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UDC 577.15; 579.66 **Research article**

Enzymatic activity of Bacillus paralicheniformis T7 strain and its application in cheese production

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Abstract

Background and Aim. The increasing demand for inexpensive milk coagulants stimulates the search for new sources of milk-forming enzymes among proteolytic microorganisms. The aim of this work was to isolate a strain with proteolytic activity.

Materials and Methods. In the present study, a proteolytic strain of *Bacillus paralicheniformis* T7 was isolated and identified. By culturing *B. paralicheniformis* T7 for three days on feather medium, an enzymatic extract with proteolytic activity of 715.7 ± 40.2U/mL was obtained. In addition to proteolytic activity, the B. paralicheniformis T7 strain also exhibited amylase (176.1 ± 16.3), esterase (24.3 ± 0.4), lipase (17.5 ± 0.8), and phosphatase (11.9 ± 0.6) activities.

Results. Zymographic analysis using casein showed that the extract contained caseinolytic proteases. Proteases from *B. paralicheniformis* T7 completely hydrolyzed of 1 mg casein in 90 s. Six proteases, as well as esterase, amylase, phosphatase were identified in the extract by HPLC-Q/TOF analysis. In addition to caseinolytic activity, *B. paralicheniformis* extract has milk-clotting activity, which was 12 and 23 U/mL for cow's and ewes' milk, respectively. Cheese was produced from fresh cow's milk using the enzymatic extract, with a yield of 17%.

Conclusion. The use of the *B. paralicheniformis* T7 strain as a new source of proteolytic enzymes, and its proteases as milk-clotting enzymes in the cheese-making industry, appears promising.
Keywords: *Bacillus paralicheniformis*; enzyme; protease; milk-clotting; chees.

Introduction

Bacilli species have a well-developed enzymatic system and secrete various enzymes into the medium. In industrial biotechnology, most enzymes are obtained through microbial synthesis, and bacteria of the genus *Bacillus* play a significant role in this process. These microorganisms are characterized by low nutritional requirements and can grow on inexpensive substrates such as feathers, tannery waste, bagasse, corn extract [1]. *Bacillus* species can be successfully cultivated in fermenters, where they are able to achieve high titers within a relatively short period of time [1, 2].

The diverse enzymatic characteristics of *Bacillus* species make them advantageous for a wide range of applications, including food processing, agriculture, biomedicine, biofuel production, hydrolysis, bioremediation, and natural polymer processing [3, 5]. Some *Bacillus* strains exhibit enhanced enzyme production capabilities [1], with *B. paralicheniformis* strains occupying a prominent position in the

enzyme production sector. For example, *B. paralicheniformis* MKU3 is known to produce proteases that efficiently degrade feather keratin [6], while *B. paralicheniformis* BL.HK produces extracellular proteases used in the enzymatic treatment of animal hides [7]. In addition, *B. paralicheniformis* HR-1 and B. haynesii HR-5 strains isolated from bottom sediments are used to produce alkaline proteases applied in the textile and leather industries [8]. Proteases obtained from *B. paralicheniformis* T7 also exhibit significant keratinase activity in the hydrolysis of bird feathers, wool, horns, hooves, and skins [9]. Along with proteases and keratinases, *B. paralicheniformis* strains produce α-amylases [10], phosphatases [11], esterases [12], and xylanases [13]. In particular, α-amylase produced by *B. paralicheniformis* ATCC 9945a efficiently hydrolyzes starch without pretreatment [10]. Furthermore, α-amylase from *B. paralicheniformis* GRA2 was integrated into a multienzyme preparation for the hydrolysis of food waste [14]. Meanwhile, the *B. paralicheniformis* strain APSO efficiently produces thermostable alkaline phosphatase [11], and the *B. paralicheniformis* strain G1, isolated from Arabian Sea sediments, secretes esterases that effectively biodegrade polystyrene [12]. The examples given indicate that bacilli in general and *Bacillus paralicheniformis* in particular are good sources of effective enzymes used in the food and processing industries.

The aim of this work was to isolate a strain with proteolytic activity. The strain of *Bacillus paralicheniformis* T7, isolated from the soil of the southern Kazakhstan, was proposed as such the strain. By culturing the strain on feather medium, an enzymatic extract was obtained, the proteases of which showed high caseinolytic and milk-clotting activity. In addition, the enzyme extract demonstrated amylase, phosphatase, myo-inositol phosphohydrolase, esterase, and lipase activities. The enzymatic extract from *Bacillus paralicheniformis* T7 was also tested in cheese production from cow's milk.

Materials and Methods

Media and reagents

Nutrient agar (HiMedia, India, cat. #M001, India) was used for the isolation and cultivation of colonies on plates. Nutrient broth (HiMedia, cat. #M002, India) was used for cultivation in flasks. Feather broth (w/v), consisting of 0.075% feather powder, 0.02975% Na_2HPO_4, 0.03475% NaH2PO4, and 0.2% yeast extract, was used to obtain the enzymatic extract, while skim milk agar (2% skim milk powder, 1% tryptone, 1% agar, and 0.1% NaCl) was used to test the proteolytic activity of the strain.

Strain isolation

One gram of a soil sample combined with 9 mL of 0.9% (w/v) NaCl and shaken for 30 min at room temperature. Then, 100 µL of the suspension was seeded onto feather agar plates, followed by cultivation at 37 °C for 48 h. Well-grown isolated colonies were isolated and tested for a proteolytic activity on milk agar plates. A single colony showing proteolytic activity was randomly selected and subjected to identification.

Identification of bacteria

The strain was identified by sequencing of the 16S rRNA gene. The strain was cultivated in nutrient broth at 37 °C for 24 h. The cells were harvested by centrifugation (6,000 × g, 4 °C, 7 min), and genomic DNA was isolated from the pellet using the Wizard® Genomic DNA Purification Kit (Promega, USA). A fragment of the 16S rRNA gene was amplified by PCR and sequenced for identification. The amplification was performed with the universal primer pair 27F (5'-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGTTACCTTGTTACGACTT-3′). The PCRs mixture (50 µL final volume) consisted of 5 µL of 10X Taq Buffer (Thermo Fisher), 3 µL of 25 mM MgCl2, 5 µL of dNTPs (a 2 mM stock solution), 1 µL of each primer (a 10 µM stock solution), 100 ng of the DNA template, 1 µL of Taq polymerase (5000 U/mL, Invitrogen), and 34 µL of nuclease-free water. The following amplification parameters were used: initial denaturation at 95 °C for 5 min; 30 cycles of 95 °C for 1 min, and 55 °C for 1 min, and 72 °C for 1 min; and final extension at 72 °C for 10 min.

Sequencing was carried out on an ABI 3730xl Genetic Analyzer (Applied Biosystems, USA) using BigDye Terminator v3.1 (Applied Biosystems). The obtained sequences were compared with GenBank data using the Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Growth of the bacterial culture and preparation of the enzyme extract

Cells of the strain were inoculated into 5 mL of the Luria–Bertani broth and cultured for 16 h at 37 °C and 170 rpm in 4000i control shaking incubator (IKA, Germany). The culture was then inoculated

into 150 mL of feather broth and cultivated for 48 h at 37 °C and 170 rpm. The culture was centrifuged at 10,000 × g and 4 °C for 10 min, and the supernatant was passed through a 0.22 μm membrane filter to remove feather microparticles and bacteria cells. The sterile enzymatic extract was stored on the ice and used for further experiments.

Enzyme assays

Proteolytic activity was assessed according to *Coêlho* et al. [15] using azocasein (Sigma-Aldrich) as a substrate in 50 mM Tris-HCl (pH 9.0) at 60 °C. Milk-clotting activity was measured following the method of *Akishev* et al. [16] using cow's milk as a substrate. Alpha-amylase activity was determined in 100 mM phosphate buffer (pH 6.0) at 85 °C by the reducing sugar method using potato starch (Sigma-Aldrich) as a substrate [17]. Esterase activity was determined at 40 °C in 50 mM phosphate buffer (pH 7.0) according to *Zhao* et al. [18] using 4-nitrophenyl acetate (Thermo Scientific, Waltham, MA, USA) or 4-nitrophenyl octanoate (Thermo Fisher, Kandel, Germany) as substrates. Alkaline phosphatase activity was assayed following the method of *Abdelgalil* et al. [11] using p-nitrophenyl phosphate disodium salt hexahydrate (PanReacAppliChem, Darmstadt, Germany) as a substrate in 100 mM phosphate buffer (pH 10.3) at 70 °C. Myo-inositol phosphohydrolase activity was assessed as described by *Choi* et al. [19] using phytic acid sodium salt as a substrate in 100 mM Tris-HCl (pH 8.0) at 60 °C. All experiments were performed in triplicate. Lactase activity was determined by measuring the amount of glucose released during lactose hydrolysis using a glucose determination kit (Vital, Russia).

Enzymatic activity measurement data were obtained from independent activity assays, and mean values, standard deviations (SD), and *p*-values were calculated using GraphPad Prism version 8.0.1 (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). All data are presented as means ± SD (n = 3).

HPLC-Q/TOF analysis

The enzymatic extract was concentrated 50-fold using a Pierce Protein Concentrator with a 10 kDa cutoff (Thermo Scientific, USA). Protein separation was performed by SDS-PAGE in a 12% polyacrylamide gel. The proteins were then extracted from the gel and digested with trypsinized. The resulting peptides were separated by nano-HPLC using an acetonitrile gradient and identified by Q-TOF mass spectrometry on a Maxis Impact II Instrument (Bruker, Germany). Mascot software was used to search against the NCBInr 20140923 database (49,710,996 sequences; 17,838,311,419 residues).

Zymographic analysis

The ability of the enzymatic extract to hydrolyze casein was evaluated by SDS-PAGE in a 4-20% gel copolymerized with 0.1% (w/v) sodium caseinate (Sigma, cat. #C8654, Germany) incorporated as a substrate. The enzymatic extract was mixed with SDS-PAGE sample buffer (125 mM Tris-HCl pH 6.8, 4% of SDS, 0.002% of bromophenol blue, and 20% of glycerol) at a sample-to-buffer ratio of 4:6. The sample was not boiled before loading onto the gel, and β-mercaptoethanol was not before electrophoresis.

Preparation of cheese from cow's milk

Laboratory-scale cheese production was performed according to ref. [20], with some modifications. Starter cultures and salt were excluded from the procedure. The purpose of this exclusion was to evaluate only the effect of the enzymatic extract of *B. paralicheniformis* T7 on the cheese production. Cheese was prepared from 1 L of fresh cow's milk. Milk components were quantified using a Lactan 600 Ultra Milk Analyzer (Sibagropribor Ltd.). The milk was pasteurized at 75 °C for 30 s. After that, 100 mL of enzymatic extract was added, and the mixture was incubated at 37 °C until a clot formed, which was then cut into 1 cm cubes. At the end of the incubation, whey was separated from curd, and the amount of whey was recorded. The curd was pressed with a 2 kg weight for 4 h at 22 °C. After pressing, the cheese yield (g) was recorded. The moisture content of the cheese was measured using an Infrared Moisture Determination Balance MD 83 and recorded.

Software and statistical analysis

All experiments were conducted independently in triplicate. For quantitative assays, mean values and standard deviations (SD) were calculated using GraphPad Prism 8.0.1 software. Specific activity is presented as the mean, whereas other parameters are shown as the mean ± SD (n = 3).

Results and Discussion

The strain isolated from the soil formed beige, large, convex colonies with irregular edges and a viscous consistency on nutrient agar after 24 h of cultivation (Figure 1a). When the strain was cultivated in nutrient broth, the cells formed a surface film and a flocculent precipitate. The skim milk agar test revealed that the strain exhibited proteolytic activity (Figure 1b).

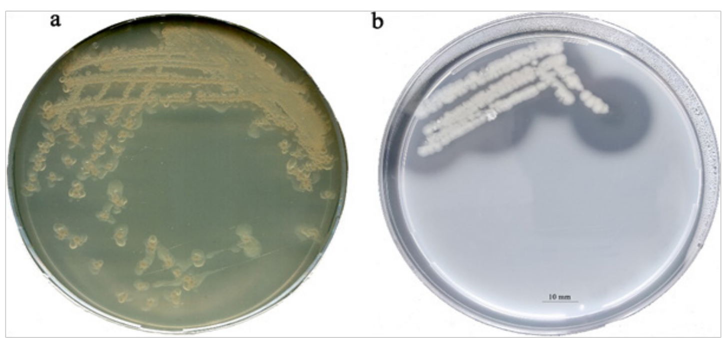


Figure 1 – Colonies of *B. licheniformis* on nutrient agar (a) and skim milk (b) plate

Clear zones measuring 11-14 mm formed around the colonies due to the hydrolysis of milk proteins such as casein and were clearly visible on the plate. The cells were found to be Gram-positive. Sequencing of a fragment of the 16S rRNA gene and comparison with GenBank data confirmed that the strain belonged to *Bacillus paralicheniformis*, with 100% identity.

Cultivation of *B. paralicheniformis* T7 on feather medium showed complete degradation of feathers within 7 days (Figure 2 a, b). Measurement of proteolytic activity in the nutrient medium showed that the accumulation of proteases in the medium was not linear. Cultivation of the strain in feather broth showed that the maximum proteolytic activity was reached after 72 h of cultivation, and the activity of the enzymatic extract from B. paralicheniformis T7 was found to be 715.7 ± 40.2 U/mL (Figure 2c).

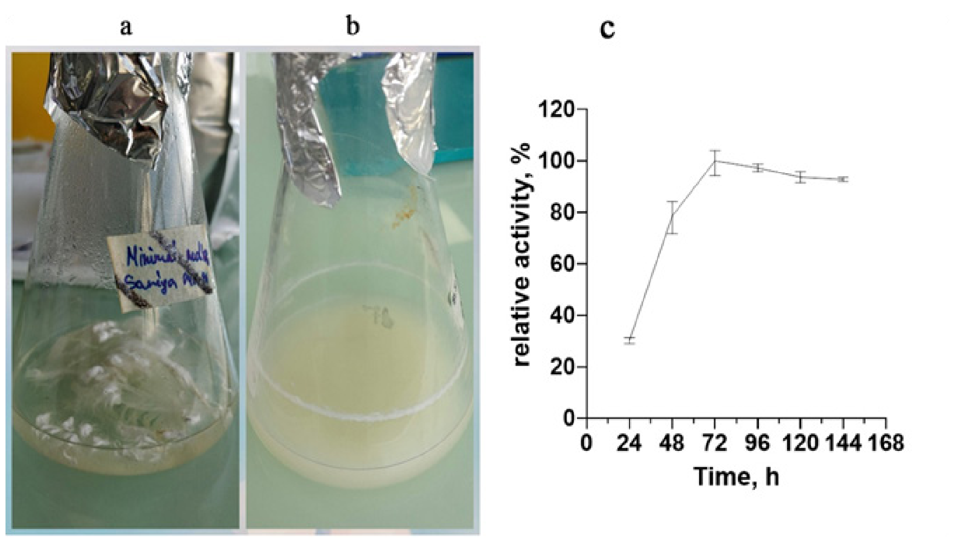


Figure 2 – Feathers at the beginner (a) and at the end (b) of cultivation *B. paralicheniformis* T7
Depending of proteolytic activity of *B. paralicheniformis* T7 on the cultivation time (c)

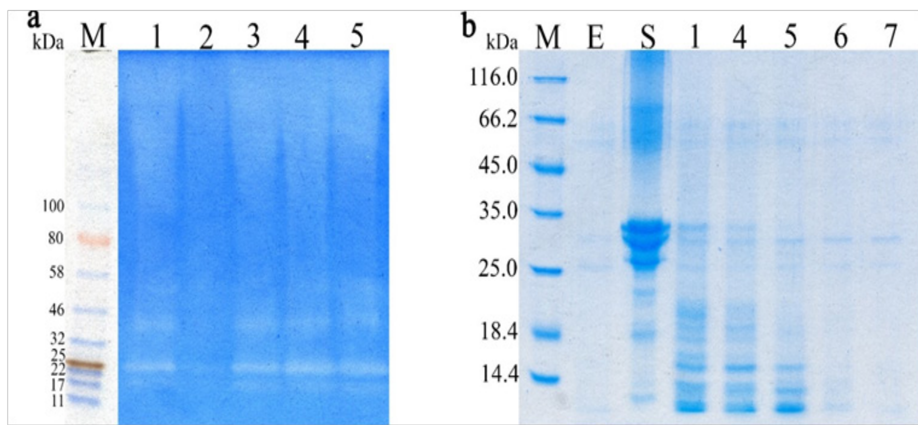
A study of the enzymatic activity of the extract showed that, in addition to high protease activity, the *Bacillus paralicheniformis* T7 strain also exhibited amylase, esterase, lipase, phosphatase, myo-inositol phosphohydrolase and lactase activity (Table 1).

Table 1 – Enzymatic activity of the supernatant from *Bacillus paralicheniformis* T7 after submerged fermentation. All measurements were performed independently in triplicate, and the mean of the three replicates is presented as the reported value with standard deviation (± SD).

Type of enzyme	Substrate	Activity (U/mL)
Protease	Azocasein	715.7 ± 40.2
Milk clotting	Cow milk	5.8 ± 0.1
Amylase	Potato starch	176.1 ± 16.3
Esterase	p-nitrophenyl acetate	24.3 ± 0.4
	p-nitrophenyl butyrate	57.8 ± 0.4
Lipase	p-nitrophenyl decanoate	17.5 ± 0.8
Phosphatase	p-nitrophenyl phosphate disodium salt 6-hydrate	11.9 ± 0.6
Myo-inositol phosphohydrolase	Phytic acid sodium salt hydrate	0.3 ± 0.03
Lactase	Lactose	0.02 ± 0.01

The zymographic analysis revealed that the enzymatic extract of *B. licheniformis* T7 contains caseinolytic proteases (Figure 3a). The activity of the enzymatic extract of *B. licheniformis* T7 was inhibited by PMSF, but not by EDTA, Pepstatin A, or E64. Six peptidases and proteases were identified in the enzymatic extract by HPLC-Q/TOF mass spectrometry and Mascot analysis: bacillopeptidase F of *B. licheniformis* (155 kDa), peptidase S8 of *B. licheniformis* (67.7 kDa), aminopeptidase of *Bacillus* spp. (38.2 kDa), peptidase M14 of *Bacillus* spp. (60.7 kDa) and serine protease of *Bacillus* spp. (48.3 kDa) and protease Subtilisin (38.9 kDa) of *B. licheniformis*. In addition, α-amylase AmyS (55.2 kDa), alkaline phosphatase (59.8 kDa), and a GDSL-type esterase/lipase family protein (26.8 kDa) were identified in the enzymatic extract by HPLC-Q/TOF mass spectrometry.

The combined proteolytic activity of these enzymes contributed to the rapid hydrolysis of casein (Figure 3b). One microgram of the sodium caseinate began to degrade after 15 s of digestion by the enzymatic extract and was completely hydrolyzed after 90 s.



with EDTA, lane 4 - the enzyme extract with Pepstatin A, lane 5 - the enzyme extract with E64
Figure 3 – The zymogram with copolymerized casein for enzyme extract of *B. paralicheniformis* T7

Along with proteolytic activity, the enzymatic extract also showed milk-clotting activity. Testing of milk-clotting activity on five types of milk showed that the enzymatic extract of *B. paralicheniformis* T7 exhibited coagulation activity toward cow's and ewes' milk, but did not show this activity toward goat's, camel's, or mare's milk. The milk-clotting activity toward cow's and ewes' milk was 12 and 23 U/mL, respectively. These results indicate that the milk-clotting activity of the extract is two times higher toward ewes' milk than toward cow's milk.

The milk-clotting activity of the enzymatic extract from *B. licheniformis* T7 was also tested for clot formation in fresh cow's milk. The milk had the following characteristics: fat - 4.76%, protein - 2.96%,

lactose - 4.36%, and total solids - 12.68%. A clot was obtained from 300 mL using 300 units of the enzymatic extract within 34 minutes at 37 °C. The clot was firm; after cutting, whey separated from the curd (Figure 4a), and after kneading and further whey separation, a cheese mass was formed (Figure 4b), which, after pressing, took the form of cheese (Figure 4c). The resulting cheese was firm, white, odorless and tasteless. Measurements showed that the yield of cheese and whey were 173 g and 780 mL, respectively. The moisture content of the cheese was 34%.

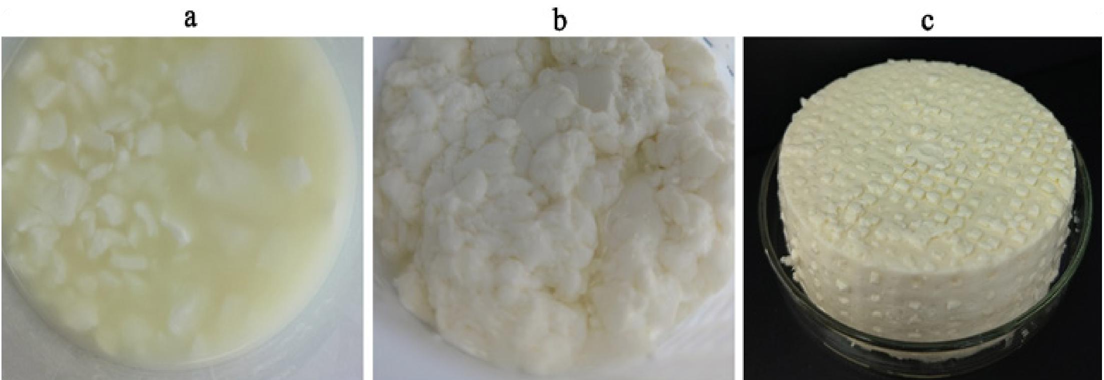


Figure 4 – Cheese from cow's milk prepared by the enzyme extract from *B. paralicheniformis* T7

Bacilli species are well-known producers of proteolytic enzymes. In nature, bacilli occupy various ecological niches, including soil, water, plants [1]. The high productivity of *Bacillus* makes these bacteria leading candidates in biotechnology as enzyme-producing strains [21].

In the present study, a strain identified by molecular genetic methods as *Bacillus paralicheniformis* T7 and possessing proteolytic properties was isolated from the soil of southern Kazakhstan.

The isolated strain showed high proteolytic activity, which was manifested in the complete degradation of the robust β-keratin protein. By culturing *B. paralicheniformis* T7 on minimal saline medium containing feathers as the sole source of organic matter, complete degradation of chicken feathers was achieved. The degradation of barbs and barbules continued throughout the incubation period and resulted in complete exposure of the shaft, while the culture became milky.

When cultured on salt medium with feathers, strain *B. paralicheniformis* T7 produced proteases with a total activity of 715.7 ± 40.2, which exceeds that reported for several known bacterial strains. For example, *Bacillus* sp. CL33A produced proteases with an activity 248 U/mL [22], *Bacillus* sp. A5.3 showed an activity of 158.8 ± 2.5 U/mL [23], the activity of *B. licheniformis* BL312 was 49.4 ± U/mL [24], the extract from *B. pumilus* D3 had an activity of 78.3 U/mL [25], and B. cereus VITSN04 showed an activity of 167.07 ± 0.30 U/mL [26]. Proteases secreted by *Deinococcus radiodurans* R1 had an activity of 110 U/mL [27]. Testing of proteases from *B. paralicheniformis* T7 with inhibitors, together with zymographic analysis, showed that proteases belong to the serine protease family, because their activity was inhibited only by PMSF and not by EDTA, pepstatin A, or E64. Serine proteases are known to be a common type of proteolytic enzyme in members of the genus *Bacillus* [1]. In addition to proteases, *B. paralichenifromis* T7 also secretes other hydrolases, including amylase, esterase, lipase, phosphatase myo-inositol phosphohydrolase, lactase, thus acting as a strain with multienzyme activity.

Among the proteases identified by proteomic analysis in the secretory proteome of *B. paralichenifromis* T7 were enzymes also found in other [28-32]. Their high activity ensures rapid hydrolysis of casein in a very short time: 1 mg of the soluble caseinate was completely hydrolysed within 1.5 min. This high activity against casein makes the enzymatic extract from *B. paralicheniformis* T7 similar to proteases from Bacillus sp. A5.3 which hydrolyzed the casein within 1 min [23]. However, unlike *Bacillus* sp. A5.3, proteases from *B. paralicheniformis* T7 also have milk-clotting activity, which is known to be substrate-specific process. The coagulation of milk requires hydrolysis of κ-casein, which ensures the stability of the entire casein complex in milk [33]. For example, the aspartic protease chymosin (EC 3.4.23.4) hydrolyzes the peptide bond between Phe105 and Met106 in κ-casein, which leads to destabilization of the casein micelle and, consequently, to milk coagulation [16]. In addition to the aspartic protease chymosin, serine proteases and pepsin-like fungal enzymes also exhibit milk-

clotting activity and are used in cheese making [34]. Among bacteria, in addition to *B. paralicheniformis* T7, proteases from *B. subtilis* YB-3 [35], *B. subtilis* MTCC 10422 [36], *B. amyloliquefaciens* D4 [37], *B. licheniformis* T7 USC13 [38] and *B. amyloliquefaciens* JNU002 [39] have also shown milk-clotting activity.

The use of the enzymatic extract in laboratory cheese production made it possible to achieve a yield of 17% at a moisture content of 34%, which is a good indicator and confirms the applicability of the extract from *B. paralicheniformis* T7 in cheese making. The presence of hydrolases such as esterases, lipases, phosphatases, and lactases in the enzyme extract allows for more thorough processing of the casein curd. Esterases preferentially hydrolyze water-soluble esters of short-chain fatty acid (<10 carbon atoms), whereas lipases exhibit activity primarily toward insoluble long-chain triglycerides (>10 carbon atoms). By breaking down short-chain substrates, esterases influence cheese aroma, while lipases, by hydrolyzing milk fat, may affect fat composition and improve cheese flavor.. Lactases hydrolyze milk sugar (lactose), releasing galactose and glucose, which also influences the flavor of cheese. Phosphatases act on milk phospholipids, which make up approximately 0.03% of the total composition, and are also involved in the biochemical processes of cheese ripening. Thus, the obtained results indicate the potential of the enzyme extract from *B. paralicheniformis* T7 as a multienzyme preparation for use in cheese production. Increasing milk volumes create a need for its processing into durable products such as cheese. The growing demand for cheese makes it necessary to find new sources of inexpensive enzymes. The high productivity of alkaline and thermostable proteases with strong proteolytic properties, combined with milk-clotting activity, allows us to propose strain *B. paralicheniformis* T7 as a new source of proteolytic enzymes.

Conclusion

Bacillus paralicheniformis T7 is a new strain that secretes proteolytic enzymes. The enzymatic extract of *B. paralicheniformis* T7 has a high caseinolytic activity of 715.7 ± 40.2 U/mL under optimal conditions and a milk-clotting activity of 12 and 23 U/mL toward cow’s and ewes’ milk, respectively. The enzyme extract contains enzymes with caseinolytic activity that belong to the serine protease family. The enzymes secreted by strain *B. paralicheniformis* T7 exhibit milk-clotting activity toward cow’s and ewes’ milk and therefore have strong potential for cheese making, while *B. paralicheniformis* T7 itself is a promising strain for the producion of proteolytic and milk-clotting enzymes.

Author Contributions

Conceptualization - K.B.; Methodology - A.S. and B.K.; Investigation- A.S., T.A., M.A., M.K. and U.A.; Writing, original draft preparation - B.K.; Writing-review and editing - K.B.; Visualization - A.S.; Supervision - K.B.; Project administration - K.B.; Funding acquisition - K.B. All authors have read and agreed to the published version of the manuscript.

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Bacillus paralicheniformis **T7 штамының ферментативті белсенділігі және оны ірімшік өндірісінде қолдану**

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Түйін

Алғышарттар мен мақсат. Қымбат емес сүт коагулянттарының қымбаттауы протеолитикалық микроорганизмдер арасында сүт түзетін ферменттердің жаңа көздерін іздеуді ынталандырады. Бұл жұмыстың мақсаты протеолитикалық белсенділігі бар штамды бөліп алу болды.

Материалдар мен әдістер. Бұл жұмыста *Bacillus paralicheniformis* T7 протеолитикалық штамы бөлініп алынды және анықталды. *B. paralicheniformis* T7 тұқымын қауырсынды ортада үш күн бойы өсіру арқылы протеолитикалық белсенділігі 715,7±40,2 У/мл ферменттік сығынды алынды *B. paralicheniformis* штамының T7 протеолитикалық белсенділігінен басқа амилаза (176,1 ± 16,3), эстераза (24,3 ± 0,4), липаза (17,5 ± 0,8) және фосфатаза (11,9 ± 0,6) белсенділігі де бар.

Нәтижелер. Казеинді зимографиялық талдау сығындысында казеинолитикалық протеазалар бар екенін көрсетті. *B. paralicheniformis* T7 протеазалары 90 с ішінде 1 мг казеинді толығымен гидролиздендірді. HPLC-Q/TOF талдауы арқылы сығындыда алты протеаза, сондай-ақ эстераза, амилаза және фосфатаза анықталды. *B. paralicheniformis* сығындысы казеинолитикалық белсенділіктен басқа сүт ұю белсенділігіне ие, ол сиыр және қой сүті үшін сәйкесінше 12 және 23 бірлік/мл болды. Сырды жаңа сиыр сүтінен шығымдылығы 17% ферменттік сығынды арқылы жасайды.

Қорытынды. *B. paralicheniformis* T7 штамын протеолитикалық ферменттердің жаңа көзі ретінде және оның протеазаларын ірімшік өнеркәсібінде сүт ұю ферменттері ретінде пайдалану перспективалы болып көрінеді.

Кілт сөздер: Bacillus paralicheniformis; фермент; протеаза; сүт коагуляциясы; ірімшік.

Ферментативная активность штамма *Bacillus paralicheniformis* T7 и ее применение в производстве сыра

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Аннотация

Предпосылки и цель. Увеличение стоимости недорогих коагулянтов молока стимулирует поиск новых источников молокообразующих ферментов среди протеолитических микроорганизмов. Целью данной работы было выделение штамма с протеолитической активностью.

Материалы и методы. В настоящей работе выделен и идентифицирован протеолитический штамм *Bacillus paralicheniformis* T7. Путем культивирования *B. paralicheniformis* T7 в течение трех суток на перьевой среде был получен ферментативный экстракт с протеолитической активностью 715,7 ± 40,2 Ед/мл. Штамм *B. paralicheniformis* T7 помимо протеолитической активности обладает также амилазной (176,1 ± 16,3), эстеразной (24,3 ± 0,4), липазной (17,5 ± 0,8) и фосфатазной (11,9 ± 0,6) активностью.

Результаты. Зимографический анализ казеина показал, что экстракт содержит казеинолитические протеазы. Протеазы B. paralicheniformis T7 полностью гидролизовали 1 мг казеина за 90 с. В экстракте с помощью ВЭЖХ-Q/TOF-анализа были идентифицированы шесть протеаз, а также эстераза, амилаза и фосфатаза. Помимо казеинолитической активности, экстракт *B. paralicheniformis* обладает молокосвертывающей активностью, которая составила 12 и 23 ед/мл для коровьего и овечьего молока соответственно. Сыр произведен из свежего коровьего молока с использованием ферментативного экстракта с выходом 17%.

Заключение. Представляется перспективным использование штамма *B. paralicheniformis* T7 в качестве нового источника протеолитических ферментов и его протеаз в качестве молокосвертывающих ферментов в сыродельной промышленности.

Ключевые слова: *Bacillus paralicheniformis*; фермент; протеаза; свертывание молока; сыр.