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Original articles

ISOLATION, IDENTIFICATION AND USAGE OF BACILLUS STRAINS IN MICROBIAL INHIBITION TEST IN MILK

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ABSTRACT

The use of antibiotics in the treatment of livestock has increased the productivity of the meat and dairy industry. The side effects of antibiotics have raised concerns about their widespread use and have necessitated the monitoring of antibacterial residues in dairy products. 14 microbial isolates identified as *B. licheniformis, B. sonorensis, B. cereus, B. simplex, B. thuringiensis, B. pumilis, B.* mojavensis, B. *subtilis, B.* atrophaeus, B. *paralicheniformis* were selected from soil samples collected in 7 regions of Kazakhstan. Sensitivity testing of the isolated strains showed that the strains were susceptible to 13 antibiotics belonging to lincosamide, ansamycin, quinolone, macrolide, fluoroquinolone, tetracycline, nitrobenzene, aminoglycoside, glycopeptide and β-lactam antibiotics. The proteolytic strain *Bacillus licheniformis* T7 seems promising as a test culture. It is highly sensitive to antibiotics, grows rapidly on a variety of nutrient media, is alkaline and spore-forming and can be cultured at 37-55°C. The peculiarity of the strain to change the pH of the medium from 5.0 to 7.0 and above makes it possible to use bromocresol purple as a growth-detecting dye. This has been shown in experiments using antibiotic milk samples on LB agar and LB broth pH 5.0. In the absence of growth-inhibiting antibiotics, the culture of *B. licheniformis* T7 grows vigorously, which causes a pH shift to 7.99 and causes the color of bromocresol purple to change from yellow to purple. The results showed promise for the strain *Bacillus licheniformis* T7 to be used as a bacterial culture in the development of a microbiological test for the detection of antibiotics in milk.

Key words: milk, antibiotic, bacteria, *Bacillus licheniformis*, sensitivity

INTRODUCTION

Antibiotics are one of the effective drugs used in the treatment of humans and animals [1], including the treatment of infectious diseases in dairy cattle [2]. The most popular antibiotics for milk cattle are β-lactam, tetracycline, aminoglycoside, sulphalamide and macrolide compounds, which differ in their chemical structure and mechanism of antimicrobial action. However, the residual content of these antibiotics in milk can cause allergic reactions in humans and contribute to the development of drug resistance in bacteria [3]. Studies have shown that antibiotic use at an early age can subsequently increase the risk of allergy and obesity, which is closely linked to the disruption of gut microflora due to antibiotic action [4]. Antibiotic residues impede milk processing (e.g. fermentation by lactic acid bacteria) and have a negative effect on the intestinal microflora of newborn animals [5]. The side effects of antibiotics are the flip side of their effectiveness.

Antibiotics are widely used to treat mastitis, reproductive diseases and hoof diseases on dairy farms. They are injected either into a vein or into the muscle and enter the milk already in very low concentrations. Antibiotic drugs have different half-lives, which depend on the chemical structure of the substance and the metabolism of the animal. For example, a residue of penicillin G can be found in milk after 9 days of treatment [6] and a residue of gentamicin after 6 days of treatment of mammary inflammation [7].

The issue of antibiotic residues in the context of antibiotic overuse by the public is of concern [8] and leads to numerous restrictions on uncontrolled antibiotic therapy in the dairy sector [9]. It should be noted that the use of antibiotics in animal husbandry is not always effective, for example, in the treatment of mastitis [10]. Additional risks cause the release of antibiotics with manure into the environment [11]. All this makes it necessary to determine the residual content of antibiotics in milk and dairy products.

Various techniques based on chromatography and immunology are used to detect different antibiotic residues [12]. Quantitative methods such as high performance liquid chromatography [13], gas liquid chromatography [14], thin layer chromatography [15], mass spectrometry [16] and electrochemical methods [17,18] are actively used to detect antibiotic residues in food. The methods have varying degrees of accuracy, specificity and detection rate, but a common disadvantage is that they require expensive equipment in laboratories and higher requirements for the qualification of laboratory personnel, which is difficult to achieve on farms and milk processing plants. At the same time, microbiological methods based on the susceptibility of bacteria to the presence of antibiotics seem to be promising [19]. These methods are inexpensive and, unlike immunological methods and receptor binding tests, cover a wide range of different antibiotics. The search for a bacterial strain characterised by antibiotic sensitivity, high growth rate and exhibiting phenotypic traits detectable in a visual way therefore seems promising.

For practical use, tests are available to determine the residual antibiotics and sulphonamides in milk and milk products: Milchtest CMT (Packhaus Rockmann GmbH, Germany), Delvotest SP-NT (DSM Food Specialties, Denmark), Copan Milk Test (Copan Innovation, Italy), Eclipse Farm 3G (Zeulab, Spain). These tests are based on the diffusion of milk in agar containing spores of the strain and the dye bromocresol purple. The method is based on the fact that when milk is added to a test microtube in the absence of substances that inhibit the growth of bacteria, spores germinate and sucrose is

fermented, which leads to a change in the pH of the medium to the acid side (pH below 5) and a change in the color of the dye from purple to yellow. Tests detect more than 50 antibiotics and inhibitory substances, including β-lactam antibiotics, tetracyclines, aminoglycosides, macrolides, sulphonamides, lincosamides and ansamycins. These tests use spores of the thermophilic strain *Bacillus stearothermophilus* var. Calidolactis capable of growth at 64-65°C. The advantages of microbiological diffusion tests are susceptibility to a large class of antibiotics and high sensitivity to antibiotics. For example, for penicillin sensitivity tests based on *B. stearothermophilus* var. Calidolactis is 1-3 μg/kg, while the EU/Codex limit is 4 μg/kg. On the other hand, excessive sensitivity of *B. stearothermophilus* var. Calidolactis to antibiotics and inhibitors may be misinterpreted. Thus, out of 200 milk samples tested with Delvotest SP-NT, 40 samples tested positive for antibiotic residues, but only 4 had an oxytetracycline concentration above the maximum limit [20], which was confirmed by HPLC-MS/MS.

Such qualities as sensitivity to a large number of known antibiotics and inhibitors, sporulation, rapid growth, fermentation of sugars with a change in the pH of the medium, thermophilicity favor the use of *B. stearothermophilus* strains as the test culture [12,21,22]. Many strains of *Bacillus* are highly sensitive to antibiotics, such as *B. cereus* [23], *B. velesensis* [24], *B. megaterium* [25]. Being soil bacteria and being exposed to antibiotics used in animal husbandry, however, bacilli have not been observed to acquire antibiotic resistance and antibiotic susceptibility persists [26]. Thus, microorganisms of the genus *Bacillus* seem to be the most promising bacteria for test culture, which was the subject of this study. The work was carried out under the project «Development of a microbiological method for monitoring the content of antibiotics in milk» of the Scientific and Technical Program «Development of methods for analytical control and monitoring of food safety».

The aim and objectives of this work were to isolate and identify bacterial strains of the genus *Bacillus*, screen them for sensitivity to β-lactam and aminoglycoside antibiotics and select the most promising strain to develop a microbial test culture for the detection of antibiotics in food.

MATERIALS AND METHODS

Mediums

Nutrient broth (0.5% peptide hydrolysate of animal tissues, 1.5% meat extract, 1.5% yeast extract, pH 7. 4±0.2) and nutrient agar (0.5% peptide hydrolysate of animal tissues, 1.5% meat extract, 1.5% yeast extract, 1.5% agar), Arret and Kirschbaum medium (0.6% peptone, 0.4% tryptone, 0.3% yeast extract, 0.15% peptone B, 0.1% glucose, 0.03% manganese sulfate, 1.5% agar, pH 6.6± 0.2), milk agar (2% skim milk powder, 0.1% NaCl, 1% tryptone, 1% agar), Luria-Bertani broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl, pH 7.0± 0.2), Luria-Bertani agar (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 1.5% agar) was used in the study. The dye bromocresol purple was used from Titan Biotech Ltd. (India). Other chemical reagents were produced by Merck, Sigma and AppliChem (Germany) with «Pure» and «For molecular biology» purity.

Antibiotics

Thirteen antibiotics produced by Titan Biotech Ltd. (India), belonging to different classes, were used in the work. Information on the antibiotics is given in Table 1.

Isolation of the strains

The strains isolated from soil samples collected from different regions of the Republic of Kazakhstan were used in this work. Soil samples were measured by 1 g and suspended in 9 ml of sterile 0.9% (w/v) NaCl. The resulting mixture was shaken for 30 minutes. In order to precipitate large soil particles, at least 30 seconds were waited between seeding on solid media and shaking. Next, 100 μl of the resulting suspension was plated onto nutrient agar. The cultures were incubated at 37°C for 24 hours. The grown individual colonies were isolated and Gram stained.

Morphology determination of strains

The isolated colonies grown on nutrient agar were checked for homogeneity by visual and microscopic inspection. Visual control determined the following characteristics of the bacterial colonies: diameter in millimeters, pigmentation, shape,

Table 1 – Information on antibiotics

Class of antibiotics	Antibiotics	Abbreviated title used in this work
Lincosamides	Clindamycin	Cd
Ansamycins	Rifampicin	Rf
Quinolones	Nalidixic acid	Nl
Macrolides	Erythromycin	Er
fluoroquinolones	Ciprofloxacin	Cip
Tetracyclines	Tetracycline	Tet
Nitrobenzenes	Chloramphenicol	Chl
Aminoglycosides	Tobramycin	Tb
	Streptomycin	Str
	Kanamycin	Kn
	Gentamicin	Gn
Glycopeptides	Vancomycin	Vn
Beta-lactam antibiotics	Penicillin	Pn
	Ampicillin	Amp

height, profile. Surface and consistency observations of the colonies were made.

For cell microscopy, smears were physically fixed and stained with Gram stains. Pure cell cultures were further screened using milk agar. The morphological characteristics of each isolate were compared with data from Bergey's Manual of Systematic Bacteriology.

Strain identification by MALDI-TOF Biotyper

Smears of three separate colonies for each strain were applied in a thin layer to separate spots on a MALDI Biotarget steel plate. Each sample was coated with 1 µl matrix solution of HCCA (α-cyano-4-hydroxycinnamic acid, Bruker Daltonics) and allowed to air dry. The analyses were then performed using matrix-assisted laser desorption ionisation (MALDI) time-of-flight (TOF) mass spectrometry (MS), spectra were obtained on a Biotyper Microflex LT (Bruker Daltonics, Bremen, Germany).

Strain identification by sequencing

Genomic DNA was isolated with Wizard® Genomic DNA Purification Kit from Promega. The 16S rRNA gene fragment was amplified by PCR with the universal primer pair 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGTTTTACCTTGTTACGACTT-3′). The amplicons were subjected to Sanger sequencing using the BigDye Terminanor v 3.1 Cycle sequencing kit. Gene fragments were separated using an ABI 3730xl Genetic Analyzer automated sequencer (Applied Biosystems). The sequences were compared with GenBank data using the Basic Local Alignment Search Tool.

Sensitivity to antibiotics

Antibiotic sensitivity was tested by the disc-diffusion method according to [27]. Briefly, each strain was cultured in LB broth for 16-18 hours. Then, 100 µl overnight culture was spread evenly over the surface of the LB-agar. The antibiotic discs were placed on the surface of the agar at an equal distance one from the other and 1-2 cm from the edge of the cup. The plates were incubated in a dry-air thermostat immediately after the discs were applied for 18-20 hours at 37°C. The diameter of the growth retention zones around the discs was measured in millimetres, including the diameter of the discs themselves.

Testing Bacillus licheniformis T7 on milk samples

A culture of *B. licheniformis* strain T7 was grown for 18 hours in an incubator shaker at 37°C in 180 rpm shaking mode. The cells of the strain were collected by centrifugation at 6000 × g 7 min 4°C. Sterile LB-agar was mixed with 100 µl of bacterial stock, 8 mm diameter wells were cut in the agar and 100 µl of each sample of reconstituted 12% milk with antibiotics Vn (30 µg), Chl (30 µg), Gn (120 µg), Tet (30 µg), Amp (10 µg), Kn (30 µg) were poured. Milk without added antibiotics was used as a control. Two variants of LB-agar were used: with pH 5.0 and 7.0. Samples were cultured at 55°C.

Testing Bacillus licheniformis T7 on milk samples with bromocresol purple

For the cultivation of *B. licheniformis* T7 on bromocresol purple medium, two variants were tested: using LB agar and LB broth. In the first variant, a culture of strain *B. licheniformis* T7 was grown for 18 hours in an incubator shaker at 37°C in shaking mode at 180 rpm. The cells of the strain were collected by centrifugation at 6000 × g 7 min 4°C. Sterile LB agar (pH 5.0) containing bromocresol purple (10 mM/L) was mixed with 100 µl of bacterial stock, 8 mm diameter wells were cut in the agar and 100 µl each of reconstituted 12% milk samples with antibiotics Vn (30 µg), Chl (30 µg), Gn (120 µg), Tet (30 µg), Amp (10 µg), Kn (30 µg) were poured. Milk without added antibiotics was used as a control. Samples were cultured at 55°C. For the second variant, the culture was introduced into LB broth with pH 5.0 and bromcresol purple (10mM/L) with 32 µg/mL gentamicin and cultured for 18 hours at 37°C in 180 rpm shaking mode. The tube with culture with bromcresol purple and without antibiotic was used as control.

RESULTS AND DISCUSSION

14 microbial isolates were isolated from soil samples of Zhambyl, Kyzylorda, Karaganda, Aktobe, Almaty, North Kazakhstan and Turkestan regions. Morphological analysis of the colonies showed that isolates cultured on nutrient agar after 16-48 hours of cultivation formed large convex colonies with jagged edges and viscous consistency. Cultivation in nutrient broth produces a film and a flocculated sludge. Gram staining showed that the bacteria were Gram-positive bacilli.

Identification by MALDI-TOF Biotyper with Score 1.832-2.212 showed that 13 of the isolates belonged to the genus *Bacillus* and one (Akt) isolated from soil of Aktobe oblast belonged to the genus *Solibacillus*. Sequencing of DNA locus encoding small subunit of 16S ribosomal RNA allowed to clarify the species identity of isolates. Table 1 provides information on the isolated strains.

Table 2 – Information on isolated strains

Strain	Origin	Biotyper result (Score)	16S rRNA sequensing result (Identity %)	Sensitivity to antibiotic
T5	Jambyl Region	*Bacillus licheniformis* (2.104)	*Bacillus licheniformis* (98.89%)	Cd, Rf, Er, Cip, Tb, Tet, Pn, Str, Chl
T6	Jambyl Region	*Bacillus sonorensis* (2.212)	*Bacillus sonorensis* (99.65%)	Cd, Rf, Er, Cip, Tb, Tet, Pn, Str, Chl
T7	Jambyl Region	Not identified	*Bacillus licheniformis* (98.67%)	Cd, Rf, Er, Cip, Tb, Tet, Pn, Str, Chl
RF1	Kyzylorda Region	*Bacillus pumilis* (2.023)	*Bacillus pumilis* (97.86%)	Cd, Rf, Er, Cip, Tb, Tet, Pn, Str, Chl

RF2	Kyzylorda Region	*Bacillus simplex* (1.934)	*Bacillus simplex* (95.27%)	Cd, Rf, Er, Cip, Tb, Tet, Pn, Str, Chl
PH5	Kyzylorda Region	*Bacillus thuringiensis* (1.952)	*Bacillus thuringiensis* (96.87%)	Cd, Rf, Er, Cip, Tb, Tet, Pn, Str, Chl
PH6	Kyzylorda Region	*Bacillus pumilis* (1.832)	*Bacillus pumilis* (95.82%)	Cd, Rf, Er, Cip, Tb, Tet, Pn, Str, Chl
PH7	Kyzylorda Region	*Bacillus cereus* (1.938)	*Bacillus cereus* (98.47%)	Cd, Rf, Er, Cip, Tb, Tet, Pn, Str, Chl
ZH1	Karaganda Region	*Bacillus cereus* (1.87)	*Bacillus paralicheniformis* (100%)	Cd, Rf, Nl, Er, Cip, Tb, Tet, Pn, Str, Chl
Akt	Aktobe Region	*Solibacillus silvestris* (1.839)	*Solibacillus* sp. (100%)	Cd, Rf, Nl, Er, Cip, Tb, Tet, Pn, Str, Chl
KK4	Almaty Region	Not identified	*Bacillus atrophaeus* (100%)	Cd, Rf, Nl, Er, Cip, Tb, Tet, Pn, Str, Chl
KR	Kyzylorda Region	*Bacillus cereus* (1.9)	*Peribacillus frigoritolerans* (100%)	Cd, Rf, Nl, Er, Cip, Tb, Tet, Pn, Str, Chl
P3	North Kazakhstan Region	Not identified	*Bacillus subtilis* (100%)	Cd, Rf, Nl, Er, Cip, Tb, Tet, Pn, Str, Chl
SH1	Turkistan Region	Not identified	*Bacillus mojavensis* (100%)	Cd, Rf, Nl, Er, Cip, Tb, Tet, Pn, Str, Chl

As shown in the table, the isolates are represented by *B. licheniformis, B. sonorensis, B. cereus, B. simplex, B. thuringiensis, B. pumilis, B. mojavensis, B. subtilis, B. atrophaeus, B. paralicheniformis*, *Solibacillus silvestris.* Identification by 16S rRNA fragment showed an identity with NCBI data of 95.27-100%.

The determination of strains sensitivity to 13 antibiotics by the disc-diffusion method (Table 1) indicated that the strains were susceptible to most of the antibiotics used in this work: Cd, Rf, Er, Cip, Tb, Tet, Pn, Str, Chl. For the remaining antibiotics sensitivity was not so apparent, however, this may be due to the need to optimize cultivation conditions.

The antibiotic sensitivity of strain *B. licheniformis* T7 was determined by the disc-diffusion method. The diameter of the inhibition zones for the antibiotics ampicillin (Amp), gentamicin (Gen), tetracycline (Tet), chloramphenicol (Chl), nalidixic acid (Nl), rifampicin (Rf) and ciprofloxacin (Cip) were 42, 39, 51, 40, 42, 48, 69 mm, respectively (Figure 1).

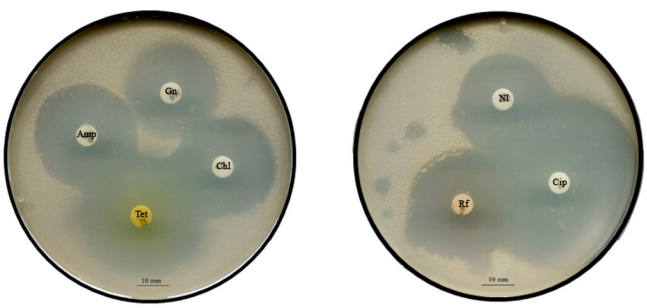


Figure 1 – Sensitivity of *B. licheniformis* T7 strain to antibiotics: Gn, Amp, Tet, Chl, Nl, Rf, Cip

Studies on cultivation conditions showed that strains *B. licheniformis* T5*, B. sonorensis* T6 and *B. licheniformis* T7 showed stable growth in the temperature range 37°C-55°C. Temperature tolerance is an important characteristic of the strains, as it allows a uniform distribution of the strain cells in the molten agar medium.

The advantage of the proteolytic strain *B. licheniformis* T7 is that no special media are required for its growth – nutrient broth and agar are optimal. Further advantages of the *B. licheniformis* T7 strain are its identical growth rate at 37°C and 55°C, which characterizes the strain as a facultative thermophile, and its tolerance to a wide pH range from 5.0 to 9.0. Studies have shown that the acidity of the nutrient medium is actively regulated by the strain itself. It was recorded that during its life activity *B. licheniformis* T7 changed the reaction of the medium to different pH values, approaching the optimal pH for its development - 7.25. An alkalinization of the medium was observed, which allows the strain to be classified as a facultative alkalophile.

Growth of the culture in nutrient broth was accompanied by clouding of the medium and the formation of a stable grayish-white film on the surface of the broth. On the surface of solid medium after 24 hours of incubation, colonies of complex shape, bumpy, smooth moist surface, whitish in colour, 4-6 mm in diameter were observed (Figure 2). In addition, strain *B. licheniformis* T7 was found to be spore-forming.

When cells of strain *B. licheniformis* T7 introduced into LB-agar were cultured after 18 hours of incubation at 55°C, a uniform lawn was observed. In experiments with reconstituted milk, growth inhibition was found around the wells with milk samples containing antibiotics, samples 1-6 in Figure 3. At the same time, a lawn of colonies is present around the well with the control sample without antibiotics. Using the two pH values, the growth inhibition due to antibiotics was found to be more pronounced at a medium pH of 5.0 (Figure 3b) than at 7.0 (Figure 3a). The zones of inhibition when using LB-agar with pH 5.0 were 32-34, whereas for LB-agar with pH 7.0 this figure was 25-28 mm.

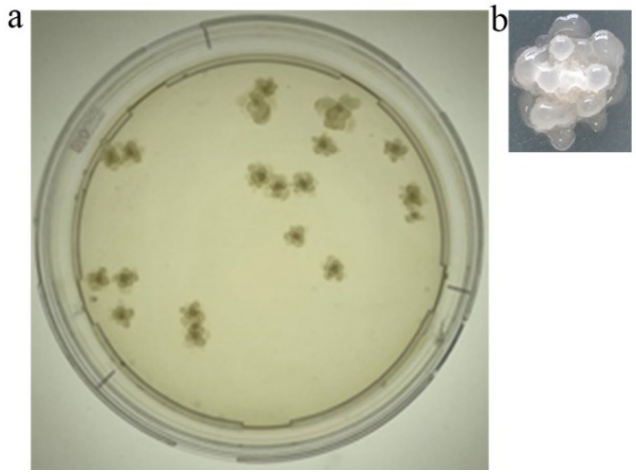
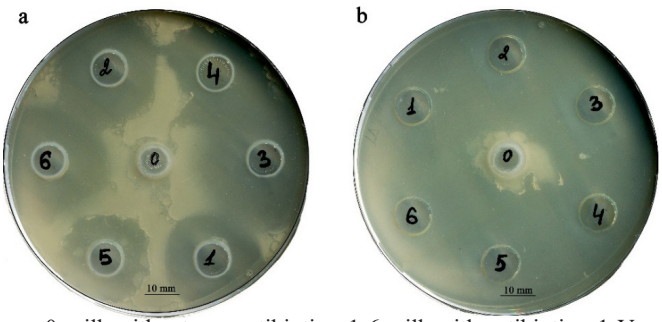


Figure 2 – Colonies of *B. licheniformis* T7 on nutrient agar after 24 h of incubation at 37°C (a) and single colony (b)



0-milk without any antibiotics; 1-6 milk with antibiotics: 1-Vn; 2-Chl; 3-Gn; 4-Tet;

5-Amp; 6-Kn

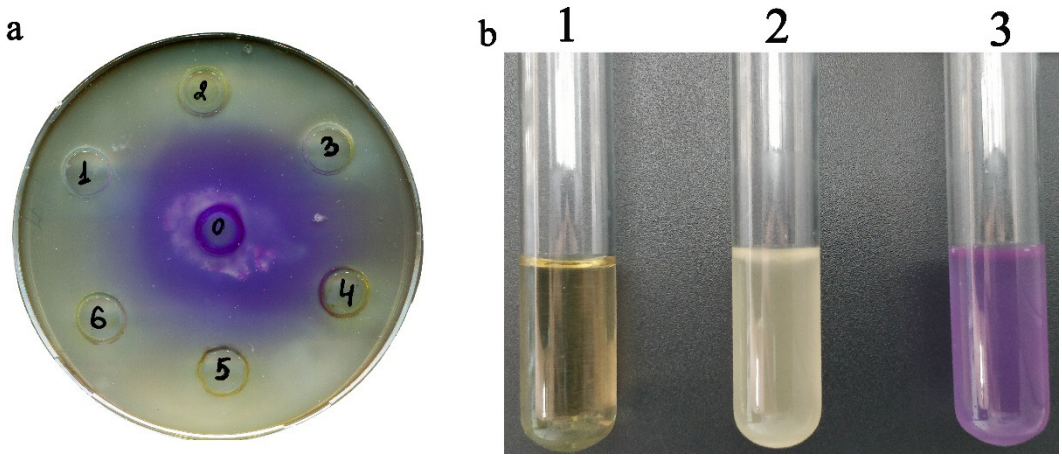
Figure 3 – Testing the milk with antibiotics on Luria-Bertani agar copolymerized with *B. licheniformis* T7 strain cells at pH 7.0 (a) and pH 5.0 (b)

Treatment of an LB plate of *B. licheniformis* T7 agar medium (pH 5.0) with bromocresol purple (BCP) showed that a color change from yellow to purple occurred around the control well (0) to which milk was added, which did not contain any antibiotic (Figure 4a). The color change is caused by a shift in the pH of the agar around the control well from 5.0 to >7.0. There is no color change around wells 1-6, since the pH of the agar around the ethyl wells still remains 5.0, which indicates the absence of growth of the bacterial culture due to the suppression of cell growth with the antibiotics vancomycin, chloramphenicol, gentamicin, tetracycline, ampicillin, kanamycin (Figure 4a).

When experimenting with LB broth (pH 5.0) with the addition of bromocresol purple, a color change was also noted in the control (without the addition of antibiotic) sample (Figure 4b). If the initial pH value in the LB-broth was 5.0, and in the sample with the addition of gentamicin (tube 1 of Figure 4b) this pH value is maintained, then in the grown culture without the addition of gentamicin (tubes 2 and 3 of Figure 4b) the pH rose to 7.99, which led to followed by the acquisition of purple medium (tube 3 of Figure 4b).

The basis of microbiological test for the determination of residual antibiotics, based on the principle of diffusion of milk into agar, is a strain that must meet the following requirements: be as sensitive as possible to most antibiotics used in veterinary medicine, be spore-forming, whose spores must withstand temperatures exceeding 50°C, have the ability to change the pH value in the process of physiological growth. The listed signs are possessed by bacteria of the genus *Bacillus*, whose representatives are susceptible to a number of antibiotics [23-25]. For example the *B. megaterium* strain makes it possible to detect 105 μg/L chlortetracycline, 100 μg/L oxytetracycline, and 134 μg/L tetracycline within 5 hours [25] and *B. licheniformis* can detect not only antibiotics, but also the preservatives nisins and monolaurin [28].

Thus, strain *B. licheniformis* T7 is of considerable interest as a test culture in the development of a microbiological test for the detection of antibiotics in milk. Another strain, *B. stearothermophilus* 953, which is sensitive to tetracycline, aminoglycosides, erythromycin antibiotics and is particularly sensitive to the 6 β-lactam antibiotic groups (penicillin-G, ampicillin, amoxicillin, cloxacillinnin, cephalexin, cefazolin) and changes colour when bromocresol purpuree is used, is described in literature [12]. However, unlike *B. licheniformis* T7, the colour change of *B. stearothermophilus* 953 is from purple to yellow, as the initial pH of the medium is 7.2, and after 3 hours of cultivation at 55-64 °C the pH of the medium shifts to acidic and the colour changes to yellow. The authors in [21] used *B. stearothermophilus* strain ATCC12980, which



a: 0-milk without any antibiotics; 1-6 milk with antibiotics: 1-Vn; 2-Chl; 3-Gn; 4-Tet; 5-Amp; 6-Kn, b: 1- bacterial culture with BCP and Gn; 2-bacterial culture; bacterial culture with BCP

Figure 4 – Testing the milk with antibiotics - on Luria-Bertani agar (pH 5.0) copolymerized with *B. licheniformis* T7 strain cells and stained with bromocresol purple (a) and Luria-Bertani broth (pH 5.0) (b)

showed its relevance in determining 34 antibiotics in milk. The strain *B. stearothermophilus* var. Calidolactis C953 was used in the detection of antibiotics in sheep milk [22]. The dye used in this test was brilliant black. *B. subtilis* strain BGA was used in a microbiological test system for the detection of fluoroquinoline and sulphalamine antibiotics in sheep milk [29].

Thus, the basis for developing an inhibitory diffusion test will be the *B. licheniformis* T7 strain, which has shown its promise in this direction. The next stage of work will be the study of spore formation of the strain, including the selection of conditions for the rapid germination of spores, the determination of the sensitivity limit of spores to antibiotics, and the development of a prototype test kit.

CONCLUSION

14 microbial isolates were selected from soil samples collected in Zhambyl, Kyzylorda, Karaganda, Aktobe, Almaty, North Kazakhstan and Turkestan regions of Kazakhstan. Based on cultural and morphological features, proteomic profile analysis and sequencing of 16S rRNA conserved locus fragment they were identified as *B. licheniformis, B. sonorensis, B. cereus, B. simplex, B. thuringiensis, B. pumilis, B. mojavensis, B. subtilis, B. atrophaeus, B. paralicheniformis*. Sensitivity testing of the isolated strains showed high sensitivity to the following antibiotics: clindamycin, rifampicin, erythromycin, ciprofloxacin, tobramycin, tetracycline, penicillin, streptomycin, and chloramphenicol. A study of strain *B. licheniformis* T7 showed that the strain grows actively at 37-55°C, is alkaline, spore-forming and proteolytic. Experiments using bromocresol purple showed that strain *B. licheniformis* T7 allowed visual detection of antibiotics in milk on solid and liquid nutrient media. The results show the perspective of *Bacillus licheniformis* strain T7 to be used as a test culture in the development of a microbiological test for the detection of antibiotics in milk.

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ВЫДЕЛЕНИЕ, ИДЕНТИФИКАЦИЯ И ПРИМЕНЕНИЕ ШТАММОВ РОДА *BACILLUS* В МИКРОБИОЛОГИЧЕСКОМ ТЕСТЕ ПО ОПРЕДЕЛЕНИЮ АНТИБИОТИКОВ В МОЛОКЕ

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АННОТАЦИЯ

Использование антибиотиков в лечении сельскохозяйственных животных позволило увеличить продуктивность мясомолочного животноводства. Побочные действия антимикробных препаратов представляют собой оборотную сторону их эффективности. Нежелательные эффекты являются причиной обеспокоенности по поводу повсеместного использования антибиотиков и обусловливают необходимость контроля их остаточного содержания в молочной продукции. Из образцов почвы, собранных в 7 областях Казахстана, выделено 14 изолятов микроорганизмов, которые были идентифицированы как *B. licheniformis, B. sonorensis, B. cereus, B. simplex, B. thuringiensis, B. pumilis, B. mojavensis, B. subtilis, B. atrophaeus, B. paralicheniformis*. Установлена чувствительность выделенных штаммов к 13 антибиотикам, относящимся к классам линкозамидов, хинолонов, макролидов, фторхинолонов, тетрациклинов, нитробензенов, аминогликозидов, гликопептидных и бета-лактамных антибиотиков и ансамицинам. Весьма интересным представляется протеолитический штамм *Bacillus licheniformis* Т7. Штамм *B. licheniformis* Т7 обладает высокой чувствительностью к антибиотикам, быстро растет на различных питательных средах, является алкалофильным и спорообразующим, его можно культивировать при температуре 37-55°С. Способность штамма к изменению pH среды от 5,0 до 7,0 и выше позволяет использовать бромкрезол пурпурный в качестве детектирующего рост красителя, что было показано экспериментах с использованием образцов молока с антибиотиками на ЛБ-агаре и ЛБ-бульоне с pH 5.0. В условиях отсутствия ингибирующих рост антибиотиков культура *B. licheniformis* Т7 демонстрирует обильный рост, что вызывает смещение pH до 7.99 и приводит к изменению цвета среды с бромокрезолом пурпурным с желтого на пурпурный. Результаты исследования показали перспективность штамма *Bacillus licheniformis* Т7 для использования в качестве бактериальной культуры при разработке микробиологического теста определения антибиотиков в молоке.

Ключевые слова: молоко, антибиотик, бактерия, *Bacillus licheniformis*, чувствительность

СҮТТЕГІ АНТИБИОТИКТЕРДІ АНЫҚТАУҒА АРНАЛҒАН МИКРОБИОЛОГИЯЛЫҚ СЫНАҚТА *BACILLUS* ТУЫСЫНЫҢ ШТАММДАРЫН БӨЛІП АЛУ, АНЫҚТАУ ЖӘНЕ ҚОЛДАНУ

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ТҮІН

Ауыл шаруашылығындағы жануарларды емдеуде антибиотиктерді қолдану мал шаруашылығының ет-сүт саласының өнімділігін арттырды. Антибиотиктердің жанама әсерлері олардың тиімділігінің кемшілігі болып табылады, олардың кеңінен қолданылуына алаңдаушылық тудырады және сүт өнімдеріндегі антибиотиктердің қалдықтарын бақылау қажеттілігін тудырады. Қазақстанның 7 облысының топырақ үлгілерінен микроорганизмдердің 14 изоляттары бөлініп алынды, олар *B. licheniformis, B. sonorensis, B. cereus, B. simplex, B. thuringiensis, B. pumilis, B. mojavensis, B. subtilis, B. atrophaeus , B. paralicheniformis*. Бөлініп алынған штаммдардың сезімталдық сынағы, штаммдардың линкозамид, ансамицин, хинолон, макролид, фторхинолон, тетрациклин, нитробензол, аминогликозид, гликопептид және бета-лактамды антибиотиктеріне жататын 13 антибиотикке сезімталдығын көрсетті. *Bacillus licheniformis* Т7 протеолитикалық штаммы сынақ дақылы ретінде перспективалы болып көрінеді. *B.licheniformis* Т7 штаммы антибиотиктерге өте сезімтал, әртүрлі қоректік орталарда тез өседі, сілтілі және спора түзуші, 37-55°С температурада дақылдандыруға болады. Штамм ортаның pH мәнін 5,0-ден 7,0 және одан жоғарыға дейін өзгерту ерекшелігі, өсуді анықтайтын боялыш ретінде бромкрезол күлгінін қолдануға мүмкіндік береді, ол pH 5,0 ЛБ және ЛБ-агар орталарында антибиотиктері бар сүт үлгілерін қолданатын тәжірибелерде көрсетілген. Өсуді тежейтін антибиотиктер болмаған кезде *B. licheniformis* Т7 дақылы белсенді түрде өседі, бұл pH мәнінің 7,99 көрсеткішіне дейін ығысуын тудырады және бромокрезолдың күлгін түсінің сарыдан күлгінге өзгеруіне әкеледі. Нәтижелер бойынша, сүтте антибиотиктерді анықтауға арналған микробиологиялық сынақты әзірлеуде бактериялық дақыл ретінде *Bacillus licheniformis* Т7 штаммының перспекивтігін көрсетті.

Түін сөздер: сүт, антибиотик, бактерия, *Bacillus licheniformis*, сезімталдық