *L. sakei* 103 cells with lysozyme); c — prototype (ultrasonic disintegration of *P. pentosaceus* 28 and *L. sakei* 103 cells); 1 — local violation of the integrity of the surface layers of the cell wall (appearance of roughness), 2 — destruction occurs evenly over the cell surface (transmission electron microscopy, JEM-1400, Jeol, Japan).

Protein content and spectra of enzymatic activity of lysates. Based on the comparison of the two methods for obtaining lysates, we used ultrasonic treatment for these purposes. Table 1 shows the results of determining the protein concentration and proteolytic activity of the samples.

1. Protein concentration (μg/ml) **and proteolytic activity** (PC units/mg protein) **in lysates of starter cultures with different cell processing methods** (*WAM*±SD)

Strain	Protein concentration		Proteolytic activity (ultrasonic disintegration)
	lysozyme*	ultrasonic disintegration*	
Staphylococcus carnosus 108	0,24±0,012	0,99±0,006	114,88±2,162
Lactobacillus acidophilus AT-41	0,81±0,015[a]	1,94±0,050[e]	67,46±0,661
Lactobacillus curvatus 2	0,80±0,020[c]	1,79±0,026	72,19±0,717
Lactobacillus curvatus 1	0,34±0,006	1,28±0,038	91,07±0,905
Lactobacillus sakei 105	0,19±0,006[b]	1,00±0,041	55,77±1,137
Pediococcus acidilactici 38	0,47±0,021	1,27±0,035	42,17±1,478
Pediococcus pentosaceus 28	0,15±0,015[b, d]	0,80±0,036[f]	45,01±0,705
Lactobacillus sakei 103	0,23±0,021	0,86±0,017[f]	51,24±1,372

N o t e. PC — proteolytic capability. A pooled sample was used for analysis (three replicates).
[a-b, c-d, e-f] Differences between lysates for treatment option (column) are statistically significant at $p < 0.05$.
* Differences between treatment options are statistically significant at $p < 0.05$.

The protein content in the biomass samples of the starter cultures before lysis was low (up to 0.05 μg/μl), but it ranged from 0.17 to 0.82 μg/μl for lysozyme treatment and from 0.79 to 1.95 μg/μl for ultrasound. Consequently, the lysis of biomass with ultrasonic treatment is more intensive than with enzymatic treatment, which corresponds to the results of electron microscopy. The data obtained are consistent with the results of Mehmeti et al. [15] who also performed ultrasonic disintegration of cells of starter cultures. In their paper, the protein concentration in lysates of *Lactococcus lactis* NIZO 0900 cells averaged 1.25±0.02 μg/μl, *Pediococcus pentosaceus* OZF 1.32±0.01 μg/μl.

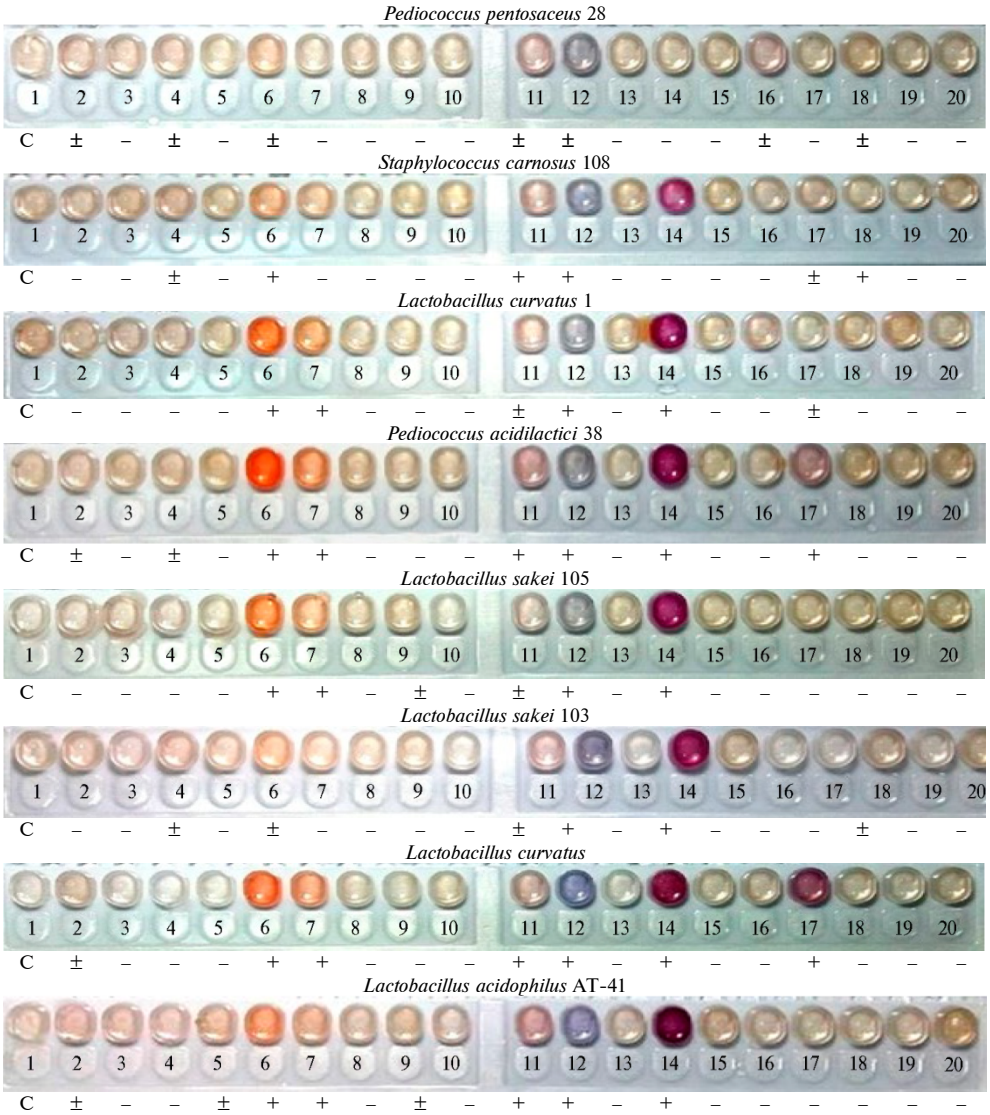


Fig. 2. Enzymatic activity of lysates of starter cultures after ultrasonic disintegration: 1 — control sample, 2 — alkaline phosphatase, 3 — esterase, 4 — esterase lipase, 5 — lipase, 6 — leucine arylamidase, 7 — valine arylamidase, 8 — cysteine arylamidase, 9 — trypsin, 10 — α-chymotrypsin, 11 — acid phosphatase, 12 — naphthol-AS-BI-phosphohydrolase, 13 — α-galactosidase, 14 — β-galactosidase, 15 — β-glucuronidase, 16 — α-glucosidase, 17 — β-glucosidase, 18 — N-acetyl-β-glucosaminidase, 19 — α-mannosidase, 20 — α-fucosidase; "+", "–", "±"— the presence, absence and weak manifestation of activity (API®ZYM test strips, BioMérieux, France).

The strains *Staphylococcus carnosus* 108, *Lactobacillus acidophilus* AT-41, *L. curvatus* 1, and *L. curvatus* 2 showed the greatest proteolytic activity. Lactic acid bacteria used as starter cultures are considered weakly proteolytic. Nevertheless, proteolysis is one of the main enzymatic reactions that occur in meat products under the influence of microorganisms. Lactic acid bacteria possess a complex proteolytic system, which consists of three components: proteases associated with the cell wall, which initiate protein breakdown into oligopeptides, peptide carriers, and intracellular peptidases, which decompose peptides into shorter peptides and free amino acids [44]. As a rule, published research results describe the proteolytic activity of lactobacilli directly in the culture medium (MRS medium, milk) [45]. In the paper of Donkor et al. [44], tripeptidase activity of lactobacilli in MRS medium was in the range of 200.0-3020.0 U/mg protein, dipeptidase activity within 50.0-

1100.0 U/mg protein. Parra et al. [46] evaluated the ability of whole cells, cell-free extracts, and cell lysates to accelerate proteolysis in curd suspensions. The obtained results allowed the suggestion that the developed model system of accelerated maturation based on cell lysates is indicative and can be used for rapid assessment of the contribution of strains to proteolysis during cheese maturation.

The ability of strains of lactic acid bacteria to produce bioactive peptides is based on the characteristics of the hydrolytic reactions of the proteins involved in them. The differences found in the proteinases of lactic acid bacteria explain the diversity of the resulting bioactive peptides, which is observed even when acting on the same protein matrix. The composition of bioactive peptides also depends on the substrate in which the hydrolytic enzymatic reaction took place [42].

To determine enzymatic activities in lysates, API®ZYM test systems were used with a visual assessment of the presence of activity by color reaction (Fig. 2) [47]. In the majority of producers, we found the enzymes naphthol-AS-BI-phosphohydrolase, leucine arylamidase, and β-galactosidase, characteristic of many lactic acid microorganisms (see Fig. 2). The obtained data are consistent with the results of other studies: the presence of β-glucosidase, β-galactosidase [48, 49], and leucine arylamidase [50] in lactic acid bacteria is known, which makes them useful starter cultures in food biotechnology.

Screening with API®ZYM showed that in the studied strains, lipolytic enzymes were either absent or contained in insignificant amounts. Using the modified method of Oto and Yamada, the authors did not reveal lipolytic activity in the lysates. In lactic acid bacteria, lipolytic activity is rarely found. Nevertheless, it has been shown that it occurs in *Lactobacillus plantarum* [51, 52]. This culture is one of the most valuable producers of many enzymes, including lipase and various esterases [53]. There are also mentions of lipase activity in the *Pediococcus acidilactici* culture, but in this species, lipase is synthesized in limited quantities and under certain conditions conducive to synthesis [54]. There are reports of lipolytic activity in strains of the species *L. helveticus*, *L. delbrueckii*, *L. bulgaricus*, *L. casei*, *L. plantarum*, and *L. acidophilus* [55].

In the lysates, no collagenase activity was detected, determined by oxyproline released as a result of hydrolysis of native collagen. Perhaps this is a consequence of the anticollagenase activity of strains. There is evidence in the literature that lactic acid microorganisms have such an activity, in particular, lipoteichoic acid, which inhibits collagen hydrolysis and activates its synthesis, can be distinguished among their metabolites [56-58].

Analysis of the biochemical composition of lysates. According to Table 2, the free amino acids in lysates are represented by the following ranges (mg/100 g of the sample): aspartic acid 0.07-0.23 (except for 1.5 mg/100 g in *L. sakei* 105), serine 0.16-0.31 (except for 8.95 mg/100 g for *L. sakei* 105), histidine 0.8-2.2, arginine 0.9-3.5, alanine 1.7-6.6, valine 0.18-0.70, phenylalanine 0.26-0.39, isoleucine 0.44-0.95, leucine 1.00-1.51, lysine 1.60-2.07, and proline 0.80-1.16. Cystine was found only in representatives of the genus *Pediococcus*, in the *P. pentosaceus* 28 and *P. acidilactici* 38 (0.010 and 0.424 mg/100 g, respectively). Methionine was also recorded in the *P. pentosaceus* 28 and *P. acidilactici* 38 lysates (0.293 and 0.782 mg/100 g), as well as in the *L. sakei* 103 strain (0.072 mg/100 g). Glutamic acid, glycine, threonine, and tyrosine were not detected in any sample. The widest range of free amino acids is presented in the lysate sample from *P. acidilactici* 38, the least – in the lysate sample from *L. sakei* 105. These data are consistent with those presented by Shaikhiev [59] with the results of amino acid analysis of total proteins in three strains of lactic acid bacteria in a culture medium.

2. Free amino acid concentration (mg/100 g of lysate) **in *Lactobacillus*, *Pediococcus*, and *Staphylococcus* strains after ultrasonic disintegration** (*WAM*±SD)

Amino acid	*L. acidophilus* AT-41	*P. pentosaceus* 28	*L. curvatus* 1	*P. acidilactici* 38	*L. curvatus* 2	*L. sakei* 103	*S. carnosus* 108	*L. sakei* 105
Aspartic acid	0.219±0.004	0.214±0.011	0.040±0.002	0.234±0.012	0.228±0.011	0.075±0.004	0.153±0.008	1.500±0.075
Glutamic acid	–	–	–	–	–	–	–	–
Serine	0.167±0.008	–	–	0.302±0.006	0.204±0.010	0.165±0.003	–	8.950±0.447
Histidine	0.898±0.045	1.093±0.055	1.298±0.065	2.166±0.108	1.074±0.054	1.106±0.055	0.874±0.044	–
Glycine	–	–	–	–	–	–	–	–
Threonine	–	–	–	–	–	–	–	–
Arginine	2.058±0.103	2.508±0.125	0.966±0.048	3.449±0.172	1.184±0.024	2.516±0.126	2.190±0.109	–
Alanine	3.260±0.163	3.821±0.191	1.799±0.090	5.139±0.257	6.598±0.330	3.882±0.194	3.235±0.162	–
Tyrosine	–	–	–	–	–	–	–	–
Cystine	–	0.010±0.001	–	0.424±0.021	–	–	–	–
Valine	0.470±0.024	0.460±0.023	0.692±0.035	0.600±0.030	0.527±0.026	0.447±0.022	0.442±0.022	0.185±0.009
Methionine	–	0.293±0.015	–	0.782±0.039	–	0.072±0.004	–	–
Phenylalanine	0.336±0.017	0.272±0.014	0.471±0.024	0.385±0.019	0.364±0.018	0.313±0.016	0.279±0.014	0.265±0.013
Isoleucine	0.452±0.023	0.448±0.022	0.943±0.047	0.930±0.047	0.515±0.026	0.565±0.028	0.575±0.029	0.795±0.040
Leucine	1.153±0.058	1.143±0.057	1.506±0.075	1.305±0.065	1.300±0.065	1.148±0.057	1.109±0.055	1.014±0.051
Lysine	1.505±0.075	1.465±0.073	0.646±0.032	1.659±0.083	2.033±0.102	2.040±0.102	1.652±0.083	0.710±0.036
Proline	1.112±0.056	0.897±0.045	1.160±0.058	0.887±0.044	0.986±0.049	0.966±0.048	0.994±0.050	–
Total amount	11.630±0.582	12.624±0.631	9.521±0.476	18.262±0.913	15.013±0.751	13.295±0.665	11.503±0.575	13.419±0.671

N o t e. A pooled sample was used for analysis (three replicates). Dashes indicate that the indicated amino acid is not detected in the lysate.

In the strains studied by Shaikhiev [59], the qualitative amino acid composition was identical (only 18 amino acids). At the same time, these strains of lactic acid bacteria practically did not differ in the content of leucine, threonine, phenylalanine, isoleucine, methionine, tryptophan, arginine, glutamic and aspartic acids, as well as proline in cultures.

3. Concentration of organic acids (mg/100 g sample) **in lysates of starter cultures after ultrasonic disintegration** (*WAM*±SD)

Strain	Malic	Lactic	Succinic	Total
Lactobacillus acidophilus AT-41	0.0038±0.0002	0.0333±0.0017	0.6915±0.0346	0.7286±0.0340
Pediococcus pentosaceus 28	0.0170±0.0009	0.0436±0.0022	0.5417±0.0271	0.6023±0.0301
Lactobacillus curvatus 1	0.0048±0.0002	0.0474±0.0024	0.0370±0.0019	0.0892±0.0045
Pediococcus acidilactici 38	0.0029±0.0001	0.0575±0.0029	0.2195±0.0110	0.2799±0.0140
Lactobacillus curvatus 2	0.1780±0.0089	0.0345±0.0017	0.5251±0.0263	0.7376±0.0370
Lactobacillus sakei 103	0.0194±0.0010	0.0375±0.0019	0.8692±0.0435	0.9261±0.0463
Staphylococcus carnosus 108	0.0076±0.0004	0.0439±0.0022	0.8223±0.0412	0.8738±0.0437
Lactobacillus sakei 105	0.0640±0.0032	0.0248±0.0012	0.0089±0.0004	0.0977±0.0049

N o t e. A pooled sample was used for analysis (three replicates).

On average, the content of malic acid in lysates (Table 3) varied within 0.002-0.02 mg/100 g, lactic acid within 0.02-0.06 mg/100 g, and succinic acid within 0.2-0.9 mg/100 g of the sample. An exception in terms of malic acid content was *L. curvatus* 2 lysate, where the content of this acid was several orders of magnitude higher than in other samples, the 0.178 mg/100 g. In *L. curvatus* 1 and *L. sakei* 105 lysates, the content of succinic acid, on the contrary, turned out to be several orders of magnitude less than in other samples, the 0.037 and 0.0089 mg/100 g, respectively. There are data in the literature on the study of the ability of lactic acid bacteria to produce organic acids. Thus, after 5 days of liquid fermentation, HPLC revealed acetate, citrate, formate, lactate, and succinate, with lactate and acetate dominating among the fermentation products in the cultures [44, 60].

Results of the proteomic study. In the studied lysates of starter cultures, a number of effects and specificity of the action on the muscle tissue of *Bos taurus* were revealed in comparison with the control (Fig. 3).

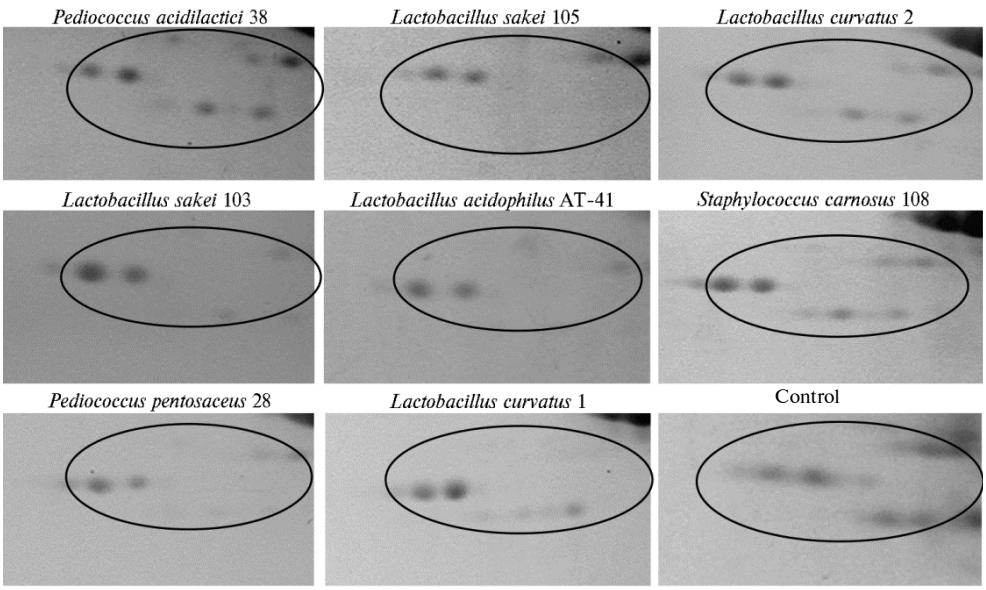


Fig. 3. Fragments of 2D electrophoretograms (IEF-PAGE) **of *Bos taurus longissimus dorsi* muscle tissue proteins after treatment with lysates of bacterial starter cultures.** The ovals enclose the zones of fractions of slow skeletal muscle troponins T. Staining with Coomassie Brilliant Blue R-250 (linear binding of the dye to the protein).

A comparative analysis of the obtained 2D electropherograms showed that when processing meat raw materials with the studied lysates of starting cultures, the amount of protein in the fractions decreased with a certain selectivity. In particular, such an effect was noted for some fractions of transcriptional variants of slow skeletal muscle troponins T (see Fig. 3) – products of the *TNNT1* gene. The position of these fractions and the results of their identification are presented in the information module Proteins of skeletal muscle of cows (*Bos taurus*) of the Proteomics of muscle organs database (http://mp.inbi.ras.ru) (Table 4).

4. Results of mass-spectrometric identification (MALDI-TOF MS and MS/MS) of **protein fractions of *Bos taurus longissimus dorsi* muscle tissue, in which changes appeared after treatment with lysates of starter cultures**

Protein (gene)	Number in Protein NCBI	S/M/C	Мм/pI	
			exp.	calculated
Musculoskeletal regulatory aggregate myosin light chain 2 (*MYL2*)	NP_001069115.1	157/12/82	240.0/5.10	19.0/4.91
Conjugated mixture of titin peptides (*TTN*)[b] (1)	DAA32835.1	59/8/< 1	160.0/6.60	3 713 421.0/6.07
Conjugated muscle peptides creatine phosphokinase (*CPK-M*)[a] (1)	NP_777198.2	216/7/23	200.0/7.40	42.9/6.63
Myoglobin conjugate (*MB*)[a] (1)	NP_776306.1	125/11/69	160.0/7.80	17.0/6.90
Fragment of L-lactate dehydrogenase A (*LDHA*)[a] (1) + Acetyl (Protein N-term)	NP_776524.1	76/24/66	27.0/7.50	36.0/8.12
Fragment of L-lactate dehydrogenase A (*LDHA*)[a] (1) + Acetyl (Protein N-term)	XP_005900750.1	127/9/30	26.5/7.50	36.0/8.12
Fragment of the light chain of myosin 6B (*MYL6B*)	NP_001069181.1	234/26/82	22.0/5.30	23.3/5.40
Musculoskeletal α-actin (*ACTA1*)	NP_001091.1	154/11/38	43.0/5.40	42.0/5.23
Fragment of a.a.s. 21-286 musculoskeletal α-actin (*ACTA1*)[a] (3) + Methyl (75H)	NP_776650.1	413/27/50	28.0/4.90	42.1/5.31
Fragment of a.a.s. 21-256 musculoskeletal α-actin (*ACTA1*)[a] (3) + Methyl (75H)	NP_776650.1	413/37/57	26.0/5.10	42.1/5.31
Fragment of a.a.s. 241-375 musculoskeletal α-actin (*ACTA1*)[a] (3)	NP_776650.1	189/18/	17.0/5.00	42.1/5.31

N o t e. a.a.s. — amino acid sequence. S/M/C: Score — an indicator of compliance, or "score" (Protein scores greater than 68 are significant, $p < 0.05$); Match peptides — number of matched peptides; Coverage — the percentage of coverage of the complete amino acid sequence of the protein by the identified peptides. Mm/pI (exp.) — molecular weight/isoelectric point based on the results of determination of 2D electrophoretic mobility, Mm/pI (calculated) — molecular weight/isoelectric point calculated on the basis of amino acid sequence data, taking into account the removal of the signal peptide using the ExPASy program Compute pI/Mw tool. [a] — msms (indication of confirmatory identification by tandem mass spectrometry, the number of tryptic peptides sequenced is indicated in parentheses). MALDI time-of-flight mass spectrometer Ultraflex (Bruker, Germany) with UV laser, positive ion mode in the mass range of 500-8000 Da.

Initially, in raw meat materials, five fractions are usually present, differing in pI and molecular weight. The higher molecular weight set contains three electrophoretic isoforms, and the lower molecular weight set contains two isoforms. Treatment with lysates of strains *Lactobacillus sakei* 105, *L. acidophilus* AT-41, and *Pediococcus pentosaceus* 28 led to the complete disappearance of low-molecular forms, and with lysates *L. sakei* 103 and *L. curvatus* 1 to a clear decrease in their number, that is, the studied lysates of starter cultures showed a selectivity of action on the actomyosin set of muscle tissue.

We did not find any specific changes in the samples treated with *Pediococcus acidilactici* 38, *L. sakei* 103, *L. acidophilus* AT-41, and *Staphylococcus carnosus* 108 lysates. The action of *L. curvatus* 2 lysate led to the formation of an atypical fraction with a molecular weight ~ of 240 kDa (Fig. 4, see Table 4), identified as an aggregate of the skeletal muscle regulatory light chain of myosin 2, which in monomeric form has a molecular weight of 19 kDa. This fraction on a 2D electrophoretogram is present in large quantities and forms a zone with characteristic mobility, but some of these molecules, under the influence of bacterial lysate, produce the oligomeric form, which consists of about 12 subunits. As a result of treatment with *L. curvatus* 2 lysate, a conjugate (~ 160 kDa) formed

by fragments of the titin protein appeared (see Fig. 4, Table 4). Titin itself has a molecular weight of about 4000 kDa and due to its size is not included in the PAGE plate. Tryptic peptides identified in the 160 kDa fraction are located in different regions of the protein molecule. Therefore, the 160 kDa fraction cannot be anything other than the resulting titin peptide conjugate. In general, it can be assumed that specific proteins and proteases are found in the lysate of the *L. curvatus* 2 strain that affect proteolysis and changes the protein folding.

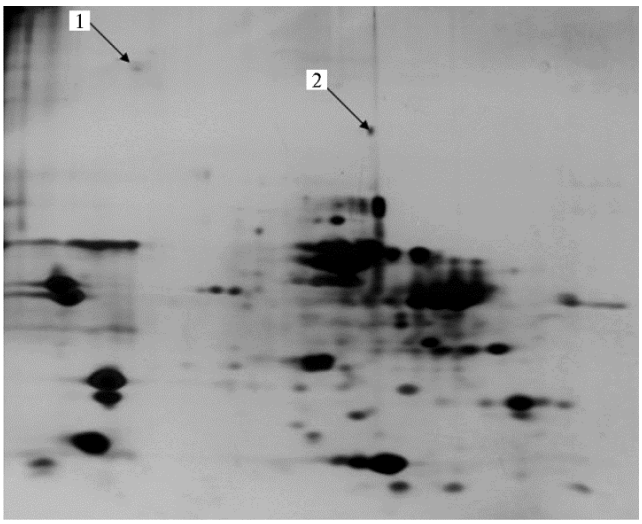
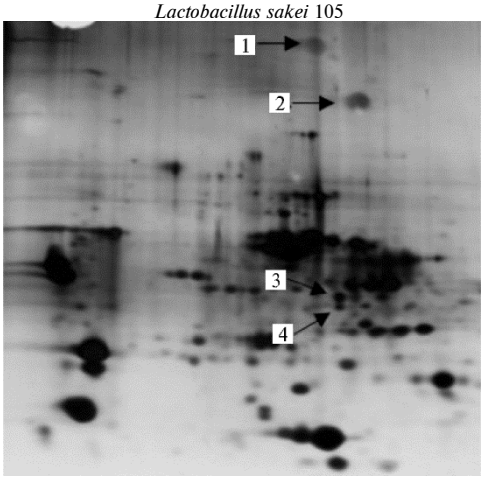


Fig. 4. 2D electrophoretogram (IEF-PAGE) **of *Bos taurus longissimus dorsi* muscle tissue proteins after treatment with a lysate of the *Lactobacillus curvatus* 2:** 1 — an aggregate of the skeletal muscle Myosin regulatory light chain 2 (*MYL2*), 2 — a conjugated mixture of titin peptides (TTN)[b] (1). Silver nitrate staining.

Lactobacillus sakei 105



Lactobacillus curvatus 1

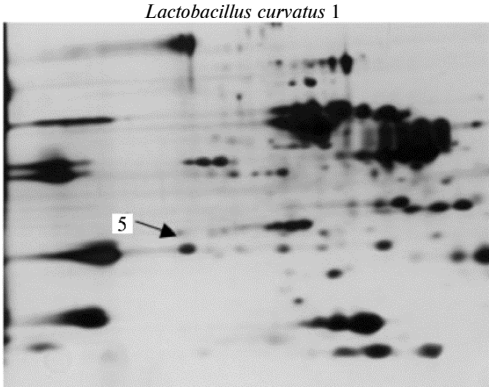


Fig. 5. 2D electrophoretogram (IEF-PAGE) **of *Bos taurus longissimus dorsi* muscle tissue proteins after treatment with a lysate of the *Lactobacillus sakei* 105 и *Lactobacillus curvatus* 1:** 1 — conjugated peptides of muscle creatine phosphokinase, 2 — myoglobin conjugate, 3 and 4 — fragment of L-lactate dehydrogenase A, 5 — fragment of the light chain of myosin 6B (high molecular weight oligomers/conjugates of proteins and fragments of L-lactate dehydrogenase A are indicated by arrows). Silver nitrate staining.

When treated with *L. sakei* 105 lysate, conjugates of muscle creatine phosphokinase and myoglobin were formed, while the monomers were preserved, and fragments of the A subunit of L-lactate dehydrogenase, acetylated at the N-terminus, appeared (Fig. 5, see Table 4). The action of *L. curvatus 1* lysate led to the appearance of a large fragment of the light chain of myosin 6B (see Fig. 5, Table 4), deaminated at several glutamine (Q) residues, which apparently served as a

protective mechanism (judging by the fact that when using other lysates, this fraction was completely destroyed).

Pronounced changes in the structure of actin of raw meat were observed when using lysates of *Staphylococcus carnosus* 108 and *Pediococcus pentosaceus* 28 (Fig. 6, see Table 4). When exposed to the enzyme complex from *Staphylococcus carnosus* 108, the actin fraction completely disappeared while preserving tropomyosins and myosin light chains, and treatment with *Pediococcus pentosaceus* 28 lysate led to the formation of three large actin fragments, two of which represented the N-terminal part of molecules of different lengths, methylated at the amino acid residue 75H. The lower molecular weight component contained a fragment of only the C-terminal part of the molecule, which indicates the presence of a specialized restriction enzyme in this species of microorganisms that recognizes a unique region of the actin amino acid sequence.

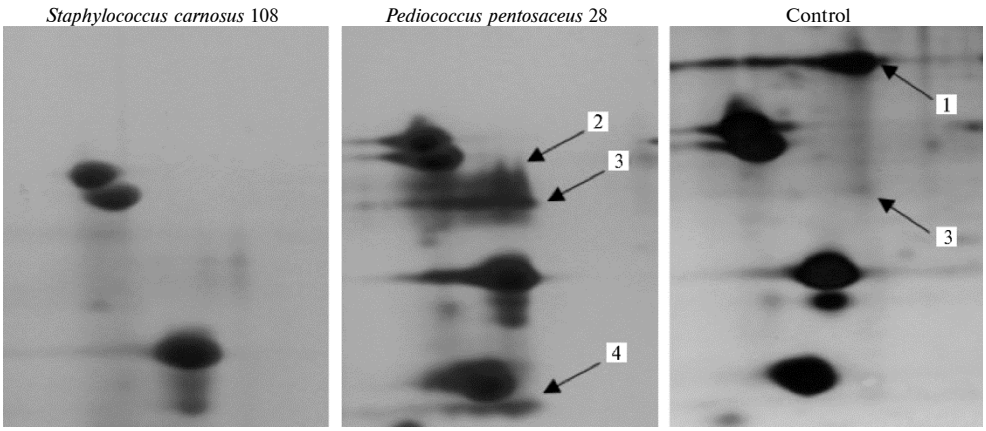


Fig. 6. Fragments of 2D electrophoretograms (IEF-PAGE) **of actin, tropomyosin areas and myosin light chains, illustrating the effect of lysates on the actin fraction:** 1 — musculoskeletal α-actin, 2 — fragment of the amino acid sequence (a.a.s.) 21-286 of musculoskeletal α-actin, 3 — fragment of a.a.s. 21-256 of musculoskeletal α-actin, 4 — fragment of a.a.s. 241-375 of musculoskeletal α-actin. Silver nitrate staining.

In general, it can be concluded that the authors have identified specific proteins − targets of enzyme preparations from different starter cultures.

Previously, we studied the effect of whole starter cultures on muscle tissue of cattle and meat products and found a number of changes in the protein composition [28], in particular, the formation of aggregates of myoglobin and troponin I, especially when using the culture of *Pediococcus pentosaceus* 31. Note that in an early study, the authors analyzed the changes that occur under the influence of living starter cultures, constantly producing sets of specific enzymes. In the presented study, enzymes released from cells during lysis using ultrasonic disintegration were used. Obviously, in preparations of cell lysates, these enzymes have a certain lifetime and a clearly lower concentration. In this case, the effect is naturally less pronounced, which was observed in our experiment, but the general mechanisms are confirmed.

In this work, under the influence of lysates of starter cultures, pronounced changes in proteins of meat raw materials occurred, including with the production of low molecular weight peptides that may have biological activity. During the fermentation of animal proteins by proteolytic enzymes secreted by *Lactobacillus helveticus*, *L. lactis* subsp. *cremoris* FT4, and *L. delbrueckii* subsp. *bulgaricus* SS1, biologically active peptides are formed, including antihypertensive peptides that inhibit angiotensin I-converting enzyme, opioid agonists, and peptide antagonists, as well as mineral binding, immunomodulatory, antibacterial, and antithrombotic

peptides. Angiotensin I-converting enzyme regulates blood pressure through the synthesis of the vasopressor angiotensin II from angiotensin I [44]. The appearance of bioactive peptides depends on the enzymatic activity of the culture; the factors affecting their production are specific for the strain. At the molecular level, the manifestation of the activity of the proteolytic system is influenced by the presence of proteins, amino acids, and carbon [61, 62]. In addition, the enzymatic activity depends on the growth phase of the microorganisms; activity persists during the exponential phase and in the initial period of the stationary phase, but decreases as the stationary phase progresses [63]; changes in enzymatic activity are also associated with the integrity of bacterial cells.

To date, the potential for obtaining bioactive peptides with the participation of starter cultures can be predicted in two ways: using genomic analysis of the components of the proteolytic system of starter cultures to identify various enzymatic activities or subelements and their strategic features [64] and based on an *in silico* approach using quantitative structure-activity relationship assessment methods to analyze the protein matrix that can release bioactive peptides [65]. To predict a large sample of starter culture strains, it is most effective to combine bioinformatics tools with experimental data [42].

The strains *Pediococcus pentosaceus* 28, *Staphylococcus carnosus* 108, *Lactobacillus curvatus* 1, *P. acidilactici* 38, *L. sakei* 103, *L. sakei* 105, *L. curvatus* 2, and *L. acidophilus* AT-41 that we selected to obtain lysates are active producers of many enzymes widely used in the food industry as starter cultures. The strains are selected in such a way that the results for lysates can be compared with those obtained earlier in terms of the effect of the corresponding live cultures on raw meat and finished meat products. For these strains, a complete scheme for obtaining bacterial lysates by the method of ultrasonic disintegration has been developed.

Thus, *Lactobacillus curvatus* 2, *L. acidophilus* AT-41, *Pediococcus acidilactici* 38, and *Staphylococcus carnosus* 108 lysates have the broadest set of intracellular enzymes, including leucine and valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and β-galactosidase. The lysates of *L. acidophilus* AT-41, *L. curvatus* 1, and *L. curvatus* 2 are characterized by the presence of alkaline phosphorylase. In the lysate of *L. acidophilus* AT-41, traces of lipase are found, as well as trypsin, in lysates of *Staphylococcus carnosus* 108 and *Pediococcus acidilactici* 38, traces of esterase lipase. All samples, except for *L. acidophilus* AT-41 lysate, contain β-glucosidase. The highest proteolytic activity was found in the lysates of *Staphylococcus carnosus* 108 (115.94 PC units/mg protein), *L. acidophilus* AT-41 (66.7 PC units/mg protein), *L. curvatus* 1 (91.03 PC units/mg protein) and *L. curvatus* 2 (72.20 PC units/mg protein). During the fermentation of raw meat with lysates of starter cultures, the formation of non-standard protein conjugates was revealed; for some cultures, specific target proteins were determined, in particular, musculoskeletal actin. The data obtained will be used in technologies for processing animal muscle tissue to increase the functionality of products. The continuation and addition of experimental studies should be genomic analysis of the components of the proteolytic system of lactic acid bacteria to identify various enzymatic activities.

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