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BACTERIOPHAGES OF *LACTOCOCCUS LACTIS* SSP. AND *STREPTOCOCCUS THERMOPHILUS* ISOLATED IN DAIRY PROCESSING PLANTS OF UKRAINE

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Aims. *Identification of Lactococcus lactis ssp. and Streptococcus thermophilus virulent bacteriophages isolated in dairy processing plants of Ukraine.* ***Methods.*** *The morphology of the viral particles was studied by transmission electron microscopy. Identification of structural proteins of phage was carried out by SDS-PAGE. The taxonomic position of phages was determined by multiplex PCR.* ***Results.*** *We presented results of the study of biological properties of 21 lactic acid bacteria phages isolated in dairy processing plants of Ukraine during 2007-2017. It was shown that phages had long, noncontractile tails, and therefore were classified to Caudovirales order, Siphoviridae family. Phages of L. lactis ssp. differed by head shape: phages with isometric heads of B1 morphotype and phages with prolate heads of B2 morphotype were detected. All analyzed S. thermophilus phages displayed specific morphology for B1 morphotype. Some of them had atypical shorter tails of 133-137 nm in length, with basal plates without globular appendages. Lytic activity spectrum of various phages was investigated and it was defined an absence of correlations between the indices of phages lytic activity and viral particles morphology. Phages of L. lactis ssp. were very heterogeneous on the basis of protein composition and formed three groups. Thus, c2 phages species differed by the fact that contained major proteins with greater molecular weights - 30, 55 and 72 kDa. Phages of S. thermophilus contained two or three major proteins with the molecular weights of 33 and 27 kDa; 42, 26, and 15 kDa, respectively.* ***Conclusions.*** *Bacteriophages of L. lactis ssp. according to their morphology, protein profile and PCR results were identified to species: 1) 38.1 % of phages belong to species 936; 2) to the species P335 – 9.5 %; 3) to the species c2 – 19.0 %. Phages S. thermophilus were identified to three groups: 1) the group of cos-type amounted to 14.3 % of the total; 2) the group of pac-type – 9.5 % and 3) 987 group – 9.5 % of phages.*

Keywords: Bacteriophages, Lactococcus lactis, Streptococcus thermophilus, Electron microscopy, Morphotype, Multiplex PCR, Protein profile.

Sensitivity of starter cultures to phage infections creates a number of complications during the production of various dairy products and cheeses and consequently leads to significant economic loss. Therefore, development of effective strategies for prevention and control of phage attacks is mandatory in dairy products manufacturing. In order to eliminate the negative effects of bacteriophages on lactic acid bacteria, precise characterization and classification of milk phages are required, as well as knowledge of the most

efficient ways of phage detection and identification, understanding of their biological properties and the process of infection [1].

It was determined that genomes of all isolated lactic acid bacteria phages contain double-stranded DNA enclosed by a capsid, which is connected to a noncontractile tail, that is a characteristic feature for viruses of *Caudovirales* order. Bacteriophage taxonomy defines three families within this order: *Myoviridae*, *Podoviridae* and *Siphoviridae*. Most lactococcal phages have a long noncontractile tail (*Siphoviridae* family) and a small isometric capsid (morphotype B1) or a prolate capsid (morphotype B2). Several phages with short tails have also been isolated (*Podoviridae* family, morphotypes C2 and C3) [2].

According to electron microscopy studies and DNA – DNA hybridization results, *Lactococcus lactis* phages have been classified into 12 species. Phages of 936, P335 and c2 species are the most common and, accordingly, the ones that cause the greatest number of fermentation processes violations [3].

The number of cases of fermentation failure caused by phages active against *Streptococcus thermophilus* strains, the main components of starters for yoghurts, other dairy drinks and many rennet cheeses, has recently increased. According to the transmission electron microscopy data, phages of *Streptococcus thermophilus* belong to *Siphoviridae* family, morphotype B1: they posess small isometric heads and long noncontractile tails (there are also phages with short tails) [4].

It was observed the correlation between the *S. thermophilus* phage DNA packaging mechanism, so-called *pac*- and *cos*-types, and the number of major structural proteins visualized by SDS-PAGE [5].

Widescale studies of lactophages are necessary to elucidate and explain the phage-host interactions. These issues are particularly urgent in Ukraine, since such studies have not been carried out earlier, despite the high risk of phage contamination during processing of raw products, in particular, in fermented dairy and cheese manufacturing. Systematic research aimed at identification of bacteriophages circulating in Ukrainian dairy industry, their isolation, study and creation of systematic phage collections have not been carried out and are urgently needed.

The purpose of this work is to study biological properties of the industrial isolates of bacteriophages active against lactic acid bacteria.

Materials and methods. *Bacterial strains, cultivation conditions.* Pure cultures of lactic acid bacteria belonging to species *L. lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris*, *L. lactis* ssp. *lactis biovar diacetylactis, S. thermophilus* from the culture collection of the Institute of Food Resources (Kyiv) were grown at 30ºC (*L. lactis*) and 41ºC (*S. thermophilus*) in 10% sterile skimmed milk or M17 broth (Oxoid, Hampshire, United Kingdom) containing 0.5% glucose (GM17; Sigma-Aldrich, St. Louis, MO, USA) for *L. lactis* or 0.5% lactose (LM17; Sigma-Aldrich) for *S. thermophilus*, renewing them every 20–25 days. They were stored at 4ºC. 1% of inoculum of 17-20 h culture was added into a tube with 9.0 ml of GM17 or LM17 broth and cultivated at the optimal temperature for 17-20 h. Then 1% of this inoculum was added into a tube with 9.0 ml of GM17 or LM17 broth and cultivated at optimal temperature until reaching the exponential phase of growth (OD_{600} = 0.6-0.7).

Bacteriophage extraction. Phage extraction and titration were carried out on solid nutrient medium GM17 or LM17, using "double" agar technique with $CaCl_2$ (Oxoid, 10 mmol l^{-1}) and glycine (Oxoid, 100 mmol l^{-1}) [6]. Plaque test was performed on Petri dishes containing mild 1.5% w⁄v agar (Merck, Darmstadt, Germany, 20 ml per dish), freshly prepared layer of lower agar and 3 ml of top agar (0.65% w⁄v agar), that contained the inoculum of $5.0x10^7$ CFU ml^{-1} culture in log-phase and 1 ml of dilution of prepared sample filtrate (0.45 μm-pore-size filters, Sartorius, Germany). To detect bacteriophages, specially selected phage-susceptible test cultures were used: *L. lactis* ssp. *lactis* $L_{1;}$ $L_{3;}$ 63 c; *L. lactis* ssp. *cremoris* C3, *L. lactis* ssp. *lactis biovar diacetilactis* 4dl; *S. thermophilus* 66/12; 27/1 from the collection of the Biotechnology Department of the Institute of Food Resources. The dishes were incubated at the optimal temperature for 24-36 h. Samples were taken from morphologically identical negative colonies of each phage isolate and was followed by the purification procedure including 5-7 consecutive cycles (1^{st} cycle: cocultivation of phages with test cultures in GM17 or LM17 broth untill visual lysis occurs and further sedimentation by centrifugation (15000 g for 10 min). The diameters of plaques formed by the purified phages were measured using a caliper and represent the average of 10 random plaques chosen over three independent experiments.

Sensitivity of cultures to phages. The phage hosts were determined using a plaque test, by placing phages on a Petri dish with GM17 or LM17 (1.5% w⁄v agar) with $CaCl_2$ (10 mmol l^{-1}), glycine (100 mmol l^{-1}) and the studied culture in log-phase of growth. Bacteriophages with the titre of 10^7 PFU ml^{-1}, 0.02 ml volume were used for tests. Dishes were incubated at the optimal temperature for 24-36 h. The strains which bacterial lawns demonstrated lysis zones after phage application were considered phagesensitive.

Phage concentration. The high titer bacteriophage lysates were prepared by consecutive infections of the host strain with the bacteriophage at an MOI (multiplicity of infection) of 0.1 to 1.0. After infection the culture was grown at optimal temperature in GM17 or LM17 supplemented with $CaCl_2$ (10 mmol l^{-1}), glycine (100 mmol l^{-1}) until completed lysis. The lysates were centrifuged for 15000 g for 10 min at temperature of 4°C for cells precipitation and the supernatant was filtered (0.45 μm-pore-size filters).

Transmission electron microscopy. For adsorption of phage particles with the titre not less than 10^9 PFU ml^{-1} to formvar surface a copper mesh with a formvar substrate film was placed on a drop of phage preparation and held for 1 h at room temperature. Non-adhered phage particles were washed in deionized H_2O for 10 min and immediately treated with uranyl acetate solution (2% w⁄v, pH 4.5) for 40 s at room temperature [7]. Microphotographs of phage particles were obtained using a transmission electron microscope JEM-1400 (Jeol, Japan) at 80 kV voltage and instrumental magnification of x50.000-100.000.

Amplification of phage DNA by multiplex PCR. To define specific genetic types of phages, PCR was performed on the DNA isolated from phage lysates. Bacteriophage DNA was isolated from lysate obtained in high titres, according to Binetti *et al.* [8]. As reference, the conserervative genes of the three basic genetic types of lactic acid phages c2, 712 (936-species) and P335 were used. Primer sequences used for multiplex PCR analysis, with PCR prod-

uct size and primer annealing temperature are the following: 936 forward, 5'-TCAATGGAAGACCAAGCGGA-3' and reverse, 5'-GTAGGAGACCAACCCAAGCC-3' (58°C, 179 bp); c2, 5'-CAGGTGTAAAAGTTCGAGAACT-3' and 5'-CAGATAATGCACCTGAATCA-3' (58°C, 474 bp); P335, 5'-GAAGCTAGGCGAATCAGTAA-3' and 5'-GATTGCCATTTGCGCTCTGA-3' (58°C, 682 bp) [9]. To identify the subtypes of phage *S. thermophilus*, multiplex PCR was used as suggested by Quiberoni *et al.* [10] and McDonnell *et al.* [11] using the following primers: *cos*, 5'-GGTTCACGTGTTTATGAAAAATGG-3' and 5'-AGCAGAATCAGCAAGCAAGCTGTT-3' (53°C, 170 bp); *pac* 5'-GAAGCTATGCGTATGCAAGT-3' and 5'-TTAGGGATAAGAGTCAAGTG-3' (53°C, 427 bp); 987 5'-CTAAGCGTTTGCCACTGTCAG-3' and 5'-GCTGCCGCTTGTTTGAAAAC-3' (55°C, 707 bp); 5093, 5'-CTGGCTCTTGGTGGTCTTGC-3' and 5'-GCGGCAACCATCTT AGACCAG-3' (55°C, 983 bp). PCR was performed on the phage samples with a titre of at least 10^{10} PFU ml^{-1}. 50 ng of the isolated DNA was used as template for PCR. DNA was amplified in 40 μl PCRs using each sequence-specific primer at 0.4 μmol l^{-1}, 0.2 mmol l^{-1} dNTPs, 1.5 U Dream Taq DNA polymerase and the manufacturer's buffer with 2.0 mmol l^{-1} $MgCl_2$ (Thermo Fisher Scientific, Inc., Vilnius, Lithuania). PCR amplifications consisted of an initial denaturing step at 95°C for 4 min, followed by 40 cycles of 95°C for 40 s, appropriate annealing temperature for 40 s, and 72°C for 1 min, with the final extension step at 72°C for 7 min. Each sample was assayed in triplicate, and each run included water blanks to eliminate the risk of contamination. PCR was performed in Applied Biosystems 2720® thermal cycler (Applied Biosystems; Thermo Fisher Scientific, Inc.). PCR products were analyzed by ethidium bromide stained 1.0 % TAE-agarose gels electrophoresis in TAE buffer (40 mmol l^{-1} Tris-acetate, 1 mmol l^{-1} EDTA) using Wide Mini-Sub Cell GT Cell and PowerPac Basic Power Supply, 100–120/220–240 V (Bio-Rad, USA). The ladder is supplied with 6XDNA Loading Dye. The product size was determined using "GeneRuler™ DNA Ladder Mix" (Fisher Scientific, Inc., Vilnius, Lithuania). Detection of the gel images was carried out at ChemiDoc™ XRS+System (BioRad Laboratories, Inc., Hercules, CA, USA).

Structural protein identification by SDS-PAGE. Protein gel electrophoresis in SDS-polyacrylamide gel (SDS-PAGE) was performed by the Laemmli method [12]. Precipitated phage particles in SM buffer with a titre of at least 10^{10} PFU ml^{-1} (approximately 50 µl) were boiled for 3 min for damage to phage DNA. Then the product was treated with 4 µg ml^{-1} DNase I (Thermo Scientific, Fermentas) at 37°C for 30 min and 5xSDS-PAGE loading buffer (250 mmol l^{-1} Tris HCl, pH 6.8, 10% (w/v) SDS, 0.5% (w/v) bromophenol blue, pH 6.8, 50% (w/v) glycerol, 0.5% (w/v) b-mercaptoethanol) was added and boiled for 10 min. Proteins were separated using SDS-PAGE (15%) using a system for vertical electrophoresis VE-10 (Helicon, Russia). Electrophoresis was performed in a buffer for electrophoresis (248 mmol l^{-1} Tris-HCl, pH 8.8, 1.92 mol l^{-1} glycine, 20 mmol l^{-1} EDTA, 1% (w/v) SDS) for 20 mA until the front of the dye reached the bottom of the gel. Proteins were stained with Coomassie brilliant blue R-250 (Sigma). Detection of the gel images was carried out at ChemiDoc™ XRS+System and proteins size were determined with Image Lab software, version 4.2.1 (Bio-Rad), using the PageRuler Prestained Protein Ladder (Thermo Scientific, Lithuania) as the standard.

Results. *Phage monitoring, phages selection, host range.* 675 whey samples were analyzed for the presence of bacteriophages. The samples were taken from dairy processing factories (20 units) from 15 different regions of Ukraine at various time points (collected in the period of 2007-2017). It was determined that about 60% of samples were contaminated with bacteriophages of lactobacteria of different taxa. From the objects of phage monitoring – the most contaminated phage-containing samples (with level II of phage contamination – from 10^2 to 10^4 PFU ml^{-1} and level III – from 10^5 and more PFU ml^{-1}), 382 phages active against *L. lactis* ssp. and *S. thermophilus* were isolated.

A systematized collection of lactic acid bacteria phages is the basis for genetic research and scientific and practical development of anti-phage measures. At the first stage of creation of such a collection, 21 phage isolates were based on their ability to effectively reproduce on selected homologous cultures of lactic acid bacteria and were accumulated at titres of $8.7x10^8$-2.8×10^{10} PFU ml^{-1} (Table 1).

Bacteriophage morphology. It was shown that phages had long, noncontractile tails, and therefore were classified to *Caudovirales* order, *Siphoviridae* family. Phages differed by head shape: phages with isometric heads of B1 morphotype and phages with prolate heads of B2 morphotype were detected. The study of morphological and morphometric properties of viral particles from the collection of biotechnology department showed that phages can be divided into 5 groups (Table 2). It was found that the majority of the studies phages

Table 1

Basic properties of phages

Phage name	Test culture	Titre[a], PFU ml^{-1}	Negative colonies morphology	Diameter zone[b], mm
Φ9/1	*L. lactis ssp. lactis* Л$_3$	$2.0x10^9$	even edge, without aureole	1.0-2.0
Φ14	*L. lactis ssp. ssp. cremoris C3*	$9.0x10^9$	even edge, without aureole	1.0-3.0
Φ4dl	*L. lactis ssp. lactis biovar diacetylactis* 4dl	$1.8x10^{10}$	even edge, without aureole	2.5-4.0
Φ11	*L. lactis ssp. ssp. cremoris C3*	$1.9x10^{10}$	homogeneous sterile zones	2.8-3.5
$ΦO_{15}$	*L. lactis ssp. lactis* Л$_1$	$2.5x10^9$	even edge, without aureole	1.5-2.8
Φ8/2	*L. lactis ssp. ssp. cremoris C3*	$2.8x10^{10}$	even edge, without aureole	2.0-2.5
$ΦO_{14}$	*L. lactis ssp. lactis* 63	$7.2x10^9$	sterile inside, cloudy at the edges	1.0-2.0
Φ8L	*L. lactis ssp. lactis* 63	$3.7x10^9$	sterile inside, cloudy at the edges	1.0-2.5
Φ8D	*L. lactis ssp. lactis* 63	$1.0x10^9$	sterile zones, small	1.0-1.5
ΦPr 3-8	*L. lactis ssp. lactis* 63	$1.1\ x10^{10}$	even edge, without aureole	2.5-3.0
ΦF/2	*L. lactis ssp. lactis* Л$_3$	$2.4x10^{10}$	even edge, aureole is large	3.0-7.0
Φy/4	*L. lactis ssp. lactis* Л$_1$	$4.0x10^9$	sterile inside, cloudy at the edges	1.0-2.5
Φy/3	*L. lactis ssp. lactis* Л$_3$	$1.1\ x10^{10}$	sterile inside, cloudy aureole	2.5-3.0
Φc/1	*L. lactis ssp. lactis* Л$_1$	$3.1x10^9$	sterile inside, cloudy at the edges	1.0-2.5
Φ3/K	*S. thermophilus* 66/12	$1.5x10^9$	sterile inside, cloudy at the edges	1.5-2.0
$ΦS_{61}$	*S. thermophilus* 66/12	$2.2x10^{10}$	homogeneous sterile zones	1.5-2.0
$ΦS_1$	*S. thermophilus* 66/12	$2.5x10^9$	blurred edge, without aureole	1.5-2.0
$ΦO_{20}$	*S. thermophilus* 66/12	$2.7x10^{10}$	homogeneous sterile zones	1.8-2.0
ΦNdan	*S. thermophilus* 27/1	$8.7x10^8$	sterile zones, small, blurred edge	1.0-1.5
$Φ36_8$	*S. thermophilus* 66/12	$1.5x10^9$	sterile zones, small	1.0-1.5
$ΦS_{27\text{-}1}$	*S. thermophilus* 27/1	$8.7x10^8$	sterile zones, small, blurred edge	1.0-1.5

[a, b], Values are the mean of three independent experiments

belonged to B1 morphotype. Phages of B1 morphotype formed 4 subgroups depending on their tail size. Virion sizes are provided in Table 2. Examples of electronograms of viruses representing each group are shown in Fig. 1.

Table 2

Ultrastructures of phages

No. group	Morphotype	Head shape	Diameter head[a], nm	Tail size[b], nm	Number of phages, %
1	B1	isometric capsid	39-51	110-142	14.3
2	B1	isometric capsid	40-55	163-207	33.3
3	B1	isometric capsid	43-54	205-291	23.8
4	B1	isometric capsid	44-46	133-137	9.5
5	B2	prolate capsid	31-40x 42-51	79-102	19.0

[a, b], The values are the mean of ten determinations.

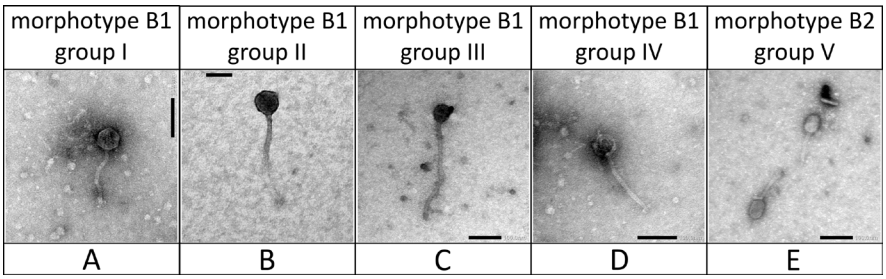

Fig. 1. Electron micrographs of phages: (A) ΦPr 3-8 (group I); (B) Φ11 (group II); (C) $ΦO_{20}$ (group III); (D) $ΦS_{27-1}$ (group IV); (E) Φy/4 (group V). Bar represents, 100 nm.

Phages of *S. thermophilus* from our samples had the structure of viral particles corresponding to B1 morphotype. It should be noted that part of phages that are active against bacteria of *S. thermophilus* species were characterized by atypical small size of tail – 133-137 nm. We have identified such phages in group B1/4 (Table 2, Fig. 1 (D).

All phages were divided into 4 groups depending on their spectrum of lytic activity. It is interesting that lytic groups I, II and IV were divided into two subgroups each correlating with phage morphotype, and finally phages were divided into 7 groups, taking into account two properties: specificity and morphological structure of viral particles (Table 3).

PCR test for the identification of phages. Based on the size of the PCR products obtained, it was found that phages ΦF/2, Φy/4 and Φc/1 are representative of c2 phages species. The electrophoregram shows (Fig. 2, lane 1, 5 and 7, respectively), the DNA of these phages forms amplicons 474 bp, which, according to other studies [9] are typical for the above-mentioned bacterial viruses.

Based on the size of the obtained PCR fragments, with a molecular weight of 682 bp, the phages Φ8D and ΦPr 3-8 were identified as P335 species (Fig. 2, lane 4 and 6 respectively). Three phages (Φ14, $ΦO_{15}$ та Φ4dl) gave a 179-bp PCR product, indicating that they belong to the 936 species of phages (Fig. 2, lane 2, 8 and 9, respectively).

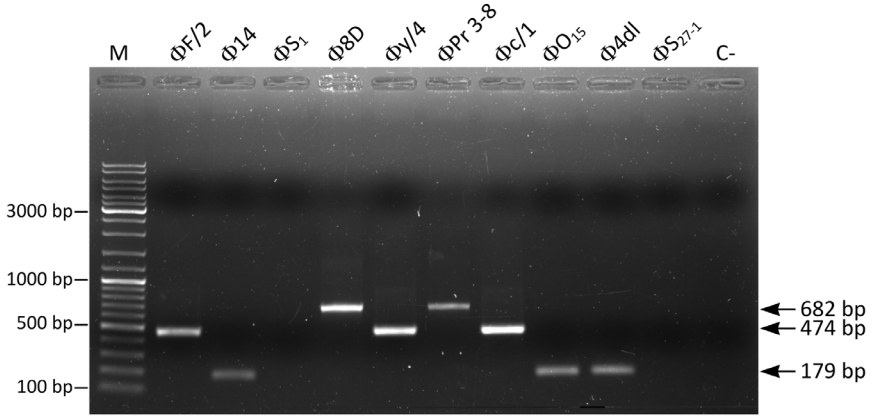

Fig. 2. Detection of *L. lactis* phages' DNA by multiplex PCR: c2-type – 474 bp, P335 – 682 bp and 936 – 179 bp. M – molecular weight marker GeneRuler™ DNA Ladder Mix ("Thermo Scientific", USA); C – negative control without DNA matrix

Table 3

Distribution of phages to lytic groups

Phages subgroup	Lytic group	Lytic activity spectrum	Morphotype
I	I	*L.lactis ssp lactis, L.lactis ssp cremoris, L.lactis ssp. lactis biovar diacetylactis*	B1 group 1
I I	I	*L.lactis ssp lactis, L.lactis ssp cremoris, L.lactis ssp. lactis biovar diacetylactis*	B2
III	II	*L.lactis ssp lactis, L.lactis ssp. lactis biovar diacetylactis*	B1 group 2
IV	II	*L.lactis ssp lactis, L.lactis ssp. lactis biovar diacetylactis*	B2
V	III	*L.lactis ssp cremoris*	B1 group 1
VI	IV	*S.thermophilus*	B1 group 3
VII	IV	*S.thermophilus*	B1 group 4

Using a set of primers for multiplex PCR to *cos*- and *pac*-types of *S. thermophilus*, fragments of 170 bp were identified which indicated the belonging of the phages ΦS_1, ΦO_{20} and ΦNdan to the *cos*-types of *S. thermophilus* viruses (Fig. 3, lane 1, 2 and 4, respectively), and fragments 427 bp for the phages ΦS_{61} and Φ3/K, which were identified as representatives of the *pac*-containing viruses (Fig. 3, lane 5 and 6, respectively).

In addition, among the phages of *S. thermophilus*, representatives of the new groups 987 and 5093 were identified, which were described by McDonnell *et al.* [11, 13]. However, among the tested phages, we found only representatives of the 987 group of *S. thermophilus* phages $\Phi 36_8$ and $\Phi S_{27\text{-}1}$, which corresponded to the PCR products of 707 bp (Fig.4, lane 4 and 7, respectively). Our sample did not contain phages similar to members of 5093 group, whose morphology is different from *cos*- and *pac*-phages, globular spherical appendages are found on baseplates of their tails. Representatives of the 5093 group (amplicon 983 bp) were not detected (Fig. 4).

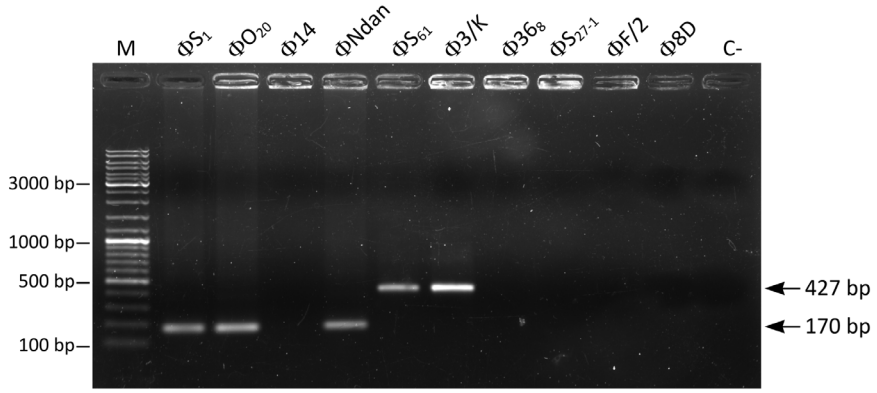

Figure 3 Detection of *S. thermophilus* phages' DNA by multiplex PCR: *cos*-type – 170 bp and *pac*-type – 427 bp. M – molecular weight marker GeneRuler™ DNA Ladder Mix ("Thermo Scientific", USA); C – negative control without DNA matrix

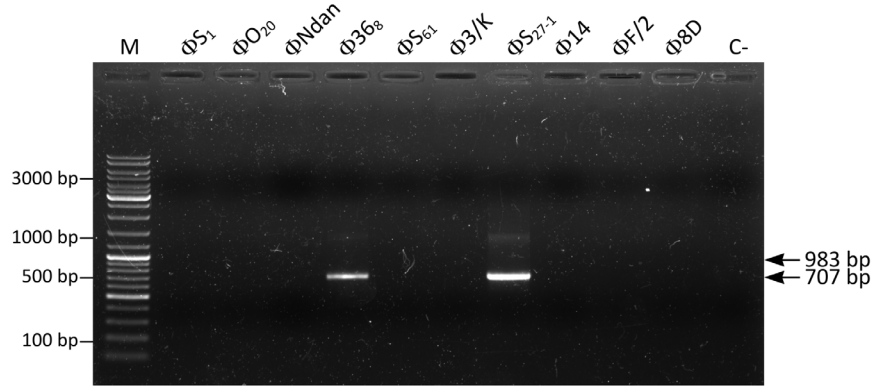

Fig. 4. Detection of *S. thermophilus* phages' DNA by multiplex PCR: 987-type – 707 bp and 5093-type – 983 bp (did not identify). M – molecular weight marker GeneRuler™ DNA Ladder Mix ("Thermo Scientific", USA); C – negative control without DNA matrix

Protein profile of phages. Phage particles derived from 3 different phages of *S. thermophilus* showed that they contain two major proteins with approximate molecular weights of 27 and 33 kDa (for example, see Fig. 5, lane 6, phage ΦO_{20}). They used *cos*- mechanism of genome packaging, so they were classified as *cos*-type. Only two phages contained three major proteins with approximate molecular mass of 15, 26 and 42 kDa (for example, see Fig. 5, lane 3, phage ΦS_{61}). They were classified into the phage group with *pac*-type of DNA packaging mechanism. Both profiles occasionally varied slightly (±1-4 kDa) from phage to phage.

In our sample *L. lactis ssp.* phages formed three groups according to their protein profiles. Thus, c2 phages species differed by the fact that contained major proteins with greater molecular weights - 30, 55 and 72 kDa (for example, see Fig. 5, lane 5 and 7, phage ΦF/2; phage Φy/3). The phage Φc/1 (c2 phages species) contained major proteins with molecular weights of 40, 53 and 72 kDa (Fig. 5, lane 9, phage Φc/1). Phages Φ8/2, ΦO_{14}

(936 phages species) contained major proteins with molecular weights of 29 and 53 kDa (Fig. 5, lane 10 (phage Φ8/2) and lane 11 (phage $ΦO_{14}$)). Phage $Φ36_8$ contained major proteins with molecular weights of 20, 29 and 53 kDa (Fig. 5, lane 1, phage $Φ36_8$).

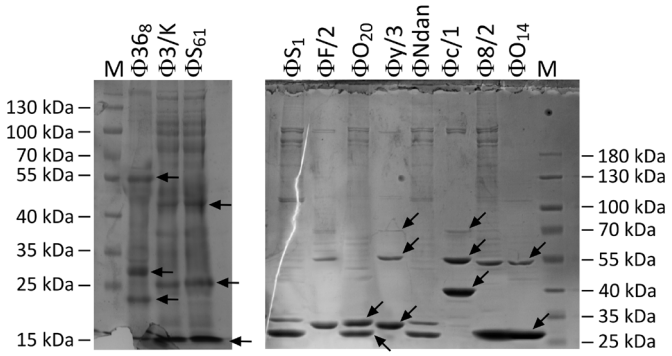

Fig. 5. Profile of *S. thermophilus* and *L. lactis ssp.* proteins of phages, separated using 15 % SDS-PAGE. Black arrows indicate structural proteins of phages. M – molecular weight marker PageRuler Prestained Protein Ladder (Thermo Scientific, Lithuania)

Discussion. In this article, we presented results of the study of basic biological properties of 21 lactic acid bacteria phages. These isolates are a representative part of a large phage collection of 382 phages isolated from samples which were obtained from dairy processing plants in various geographical locations in Ukraine and at various time points. Representative members of the phage collection used in this study were selected for identification analysis.

In accordance with the requirements of the International Committee on Taxonomy of Viruses, morphological and morphometric analysis of virus particles using the method of transmission electron microscopy was carried out for phage identification. It was defined that the majority of the examined phages belonged to B1 morphotype – 81%. According to literature data phages of B1 morphotypes were mostly mentioned abroad, although descriptions of B2 morphotype phages can often be found. In Byelorussia there was determined the distribution of B2 morphotype, c2 species phages – 63.6% of the examined viruses [14], in Germany c2 species phages was also dominant [15].

Distribution of B1 morphotype phages in Ukraine coincides with the data of researches of many scientists. So, according to Szczepanska *et al.* [16] in Poland phages of 936 species (B1 morphotype) were identified more often – in 69% of cases. Similar results were obtained by scientists from European countries, New Zealand, USA, Canada – lytic type of 936 species phages (B1 morphotype) predominated over c2 species phages (B2 morphotype) [7; 17-18].

All analyzed *S. thermophilus* phages displayed specific morphology for B1 morphotype, had isometric heads (43-54 nm in diameter) and noncontractile tails (205-291 nm in length). Also we observed phages that had isometric icosahedral heads of 44-46 nm in diameter, and noncontractile tails of a slightly shorter length, from 133 to 137 nm, with basal plates on the tails without globular appendages. Such a morphology was described for *S. thermophilus* phages of 987-group by McDonnell *et al.* [13], Szymczak *et al.* [19].

Lytic activity spectrum of various phages was investigated and it was defined an absence of correlations between the indices of phages lytic activity and viral particles morphology. Binetti *et al.* showed, that phages *S. thermophilus* also are not possible to classify on the basis of traditional criteria only, since there is no correlation between morphology and host range of viruses [8].

As it is known, by number of major structural proteins, *S. thermophilus* phages are classified into two groups. Phages with *cos*-type of packing the genome contain two major proteins with a molecular weight of 26 and 32 kDa; phages with *pac*-type contain three major proteins with a molecular weight of 13; 25 and 41 kDa [5]. In our collection three phages were attributed to *cos*-type, and two phages used *pac*-type of DNA packing.

The phages *L. lactis ssp.* were very heterogeneous on the basis of protein composition. Presence of three major proteins with relatively high molecular weight in polypeptide profiles of c2 phages was shown also by Moineau *et al.* [7], Azaïez *et al.* [20], as well as a variable amount of minor proteins.

Conclusion. It was determined that phages of *L. lactis ssp.* according to morphological structure, polypeptide composition and PCR identification belonged to species: 1) species 936 (morphotype B1) – 38.1% of the total; 2) species P335 (morphotype B1) – 9.5% and 3) species c2 (morphotype B2) – 19.0% of phages.

Based on the study of morphology, protein profile, PCR-analysis phages of *S. thermophilus* have been identified to three groups: 1) group of *cos*-type was 14.3% of the total; 2) group of *pac*-type was 9.5% and 3) 987 group – 9.5% of phages.

Thus, significant biodiversity of lactic acid bacteria phages is determined, as well as specific features of certain phages circulation in Ukrainian dairy processing plants. Reliable identification and proper determination of phages taxonomic position is very important for monitoring the distribution of phages during production, introduction of optimized phage monitoring methods, development of adequate rotational and selective phage-protective strategies.

БАКТЕРІОФАГИ *LACTOCOCCUS LACTIS* SSP. І *STREPTOCOCCUS THERMOPHILUS*, ВИДІЛЕНІ НА МОЛОКОПЕРЕРОБНИХ ПІДПРИЄМСТВАХ УКРАЇНИ

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Резюме

Мета. Ідентифікація вірулентних бактеріофагів *Lactococcus lactis* ssp. і *Streptococcus thermophilus*, виділених на молокопереробних заводах України. **Методи.** Морфологію вірусних часток вивчали методом трансмісійної електронної мікроскопії.

Ідентифікацію структурних білків фагів проводили за допомогою гель-електрофорезу в денатуруючих умовах. Таксономічне положення фагів визначали методом мультиплексної ПЛР. **Результати.** Наведено результати вивчення біологічних властивостей 21 фага молочнокислих бактерій, виділених на молокопереробних заводах України впродовж 2007-2017 рр. Було показано, що фаги мали довгі, нескоротливі хвости, і тому були класифіковані до порядку *Caudovirales*, сімейства *Siphoviridae*. Фаги *L. lactis* ssp. відрізнялись формою головки: виявлено фаги з ізометричними головками морфотипу B1 та фаги з витягнутими головками морфотипу B2. Всі проаналізовані фаги *S. thermophilus* мали морфологію, характерну для морфотипу B1. Деякі з них мали атипові короткі хвости довжиною 133-137 нм з базальними пластинами без глобулярних відростків. Вивчено спектр літичної активності різних фагів. Встановлено відсутність кореляції між показниками літичної активності та морфологією вірусних часток. Фаги *L. lactis* ssp. були дуже гетерогенними і утворювали три групи за білковим складом. Зокрема, фаги виду c2 відрізнялись тим, що містили мажорні білки з більшою молекулярною масою − 40, 53 і 72 кДа. Фаги *S. thermophilus* містили два або три мажорні білки з молекулярною масою 33 і 27 кДа; 42, 26 і 15 кДа відповідно. **Висновки.** Бактеріофаги *L. lactis* ssp. за їх морфологією, білковим профілем та результатами ПЛР ідентифіковані до видів: 1) до виду 936 віднесено 38.1 % фагів; 2) до виду P335 – 9.5 %; 3) до виду c2 – 19.0 %. Фаги *S. thermophilus* були ідентифіковані до трьох груп: 1) група *cos*-типу становила 14.3 % від загальної кількості фагів; 2) група *pac*-типу – 9.5 % і 3) 987 група – 9.5 % фагів.

Ключові слова: бактеріофаги, *Lactococcus lactis*, *Streptococcus thermophilus*, електронна мікроскопія, морфотип, мультиплексна ПЛР, білковий профіль.

БАКТЕРИОФАГИ *LACTOCOCCUS LACTIS* SSP. И *STREPTOCOCCUS THERMOPHILUS,* ВЫДЕЛЕННЫЕ НА МОЛОКОПЕРЕРАБАТЫВАЮЩИХ ПРЕДПРИЯТИЯХ УКРАИНЫ

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Резюме

Цель. Идентификация вирулентных бактериофагов *Lactococcus lactis* ssp. и *Streptococcus thermophilus*, выделенных на молокоперерабатывающих заводах Украины. **Методы.** Морфологию вирусных частиц изучали методом трансмиссионной электронной микроскопии. Идентификацию структурных белков фагов проводили с помощью гель-электрофореза в денатурирующих условиях. Таксономическое положение фагов определяли методом мультиплексной ПЦР. **Результаты.** Приведены результаты изучения биологических свойств 21 фага молочнокислых бактерий, выделенных на молокоперерабатывающих заводах Украины в течение 2007−2017 гг.

Показано, что фаги имели длинные, несокращающиеся хвосты и поэтому были классифицированы в порядок *Caudovirales,* семейство *Siphoviridae*. Фаги *L. lactis* ssp. отличались по форме головки: выявлены фаги с изометрическими головками морфотипа B1 и фаги с вытянутыми головками морфотипа B2. Все рассматриваемые фаги *S. thermophilus* имели морфологию, характерную для морфотипа B1. Некоторые из них имели атипичные короткие хвосты длиной 133−137 нм с базальными пластинами без глобулярных отростков. Изучен спектр литической активности различных фагов. Установлено отсутствие корреляции между показателями литической активности и морфологией вирусных частиц. Фаги *L. lactis* ssp. были очень гетерогенными и образовывали три группы по белковому составу. В частности, фаги вида c2 отличались тем, что имели мажорные белки с большей молекулярной массой − 40, 53 и 72 кДа. Фаги *S. thermophilus* содержали два или три мажорных белка с молекулярной массой 33 и 27 кДа; 42, 26 и 15 кДа соответственно. **Выводы.** Бактериофаги *L. lactis* ssp. в соответствии с их морфологией, белковым профилем и результатами ПЦР идентифицированы до видов: 1) к виду 936 отнесено 38.1 % фагов; 2) к виду P335 – 9.5 %; 3) к виду c2 – 19.0 %. Фаги *S. thermophilus* были идентифицированы в три группы: 1) группа *cos*-типа составляла 14.3 % от общего количества фагов; 2) группа *pac*-типа – 9.5 % и 3) 987 группа – 9.5 % фагов.

Ключевые слова: бактериофаги, *Lactococcus lactis*, *Streptococcus thermophilus*, электронная микроскопия, морфотип, мультиплексная ПЦР, белковый профиль.

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