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DEOXYRIBOZYMES IN DETECTION OF PATHOGENIC BACTERIA

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Aim. The purpose of the review was to analyze the use of DNAzyme biosensors for the detection of pathogens. In the recent years, deoxyribozymes (DNAzymes) have a significant impact as biosensors in diverse fields, from detection of metal ions in the environment to theranostic applications and detection of microorganisms. Although routinely used sophisticated instrumental methods are available to detect pathogenic bacterial contamination, they involve time-consuming, complicated sample pre-treatment and expensive instruments. As an alternative, pathogen-specific DNAzymes have demonstrated a series of advantages: a non-destructive rapid analysis technique with *in situ* and real-time detection of bacteria with high sensitivity and selectivity. A wide range of pathogen-specific DNAzymes has been developed using colorimetric and fluorescence-based detections for pathogenic bacterial contamination in various samples. The current review summarizes the *in vitro* selection of pathogen-specific DNAzymes, various strategies utilized in the sensor designs, and their potential use in theranostic applications.

Key words: pathogen, DNAzyme, biosensors, peroxidase mimicking DNAzyme.

The emergence of pathogen outbreaks imposes a significant threat to humankind and is responsible for global deaths, hospitalizations, and economic down growth. Therefore, early detection and prevention of these outbreaks areessential. Detecting and preventing pathogen outbreaks at the earliest possible stage requires highly sensitive, specific, and rapid analytical methods. Conventional detection methods such as cell cultures, enzyme-linked immunosorbent assay (ELISA) [1], and polymerase chain reactions (PCR) [2] are available to detect pathogens. Still, these methods are associated with drawbacks such as high time consumption, the requirement of expert knowledge, and highly sophisticated laboratories.

Furthermore, in the case of severe pathogen outbreaks, these techniques cannot be used to detect and prevent the spreading since the detection process requires a longer time to analyze the samples [3]. PCR-based detection methods are capable of detecting the pathogen bacteria in less time compared to the other methods. But it requires multiple sample preparation steps such as extraction, isolations, and purification of the bacterial DNA samples. The use of thermocycler for the PCR amplification makes it inconvenient to use it effectively. The detection of pathogen bacteria by ELISA is based on the specific antigen-antibody interactions and it provides a wide range of pathogen bacteria detection. Even though, ELISA contains drawbacks such as expensiveness in synthesizing antibodies and less sensitivity due to the cross contaminations. Therefore, compared to the conventional detection methods, effective analytical methods are required to detect and

prevent pathogen outbreaks earliest stage. As an alternative analytical method, DNAzyme based pathogen detection arose due to its ability to identify the target molecule rapidly, accurately, and effectively compared to the other available technologies.

Ronald R. Breaker and Gerald F. Joyce [4] discovered the first RNA cleaving DNAzyme (RCD) in 1994 via the *in vitro* selection method, which exhibits catalytic cleavage of the ribonucleotide linkage in the presence of Pb^{2+} ion. The discovery of the DNAzyme revolutionized the detection of metal contamination due to its remarkable catalytic activity. DNAzymes are isolated by the *in vitro* selection method using a catalytic strand consist of a DNA library containing 10^{14} random DNA sequences and the unique substrate strand embedded with a single ribonucleotide (rA) linkage. The incubation of specific metal ions with the DNAzyme induced the catalytic activity and promoted the ribonucleotide linkage's cleavage. The desired DNA fragment is isolated and purified by the biotin-streptavidin chromatography [5, 6] or denaturing polyacrylamide gel electrophoresis (dPAGE) [7, 8]. Finally, the PCR is carried out to amplify the cleaved DNA sequences and regenerate the DNA pool. The above procedures are repeated for 5–20 rounds until the activity of the DNA pool is sufficiently saturated with catalytically active sequences. At the end of the selection process, individual sequences in the collection will be cloned and sequenced to identify the secondary structure of the DNAzyme [9]. DNAzyme exhibits remarkable specificity for the DNA sequence of the substrate strand, and a single mismatch of deoxyribonucleotide affects the DNAzyme activity. Higher sensitivity, selectivity, signal amplification ability, and catalytic activity, thermal stability, the cost-effectiveness of the DNAzyme made it an exceptional recognition molecule for biosensing.

Initially, the activity of the RCD was investigated using the denaturing polyacrylamide gel electrophoresis (dPAGE) since the cleaved DNA fragment migrates a greater distance than the remaining DNAzyme due to the smaller size. However, the use of dPAGE is a time-consuming process and unable to detect the catalytic activity rapidly. Therefore, as an alternative method, researchers incorporated radioactive elements (^{32}P) and organic fluorophores into the DNAzyme strand for easy identification since nucleic acids are non-fluorescent biomolecules. However, the use of radioactive labels for the tagging process contains drawbacks due to its hazardous nature and limited lifetime [10]. Therefore, radioactive labels were replaced by organic fluorophores to overcome the disadvantages associated with the radioactive labels. However, organic fluorophores provide less sensitive detection compared to radioactive labels. Still, they provide several advantages over radioactive labels, such as the real-time monitoring of the ion concentration fluctuations due to its fast time scale and the non-radioactive behavior of the fluorophore facilitates easy disposal and waste management [11].

In the presence of the target molecule, the catalytic activity of the RCD is induced, and it facilitates the release of fluorophore-tagged DNA fragments due to the cleavage of the ribonucleotide linkage embedded in the substrate stand. The release of the fluorophore tagged DNA fragment generates a fluorescence signal used to detect the target molecule. The strategy of releasing fluorophore-tagged DNA fragments intrigues scientists' research interest, and this strategy was incorporated to develop novel fluorescence-based DNAzyme biosensors. Usually, fluorescent-based DNAzyme sensors were tagged with an external fluorophore and quencher molecule to act as fluorescence "Turn On/Turn Off" sensors upon the cleavage of the ribonucleotide linkage in the presence of a specific analyte [12]. Most of the DNAzyme based biosensors utilized the catalytic activity of the DNAzyme to detect metal ions. In the past few decades, novel DNAzyme based biosensors were developed, such as metal contamination detectors, cancer therapeutic drug delivery systems, etc. [13, 14].

The applications of DNAzymes are not confined to a particular field, and researchers are still exploring its capacity due to its unique characteristics. The recent advancement of biosensors based on DNAzyme to detect different pathogen bacteria proves that there is plenty of room available for the development of the DNAzyme based sensors. In the past decade, various types of DNAzyme based biosensors were developed to detect the *E. coli* bacteria contaminations which provide higher sensitivity and selectivity. However, the recent development of DNAzyme based sensors to detect other pathogen bacteria is promising due to their lower detection limits. Most of these pathogens cause severe health problems and affect the world economy. Therefore, to detect these pathogens' development of accurate, rapid analytical methods are

essential. The incorporation of the DNAzyme to detect pathogen bacteria provided a novel platform. Several biosensors were developed to detect *Escherichia coli, Klebsiella pneumoniae*, *Vibrio anguillarum*, *Helicobacter pylori*, *Cronobactersakazakii, Legionella pneumophila,* and *Salmonella*. This review analyses the recently developed DNAzyme based biosensors to detect pathogen bacteria, the different strategies utilized in the sensor designs, the importance of detecting pathogen bacteria, and how it affects global health and the economy.

Development of DNAzyme-based biosensors

Development of the DNAzyme based biosensors uses the two different activities exhibited by the DNAzyme known as catalytic activity and peroxidase mimicking activity. DNAzymes exhibiting catalytic activity (RCD) was utilized for the specific target recognition upon the ribonucleotide cleavage reaction. In the presence of the specific target, DNAzyme promotes DNA fragment release due to its catalytic activity. This released DNA fragment was utilized to incorporate signal transduction pathways such as fluorescence and colorimetry to detect the target easily. The development of the fluorescence-based DNAzyme sensor used the incorporating fluorophore/quencher systems, amplification of the released DNA fragment by rolling cycle amplification (RCA) [15] enzymatic process mediated by certain DNA polymerases in which long singlestranded (ss, and loop-mediated isothermal amplification technique (LAMP) [16]. Colorimetric-based DNAzyme sensors used the release of the urease-tagged DNA fragment to hydrolyze the urea, leading to colorimetric signals. Also, different types of signal transduction technologies were incorporated to develop novel DNAzyme based sensors. DNAzymes exhibiting peroxidase mimicking activity are usually used to develop the colorimetric sensors due to their peroxidase behavior.

This peroxidase activity mainly depends on the formation of the G-quadruplex (G4) from the guanine-rich DNA sequence. Upon binding to the hemin, G4 exhibits the peroxidase activity. It is capable of catalyzing the oxidation of H_2O_2/2,2′-azino-bis diammonium salts ($ABTS^{2-}$) to ($ABTS^{\cdot+}$) and H_2O_2/3,3′,5,5′-tetramethylbenzidine sulfate (TMB) to oxTMB to generate a color change which can be observed by the naked eye [17]. G4 were extensively explored as molecular tools in different fields for various applications to develop recognition elements. However, DNAzyme based biosensors are mainly focused on the RCD due to its target specificity, selectivity, easy modifications, ability to act as a molecular recognition element, design convenient signal transduction systems, and rapid response upon the incubation of the target.

Specificity and selectivity of RNA-Cleaving DNAzyme

Instead of utilizing metals to isolate specific RCD, alternative strategies were developed to isolate RCD specific towards a nundefined, small molecular target/cellular mixtures. To date, various successful approaches were reported about the *in vitro* selection of RCDs specific to bacteria by using the crude extracellular materials (CEM) of the bacteria as the candidate to trigger the cleavage of the ribonucleotide linkage of the DNAzyme. The successful isolation of highly specific and selective RCD depends on the negative and positive selection steps. Initially, in the negative selection step, the DNAzyme library will be incubated with the nonspecific bacterial CEM. The cleaved fragments will be discarded, and uncleaved library fragments will be isolated and purified for further selection. In the positive selection, uncleaved library fragments from the negative selection are incubated with desired bacterial CEM, and cleaved DNA library fragment is isolated and amplified. This cycle is repeated several times to achieve highly specific and selective RCDs. The specificity and selectivity of the pathogen bacteria-specific RCDs mainly depend on the two factors: the catalytic core of the RCD and the triggering factor. According to Table 1, the catalytic strand sequence of the different RCDs exhibits unique sequences specific to the different pathogen bacteria. The sequence analysis provides evidence that the selectivity and specificity are based on the catalytic core of the DNAzyme. Experiments were carried out to identify the triggering factor in the CEM. It was found that a specific protein is responsible for the induction of the catalytic activity of the RCD. Scientists also identified the trigger protein size to be around 30–100 kDa, and the catalytic activity is not based on the ribonucleases (RNases) activity. The mechanism and interaction associated with the cleavage of ribonucleotide linkage upon the CEM incubation is still a mystery and yet to be identified. The protein tertiary structure and

its functional groups may play an essential role in cleaving the ribonucleotide linkage of the DNAzyme. Since catalytic cores of the different DNAzymes are unique, it can be hypothesized that the tertiary structure of the specific protein induces the active DNAzyme and promotes catalytic activity.

Escherichia coli detection *via* DNAzyme

Escherichia coli (*E. coli*) is a gram-negative, rod-shaped, nonsporulating, facultatively anaerobic bacteria that reside in the lower intestines of humans and animals. Most *E. coli* strains are harmless, and the symbiotic relationship provides aid for the digestive process and keeps the digestive tract healthy for humans and animals [3]. However, few serotype strains are capable of causing respiratory illness, diarrhea, and urinary tract infections. Usually, the detection of *E. coli* infection is carried out using culture-based methods or PCR, fluorescently tagged enzymes, monoclonal antibody labeled gold-nanoparticles, enzyme-linked immunosorbent assay (ELISA), Laser-induced fluorescence coupled with flow cytometry, microarrays, and molecular beacon (MB) [23–25]. Even though these methods provide sensitive detection of the bacteria, certain drawbacks such as time consumption, low selectivity, sensitivity, and accuracy due to the cross contaminations, cost-ineffectiveness, and highly labor-intensive due to the pre-treatment process are observed [23, 24]. Therefore, it is essential to develop sensors to detect bacterial contaminations more rapidly and effectively to prevent *E. coli* outbreaks. Recent developments of DNAzyme based sensors attracted scientists to develop DNAzyme based biosensors to detect microorganisms. Ali and co-workers [3] initiated the development of DNAzyme based fluorescent sensors to detect *E. coli* contamination. Later, many research groups followed Ali and co-workers'footstepsand developed different biosensors utilizing various techniques to see not only *E. coli* but alsos everal other pathogenic bacteria. These biosensors exhibited promising detection of the bacteria compared to the conventional methods [3, 23–25].

Fluorescence-based DNAzyme sensing of the *E. coli*

Ali and co-workers [3] used the principle behind the fluorescence-based DNAzyme biosensors and developed a "mix and read" type fluorescent-DNAzyme based sensors to detect bacteria. Instead of using specific metal ions,the research group used the crude extracellular mixture (CEM) obtained from the *E. coli* K12 non-pathogen bacteria for the *in vitro* selection of the DNAzyme, as shown in Fig. 1, *A*. An *E. coli* specific DNAzyme, RNA cleaving fluorogenic DNAzyme-EC1 (RFD-EC1), was isolated and reported as a *cis*-acting DNAzyme where the substrate is covalently bound to the DNAzyme catalytic strand. The RFD was modified by incorporating fluorophore (F) and quencher (Q) molecules, and this F/Q pair flanked the ribonucleotide

Table 1. **Catalytic strand sequence of the different pathogen bacteria-specific RCDs. The catalytic core sequences are denoted in red color**

DNAzyme	DNA Sequence of the DNAzyme catalytic strand	Ref
RFD-EC1	5′-CACGGATCCTGACAAGGATGTGTGCGTTGTCGAGACCTGCGACCGGAACA CTACACTGTGTGGATTTCTTACAGTTGTGTGCAGCTCCGTCCGACTCTTCCTA GC FRQ GGTTCGATCAAGA-3′	[3]
VAE-2	5′-TTTCGCCATCTTAGCGAAGCGGGTGGTATCGCAGATGGGAGCTGAGTAAA CGTAGTGACGGTAAGCTT-3′	[18]
DHp3T4	5′ATGCCATCGATGGTCTTTGGTATGTGGGGTCCGAGGGTAGAGCTCTGAACT CGTTTTTTTTTTB-3′	[19]
RFD-KP6	5′-ATGCCATCCTACCAACCATGACTGGTTTGTACTAAGAGATTTCAGGCATCG CTGCACGTCGTAGGTGAGCTCTGAACTCG-3′	[20]
LP1FQ	5′-CTATGAACTGAC QRFGACCTCACTACCAAGCAAGCATGGACAATACCGA GCCTTTCATTTCAGCCGATCATACCTCAATGTAGATAAGCACATCTTGTCATC GGAGGCTTAG-3′	[21]
DAh1T1	5′-GAAAAGCGGTCTGCTGCGCTTCTTCCTCTAGTCTGTATACCTATGTTCACT TATGAGCGA-3′	[22]

Abbreviations: Fluorophore — F; Quencher — Q; Ribonucleotide — R; Substrate sequence — underlined.

linkage. To remove the nonspecific cleavage of the DNAzyme, the DNA library was initially incubated with the CEM extracted from *Bacillus subtilis*. The uncleaved sequences were incubated with the CEM obtained from the *E. coli* K12 (CEM-EC), enhancing the specificity and sensitivity of the DNAzyme towards the *E. coli* K12 bacteria. The specificity of the DNAzyme towards the *E. coli* K12 was investigated by incubating the CEM extracted from nine gram-negative pathogen bacteria and five gram-positive bacteria. None of them could activate the RFD-EC1 and facilitate the cleavage of the ribonucleotide linkage. Therefore, RFD-EC1 was shown to be highly specific for the *E. coli* K12 [3]. Ali and co-workers treated the CEM extracted from *E. coli* K12 with two types of protease enzymes known as trypsin and proteinase K to identify the RNA cleavage triggering factor of the CEM. It was found that the protease treated CEM was unable to activate the DNAzyme and suggested that the triggering factor was a protein. Further investigations were carried out to identify whether the cleavage activity was due to the ribonucleases (RNases) in the CEM. However, the cleavage activity of the DNAzyme was unaffected when CEM was treated with RNase inhibitors indicating that the cleavage activity of the DNAzyme was independent of the RNases activity.

Ali and co-workers utilized molecular sizing columns (3–100 kDa) to separate and isolate the protein responsible for the cleavage activity of the DNAzyme and reported that the filtrate from the 50kDa and 100 kDa successfully cleaved the ribonucleotide linkage of the DNAzyme, indicating that the triggering protein is in the range of 30–50 kDa [3]. The detection of *E. colivia* RFD-EC1 still requires the culturing step, and RFD-EC1 could detect a single colony-forming unit (CFU) with a robust signal after a minimum of 12 h culturing step. However, compared to the other methods such as immunoassay, PCR, and phenotypic identifications, RFD-EC1 based method is faster. In 2013, Aguirre and co-workers [24] developed a novel DNAzyme-based fluorescent assay by optimizing the previously reported RFD-EC1 DNAzyme. The *cis*-acting RFD-EC1 DNAzymewas modified to a *trans*-acting RFD-EC1 (EC1T/FS1) by detaching the substrate strand covalently attached to the catalytic strand of the DNAzyme. The modified DNA provides the advantages such as cost-effectiveness, ease of synthesis, and inexpensive modifications. Since the RFD-EC1 DNAzyme was isolated by *in vitro* selection by utilizing the CEM from *E. coli*, the possibility of achieving the cleavage activity of *trans*-acting RFD-EC1 via the crude intracellular mixture (CIM) was also investigated. Cleavage activity of the EC1T/FS1 with CIM was increased by 45% compared to CEM. Aguirre and co-workers identified super optimal broth and super optimal broth with catabolic repressor as effective growth media to extract the CIM. The CIM extracted from these culture media enhanced the

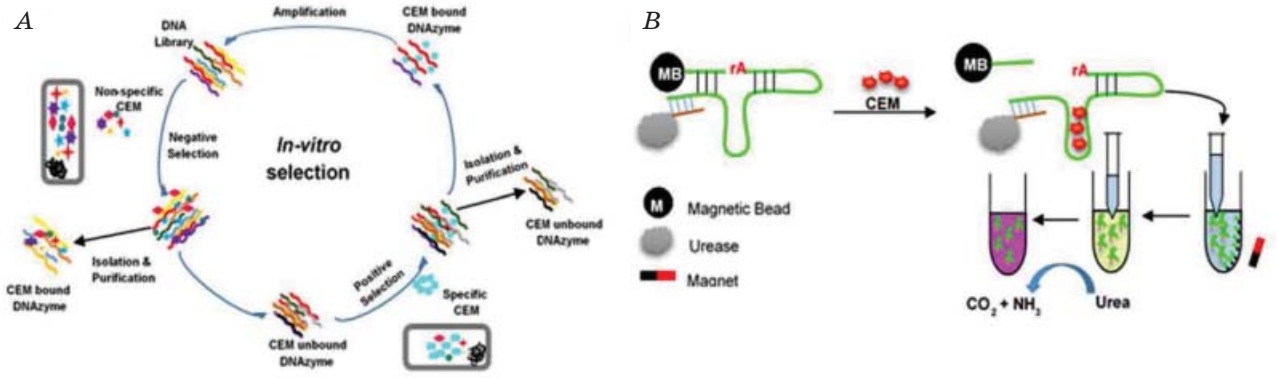

Fig. 1. ***A* — Schematic illustration of the *in vitro* selection of bacteria-specific DNAzyme using CEM**

In this process, CEM extracted from the bacterial culture was utilized as the specific analyte. It was incubated with the DNAzyme library to isolate novel DNAzyme specific to the bacterial CEM

***B* — Schematic illustration of the colorimetric detection of bacterial contamination [26]**

Upon the incubation of the specific CEM with the DNAzyme, cleaved DNAzyme fragments were purified by magnetic separation. The remaining DNAzyme fragments containing the urease enzyme hydrolyzed the urea into ammonia. This conversion results in increased pH in the medium, which was reported by the color change of the phenol red

cleavage activity of EC1T/FS1 DNAzymeby 26% compared to the other culture broth media [24]. Therefore, the nutritional factors in the growth media affect the cleavage activity by inducing bacterial growth, reducing growth time, and facilitating the rapid detection of *E. coli* compared to the other methods.

The specificity of the EC1T/FS1 for the *E. coli* was further investigated by incubating the CEM extracted from several gram-negative and gram-positive bacteria such as *P. peli*, *Y. rukeri*, *H. alvei*, *A. xylosoxidans*, *L. mesenteroides*, *L. planturum*, *P. acidilactici* and *B. subtilis*. However, the cleavage activity showed that no pathogenic bacteria could cleave the ribonucleotide linkage in the DNAzyme except the *E. coil*, proving that the trans-acting DNAzyme is highly specific for *E. coli*. The optimized assay could detect 10^3 CFU of *E. coli* without the culturing step and detect 1 CFU within a shorter time than the earlier version, which requires 12 h culturing of the sample to achieve a signal [24].

In 2019, Cao and co-workers [25] reported a simple, cost-effective bacterial detection method for *E. coli* based on the modified DNAzyme. Compared to the earlier DNAzyme based methods, Cao and co-workers used a molecular beacon (MB) as the signal molecule to report the cleavage activity of the DNAzyme. The DNAzyme was modified by removing the 5′ end primer binding site and using an intact single DNA strand consisting of the catalytic and substrate strands (s-DNAzyme-*E. coli*). The ribonucleotide linkage was placed within the substrate strand. The MB loop was designed using the complementary sequence of the cleavage site of the substrate strand (MB-rA). The 5′ and 3′ end of the MB were modified with the fluorophore and quencher molecules. In the presence of the s-DNAzyme *E. coli*, MB-Ra was hybridized with the complementary sequence and facilitated the loop's opening, enhancing the fluorescence emission. The CIM of *E. coli* induced the cleavage of the s-DNAzyme *E. coli* and facilitated the release of MB-rA. Since MB-rA consists of a partial substrate sequence, it cannot remain in open form due to the cleavage of the ribonucleotide linkage. Therefore, MB-rA reassumes the loop shape and causes the fluorescence turn-off due to the close proximity of fluorophore and quencher [25].

Most of the previously developed fluorescence-based DNAzyme detection methods use fluorescence emission due to the cleavage of the ribonucleotide linkage. Still, the detection of fluorescence emissionsis difficult. In contrast, the quenching of the fluorescence is more distinguishable and can be utilized to develop sensitive sensors. Also, compared to the traditional DNAzyme, the molecular beacon method is cost-effective and simplifies the overall detection process, providing a platform for developing culture-independent bacteria detection *via* novel DNAzyme based sensors.

Paper-based DNAzyme sensing of the *E. coli*

Tram and co-workers [26] further developed the RFD-EC1 DNAzyme to develop a colorimetric detection of *E. coli* contamination. The enzyme urease was used as the signal transducer due to its hydrolytic activity to convert urea into carbon dioxide and ammonia. A sensor was designed by using litmus dyes. Tram and co-workers modified the RFD-EC1 DNAzyme into a 5′-biotinylated DNAzymeby biotinylation process, and 3′ end of the DNAzyme was modified by sequence extension. The attachment of the biotin facilitates the immobilization of the DNAzyme onto the streptavidin-coated magnetic beads, which provide the easy separation of DNA. The 3′ sequence extension facilitates the hybridization of urease conjugate DNA oligonucleotide. The urease enzyme was conjugated onto the DNA oligonucleotide via the maleimidobenzoic acid N-hydroxysuccinimide ester (MBS) linker molecule. The simple mixing of the modified DNAzyme attached magnetic bead swith the urease conjugate DNA oligonucleotide induced the formation of functional DNAzyme. The formed functional molecule releases the urease conjugate DNA oligonucleotide in the presence of the CEM extracted from the *E. coli* due to the cleavage of the ribonucleotide linkage. After the magnetic separation, the cleavage solution was treated with urea containing solution with phenol red. As indicated in Fig. 1, *B*, hydrolysis of urea in the solution facilitates the release of ammonia. It results in increased pH in the medium, and this conversion is indicated by the color change from yellow to red in the solution. The design of the functional DNAzyme can further be used with other dyes such as bromothymol blue, neutral red, cresol red, m-cresol purple, and o-cresolphthaleincomplexone [26] since all of them are sensitive to the pH.

The functional DNAzyme was further studied using commercially available litmus papers and discovered that a color change could

be observed within 10 minutes for the *E. coli* sample and detect $5 \cdot 10^5$ bacteria cells that are on par with the detection limits of the PCR and ELISA [26]. Furthermore, detecting the single CFU of bacteria by the culture/litmus-based method provides rapid results compared to the PCR and ELISA. Compared to the other DNAzyme based *E. coli* detection methods, the current method provides advantages such as rapid detection, fewer sample preparation steps, cost-effectiveness, and efficient detection of bacterial contamination without high-end equipment such as fluorimeter, PCR machine as the detection can be carried out using litmus paper. The color change is observable by the naked eye [26].

Nanotechnology-based DNAzyme sensing of the *E. coli*

The recent development of nanoscience and nanotechnology provided a novel platform for developing cost-effective biosensors with higher sensitivity to detect microorganisms via their unique physical and chemical characteristics. Usually, most of the nanomaterial-based sensors utilize the bio-conjugation strategy to attach the biomolecules onto the nanomaterials. However, these techniques are expensive, time-consuming, and irreproducible. Further studies showed that graphene could form non-covalent bonds with biomolecules such as DNAzymes, antibodies, and aptamers. The sp^2-conjugated sites of the graphene facilitated the interfacing of nucleic acid on the graphene sheets. In 2018, Liu and co-workers [27] incorporated the same technology to develop a novel DNAzyme based fluorescent sensor to detect the *E. coli* K12 bacteria, as shown in Fig. 2, *A*. Liu and co-workers used the previously isolated *E. coli* specific RNA cleaving DNAzyme (RCD-EC) and graphene to develop the senor. The hybrid sensor performed self-assembly due to the non-covalent adsorption of RCD-EC on the graphene and resulted in low fluorescence intensity due to the super quenching ability of the graphene. In the presence of *E. coli* CEM/CIM, the cleavage reaction of the DNAzyme was induced and resulted from the release of fluorophore tagged DNA fragments. The release of the DNA fragment enhanced the fluorescence intensity drastically.The sensor's limit of detection (LOD) was estimated as 10^5 CFU/mL in CEM and 10^4 CFU/mL for the CIM extracted from the *E. coli*. Earlier studies showed that a specific protein range from 30–100 kDa is responsible for the cleavage activity of the RCD-EC, and the tenfold sensitivity occurs due to the abundant availability of the triggering protein inside the cell compared to the crude extracellular mixture isolated from the culture. The hybrid sensor can detect *E. coli* in complex matrices such as blood with a LOD of 10^5 CFU/mL [27]. The quenching molecule has been removed from the DNAzyme sequences in the sensor compared to the earlier fluorescence-based methods. It provides advantages such as cost-effectiveness, higher sensitivity due to the super quenching of the graphene, less chemical consumption due to the self-assembly of the nucleic acid and graphene. A fluorescence enhancement was utilized to detect *E. coli* and can be further modified to develop a fluorescence quenching sensor using graphene due to its super quenching properties and incorporating the rolling circle amplification (RCA) technique to enhance the detection of bacteria.

Zheng and co-workers [28] reported a novel strategy to detect *E. coli* via a specific DNAzyme as the recognition molecule and the DNA templated fluorescent silver nanoclusters (AgNCs) as the reporter molecule. As shown in Fig. 2, *B*, the DNAzyme was modified by covalently attaching acetylcholinesterase (AChE) and attaching to the magnetic beads forming a complex (MNP-DNAzyme-AChE). Cleavage of the ribonucleotide linkage of the substrate strand was induced in the presence of the CEM-EC and facilitated the release of the AChE into the solution. After the magnetic separation, free AChE was transferred into the solution containing Acetylthiocholine (ATCh) iodide and DNA-templated AgNCs. The AChE catalyzes the hydrolysis of ATCh to thiocholine (TCh). The formation of the TCh enhances the fluorescence emission of the DNA-templated AgNCs via the silver-sulfur bond formation.

The individual fluorescence intensity measurement at 635 nm proves that ATCh cannot improve the fluorescence without the AChE. The LODis 60 CFU/mL compared to the other methods, and it can even detect *E. coli* ranging from $1 \cdot 10^2$ CFU/mL to $1 \cdot 10^7$ CFU/mL [28]. The current design could be used as an ultrasensitive fluorescence turn-on sensor to detect *E. coli*. However, this method contains several steps, such as separation and transfer; therefore, it cannot be used as a rapid method to detect *E. coli* contaminations. However, this novel strategy can be utilized to develop a versatile microorganisms detection tool.

Following the previous research on DNAzyme based detection of *E. coli*, Zhou and

co-workers[29] developed an ultrasensitive method to detect *E. coli* O157:H7, capable of causing diarrhea and hemorrhagic enteritis. An ultra-sensitive magnetic sensor was developed by a novel strategy to improve the sensitivity and signal amplification via triple signal amplification (1) DNAzyme detection, (2) RCA amplification, (3) copper nanoclusters (CuNCs) formation to achieve ultra-sensing of *E. coli*. The *E. coli* O157:H7 specific DNAzyme was isolated via *in vitro* selection and hybridized with a supportive chain modified with biotin to facilitate the immobilization of the DNAzyme on the magnetic beads by using biotin-streptavidin bio-conjugation. The incubation of CIM extracted from *E. coli* with the DNAzyme induced the cleavage reaction. After the magnetic separation, the remaining DNAzyme fragments facilitate the RCA reaction to form thymine (T) rich sequences (poly-T). These sequences provide the specific sites to facilitate the formation of CuNCs in copper sulfate and sodium ascorbate. CuNCs emit red light under the ultraviolet (UV) irradiation (345 nm), and the fluorescence intensities were used to analyze the *E. coli* O157:H7 contamination in the sample quantitatively. The LOD of 1.57 CFU/mL in 1.5 h was reported for the novel sensor with an excellent linear range from 10·1 000 CFU/mL. The sensor could detect the *E. coli* O157:H7 contaminations in drinking water and apple juice [29].

DNAzyme and rolling-circle amplification (RCA) based detection of *E. coli*

Signal amplification of the biosensors is essential to enhance sensitivity and LOD. Biosensors' activity is usually based on the receptor-ligand interactions and is not highly sensitive to detect the contaminant in the environment due to low concentrations.

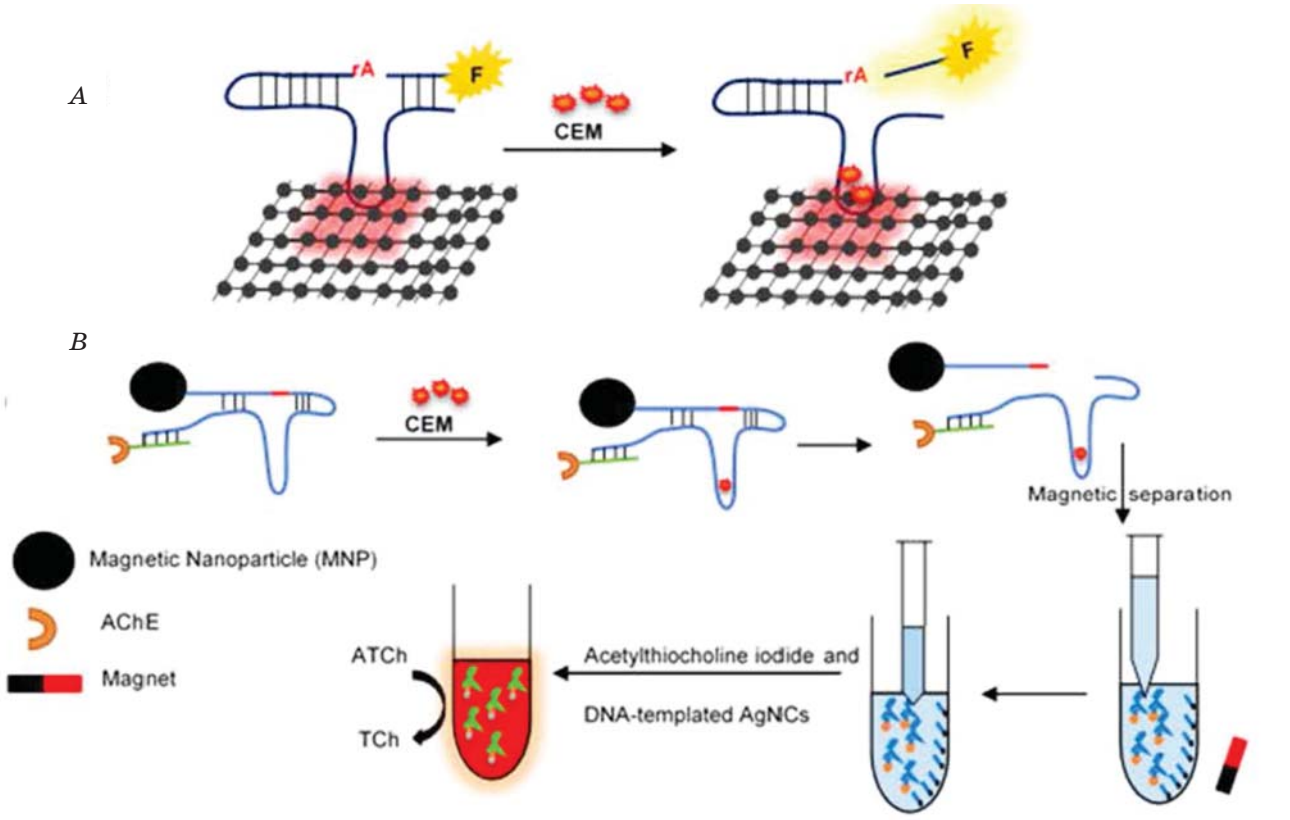

Fig. 2. A — **Schematic illustration of the graphene-based DNAzyme biosensor to detect *E. coli* bacteria [27]**
Attachment of the DNAzyme onto the graphene quenched the fluorescence emission of the fluorophore. Upon the cleavage reaction,the release of fluorophore tagged DNA fragment enhanced the fluorescence emission, suddenly causing a detectable signal

B **— Schematic illustration of the DNA-templated fluorescent silver nanoclusters sensor integrated with MNP-DNAzyme-AChE complex for detecting *E. coli* bacteria [28]**
Upon the incubation of the specific CEM with the DNAzyme, cleaved DNAzyme fragments were purified by magnetic separation. The remaining DNAzyme fragments containing the AChE enzyme convert the ATCh into TCh, which enhances the fluorescence emission of fluorescent AgNPs

Therefore, to detect these contaminations, culturing step is required to increase the pathogen's concentration, which causes a delay in the early detection of the pathogen microorganisms via biosensor. Traditional *in vitro* DNA amplification techniques such as PCR provide powerful amplification of the target. Still, it requires expensive instruments, reagents, different thermal conditions, trained personnel, and it can produce false readings due to nonspecific bindings.Therefore, rolling circle amplification (RCA) [15] of DNA attracted the interest of researchers due to its unique characteristics such as isothermal process and simplicity, as shown in Fig. 3, *A*. Here, a particular DNA polymerase (phi29 DNA polymerase (PolΦ29) is used to extend the short DNA primer around the circular DNA template several times to generate a single-stranded DNA sequence (ssDNA). Liu and co-workers [30] developed a DNAzyme feedback amplification strategy to detect *E. coli* using RCA technology with the SYBR Gold.

The design contains three major components; 1) short DNA primer (DP), 2) Circular DNA template (CDT) integrated with the antisense sequence of the RNA cleaving DNAzyme catalytic strand (RCD), 3) RNA cleaving DNAzyme substrate strand (RDS) where the 5′ end modified with a sequence similar to the DP. The hybridization of these sequences generates two complexes designated as complex I (DP and CDT hybrid) and complex II (RDS and CDT hybrid). Formation of the complex I induces the generation of long RCA products containing RCD repetitive sequences in the presence of PolΦ29. The resultant RCD hybridized with the 3' end of the RDS. In the presence of *E. coli*, the cleavage reaction occurs and facilitates CDT formation with a 5′ cleavage fragment. The PolΦ29 polymerase removes the unpaired deoxyribonucleotide from the cleaved DNA fragment and enables the complex I, which is used to feedback the RCA process to amplify the DNA. The RCA product (RP) generation was measured using the SYBR gold stain, which generates enhanced fluorescence emission upon the binding to the ssDNA. RCA-based DNAzymescan detect *E. coli* with a LOD of 10 CFU/mL using a 60 min reaction time and exhibit 1 000-fold improvement compared to the other DNAzyme signal amplification methods. The detection time is significantly reduced in RCA-based DNAzyme detection for *E. coli* than the different DNAzyme based approaches. Furthermore, RCA-based DNAzyme autonomously converts small molecular recognition signal into a distinct signal due to its amplification process and causes higher sensitivity and simplicity to detect contamination in the environment [30].

Paper/RCA based DNAzyme detection of *Escherichia coli*

Paper-based analytical devices to detect bacterial contamination [26] recently received more attention due to their simplicity, user-friendliness, cost-effectiveness, and rapid detection. Scientists have already developed some DNAzyme based paper sensors, and

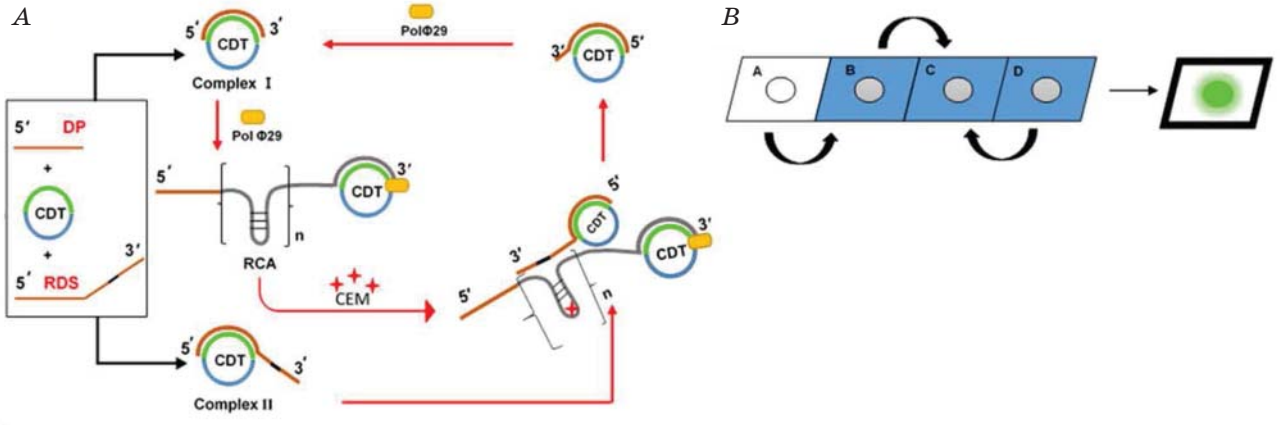

Fig. 3. ***A* — Schematic representation of RCA-based DNAzyme detection of *E.* coli [30]**

In this process, the desired DNA fragment was isothermally amplified repeatedly to synthesizethe ssDNA molecule.

***B* — Schematic illustration of the origami paper device (oPAD) [31]**

In this design, folding of the paper sensor in the correct sequence provides the colorimetric detection of the *E. coli* K12

these sensors require the pre-treatment of the sample for the analysis. Sun and co-workers studied and produced a fully integrated paper-based analytical device known as origami paper device (oPAD)to detect *E. coli* K12 by utilizing previously isolated EC1 DNAzyme [31]. The design of the sensor consists of 4 different panels; Panel A: an adsorbent pad for the purification and washing, Panel B: Whatman 3MM CHR chromatography paper for the *E. coli* cell lysis, Panel C: Whatman filter paper for the immobilization of the 3D-EC1 DNAzyme, Panel D: Nitrocellulose membrane for the RCA process as shown in Fig. 3, *B*. The folding of Panel B onto Panel A facilitates the extraction of proteins from the cell lysate directly. Upon the contact of Panel B and Panel C, the purified protein is transferred to Panel C and mediate the cleavage reaction of 3D-EC1 DNAzyme and facilitates fluorophore tagged DNA substrate release. The research group used the 3D DNA developing strategy discovered earlier to synthesize the 3D-EC1 DNAzyme [32]. The RCA-associated process was used to develop the 3D nanostructure; initially, a circular DNA template (CDT) containing anti-EC 1 sequence was synthesized. It was subjected to the RCA process to amplify the sequence. The resultant RCA products were aged 12 h at room temperature to assemble into 3D nanostructure of the DNA.

The 3D-DNA nanostructure was printed on Panel C, and after the immersion in a blocking buffer, the bioactive paper was dried at room temperature. After that, the fluorophore tagged RNA substrate (F-RS28) was mixed with pullulan solution and printed on Panel C. The cleaved 5′ fluorogenic DNA fragment migrates from Panel C to Panel D and acts as a DNA template for the RCA process. The RCA process facilitates the formation of repetitive units of peroxidase-mimicking DNAzyme known as PW17, and it facilitates the oxidation of chromogenic 3,3,5,5′-Tetramethylbenzidine (TMB) in the presence of hemin and H_2O_2. The oxidation of TMB generates a colorimetric signal which can be observable by the naked eye. The research group reported that the sensor could detect *E. coli* with a 10^3 CFU/mL LOD within 35 min [31]. Compared to the other DNAzyme based methods, the 3D-DNA nanostructure strategy provides rapid, cost-effective, user-friendly, and susceptible bacterial detection. Also, the colorimetric changes can be observed from the naked eye. Therefore, sophisticated instruments for the analysis are not longer required. Since the sensor was based on paper, the discarding of the sensor did not affect environmental pollution.

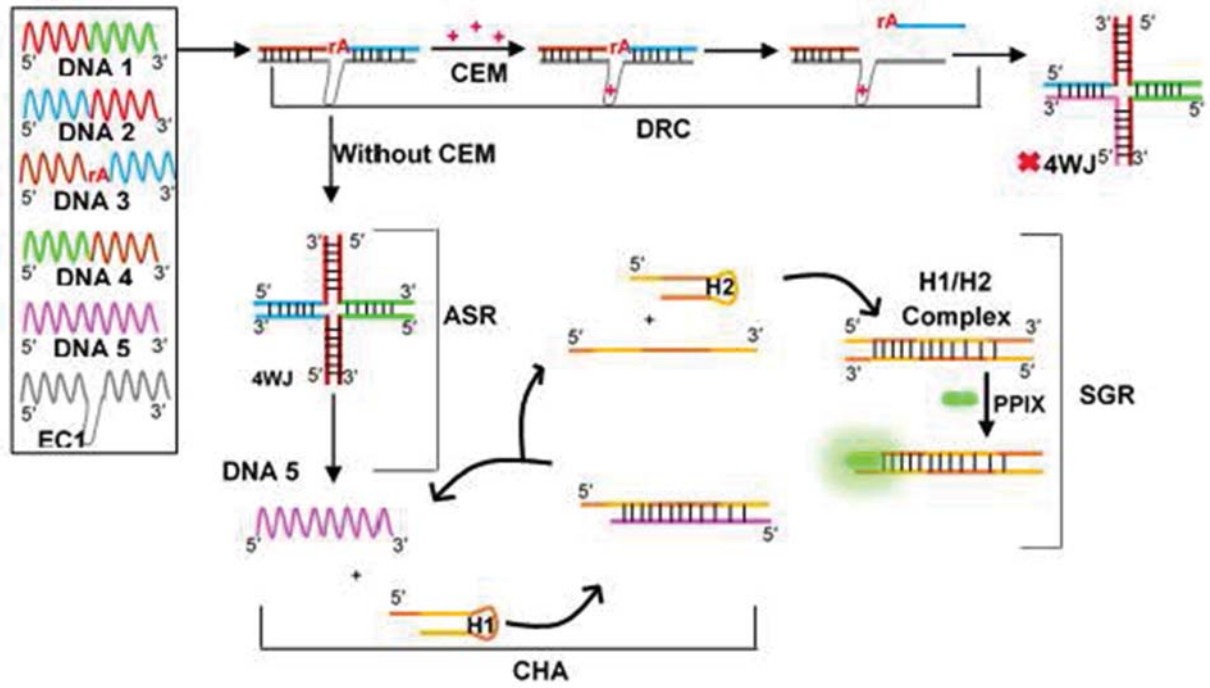

Fig. 4. **Multi-component all-DNA biosensing system based on DNAzyme for *E. coli* detection [33]**

Upon the incubation of CEM extracted from *E. coli*, the formation 4-WJ complex facilitates the formation of the H1/H2 complex, and the binding of the PPIX enhances the fluorescence emission leading to the detection of the *E. coli* K12

DNAzyme based amplification system for the detection of *E. coli*

Early detection of bacterial contaminations consists of certain drawbacks: inefficient detection due to the low bacterial concentration, cross-contamination, etc. Therefore, different types of amplification methods, such as PCR and RCA, have been used to enhance the detection limit of biosensors. Due to pre-treatment samples, specific reagents, thermal conditions, and instruments, these amplification methods impose some difficulties in practical applications. Zhou and co-workers [33] developed a novel amplification strategy by utilizing a multi-component DNA system to detect the *E. coli* bacteria contamination, as shown in Fig. 4. The system was integrated with different reactions such as (1) DNAzyme mediated RNA cleavage (DRC), (2) assembly-mediated strand release (ASR), (3) catalytic hairpin assembly (CHA), and (4) split G-quadruplex reassembly (SGR).

Previously isolated *E. coli* specific EC1 DNAzyme (RCD) and the fluorophore tagged substrate (RDS1) were used as the bacterial recognition elements. A four-way junction (4WJ) was designed using five different DNA sequences named DNA 1–DNA 5. DNA-4 was partially hybridized with DNA-5. The unpaired fragment of the DNA-4 consists of the complementary sequences to facilitate the hybridization of DNA-3. In the absence of *E. coli* CEM/CIM, hybridization of DNA-3 (RDS1) on DNA-4 facilitates the release of DNA-5 forming 4WJ. The released DNA-5 acts as a catalyst for the CHA by hybridizing with the tail containing hairpin-shaped DNA molecule (H1) and facilitating the loop's unfolding. The unfolding process exposes its sequence for the hybridization process. Another tail contains a hairpin-shaped DNA molecule (H2) bound with the exposed sequence of the H1 and forms H1/H2 complex by releasing DNA-5. Therefore, DNA-5 acts as an input signal for the formation of the H1/H2 complex. The accumulation of the H1/H2 complex increased with time due to the cyclic CHA process. Both H1 and H2 were designed to undergo intra-complex interaction to form an SGR, and it interacts with the protoporphyrin IX (PPIX) and enhances the fluorescence emission. However, in the presence of the target (*E. coli* CEM/CIM), the cleavage of the DNA-3 prevents the formation of 4WJ and terminates the entire amplification process. Therefore, the formation of the 4WJ complex acted as a "Turn on or Turn Off" switch for the CHA process and demonstrated a LODof 50 CFU/mL within 85 minutes for *E. coli* bacteria [33].

The RCA-based isothermal amplification method emerges as an alternative to PCR due to its rapid, cost-effective, and efficient amplification of nucleic acid sequences at a constant temperature. The isothermal amplification method omits the thermocycling requirement in PCR, such as denaturing, annealing, and extension, and this reaction can be performed under normal conditions without specific instruments. Since, 1990's ample isothermal amplification techniques have been developed for nucleic acids, cells, ions, and protein detection. Isothermal amplification is categorized into two sub-categories such as enzyme base amplification and enzyme-free amplification. Enzyme-based isothermal amplification uses nucleic acids and enzymes such as polymerase, exo-nuclease for the recycling process. The isothermal amplification method is utilized in various fields to develop nanomaterials for bio-imaging and bio-sensing applications. DNAzyme based isothermal amplification strategy has facilitated the platform to generate DNA amplicons faster without using specific enzymes. Compared to the other DNAzyme amplification-based methods, DNAzyme based isothermal amplification strategy provides rapid, cost-effective, sensitive, highly effective, and easy amplification detection of the bacteria. However, the method of detection can further be improved with the incorporation of modifications such as the conversion of the fluorescence detection to a colorimetric detection by utilizing the peroxidase activity of the DNAzyme, designing the DNA 1-5 with different RCD sequences specific for a particular pathogen for the effective detection of multiple bacteria using a single sensor.

Helicobacter pylori detection *via* DNAzyme

Among the pathogenic bacteria, *Helicobacter pylori* (*H. pylori*) infection affects more than 50% of the global population. Gastric *Helicobacter* species can adapt to the harsh environment found at the gastric mucus layer and are mainly responsible for gastric carcinoma and diseases such as circa, peptic ulcers, and MALT lymphoma. *H. pylori* is a gram-negative, microaerophilic, spiral-shaped bacterium. However, *H. pylori* can exhibit different shapes such as coccoid and rod shapes. *H. pylori* is genetically heterogeneous and lacks the cloning ability compared to the other microorganisms [34]. In the gastric environment, *H. pylori* exhibit urease activity

and survive in the highly acidic (pH = 1) gastric juice. The flagella regulate pathogen movements and allow rapid migration toward the neutral pH of the gastric mucus layer. The spiral shape and flagella activity facilitates the penetration of the pathogen bacterium into the mucus layer. In the mucus layer, pH neutral environment promotes the growth and colonization of the gastric *Helicobacter* species. Penetration of *H. pylori* weakens the protective mucus coating layers of the stomach and duodenum and causes the gastric acid to drift through mucus coating to the sensitive lining tissues. Therefore, these sensitive linings get irritated and develop sore or ulcers in pathogen bacteria and gastric acid [34, 35].

Diagnostic methods of *H. pylori* can be categorized as invasive and non-invasive. The invasive detections contain histology, culture biopsy, rapid urease test (CLO), and PCR. Although histology is the gold standard to detect pathogen infection, these techniques require expert pathologists, antibodies, and additional confirmation tests. Even though these detection methods provide sensitive results, these techniques are time-consuming and expensive. Non-invasive methods include urea breath test, fecal antigen test, and serology test [35]. The urea breath test is considered an alternative gold standard test to detect pathogen infection, which provides reliable information to evaluate the success of the treatment of *H. pylori*. However,using a urea breath test is limited due to the requirement of sophisticated instruments and the production of false results due to low sensitivity. Therefore, developing rapid, sensitive, and accurate analytical methods to detect *H. pylori* is essential [34, 35].

Ali and co-workers [19] developed an *H. pylori* bacterium-specific RNA cleaving DNAzyme via *in vitro* selection. The CEM from the *H. pylori* (CEM-HP) was used to isolate a new DNAzyme, DHp3T4. The catalytic activity of DHp3T4 was tested using *Escherichia coli* O157:H7, *Clostridium difficile, Salmonella, Typhimurium, Bacillus subtilis* and *Listeria monocytogenes*. DHp3T4 was highly specific to the *H. pylori* pathogen, and the other pathogenic CEM mixtures were unable to cleave the ribonucleotide linkage within the RNA/DNA chimera. Ali and co-workers further studied the *H. pylori* CEM to discover the triggering factor responsible for the cleavage of the DNAzyme. The CEM-HP was denatured at high temperatures providing evidence that the triggering factor is a protein. Another study also indicated that the cleavage activity is not based on the ribonuclease (RNase) activity and is affected by the availability of divalent ions in the mixture. The available proteins for the function of CEM-HP were studied using the molecular weight size exclusion columns of 30–100 KDa and discovered that the proteins with molecular weight in the 50–100 KDa range could cleave the proteins DNAzyme successfully [19]. Two biosensors using the DHp3T4 were developed, and Ali and co-workers incorporated the fluorophore and quencher close to the RNA linkage via modified thymine residues. The LOD of the fluorescence-based sensor was reported as 10^4 CFU/mL,which is better than the LOD of the faces antibody-based lateral flow devices (LFD) or dipstick test [19]. Even though fluorescence-based devices provide much sensitive detection of the pathogen, the research group utilized the DHp3T4 to develop a paper-based colorimetric biosensor. The fluorescence detection methods require expensive instruments fluoro-spectrophotometer. Urease was used with a DHp3T4 tagged in the biosensor, and urease remains in the cleavage fragment of the DNAzyme. In the presence of the CEM-HP, the cleavage of the ribonucleotide linkage is facilitated and causes the release of DNA fragmentsto consist with urease, as shown in Fig. 5. After completing the cleavage reaction, the cleaved products were separated using centrifugation and added to a red phenol solution containing urea. Once urea is hydrolyzed into ammonia, phenol red will turn from yellow to red.

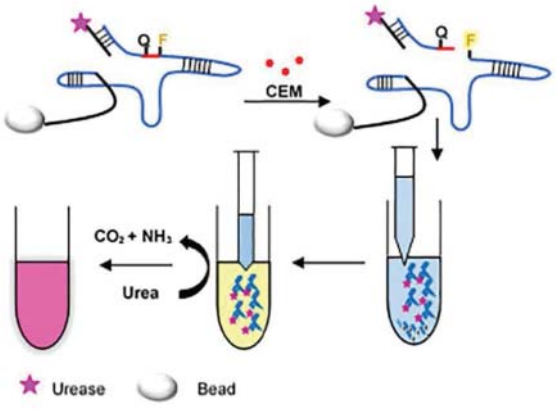

Fig. 5. **Schematic illustration of the *H. pylori* pathogen detection by the DNAzyme [19]**

Upon the CEM incubation, the urease tagged DNA fragment from the DHp3T4 DNAzyme complex triggers the conversion of urea into ammonia, which was reported by the color change of phenol red

Table 2. **Different types of bacterial pathogen detection via DNAzyme based sensors**

Bacteria	Signaling method	LOD	Linear range	References
Vibrio anguillarum	Fluorescence	$4 \cdot 10^3$ CFU/mL	–	[18]
Klebsiella pneumoniae	Fluorescence	10^5 CFU/mL	–	[20]
Legionella pneumophila	Fluorescence	10 CFU/mL	–	[21]
Aeromonashydrophila	Fluorescence	36 CFU/mL	–	[22]
Cronobactersakazakii	Colorimetric	1.2 CFU/mL	–	[36]
Salmonella	Colorimetric	1.5 copies/μL	–	[37]
Staphylococcus aureus	Colorimetric	30 nM	10^5 to 10^7 CFU/mL	[38]
Salmonella typhimurium	Colorimetric	1 nM	–	[39]
Salmonella paratyphi	Fluorescence	5 ng/mL	–	[40]
	Spectrophotometry	20 ng/mL	–	
Clostridium difficile	Colorimetric and Fluorescence	~ 10 μm	–	[41]
Listeria monocytogenes	Colorimetric	47.5 CFU/mL	–	[16]
Vibrio parahemolyticus	Colorimetric	10 CFU/mL	10^2 to 10^7 CFU/mL	[42]
Pseudomonas aeruginosa	Fluorescence	1.2 CFU/mL	–	[43]

The LOD for the paper-based colorimetric sensor was also reported as 10^4 CFU/mL, and it was identical to the fluorescence assay. The detection of *H. pylori* was completed in less than an hour without using sophisticated equipment. Also, Ali and co-workers claimed that the biosensor activity remains unaltered for at least 4 months at room temperature [19]. These biosensors provide cost-effective, rapid, portable, reliable, and sensitive detection via DNAzyme compared to the traditional detection methods and use in areas where the resources for pathogen detection are limited. Several studies have reported several other pathogenic bacteria detections based on the DNAzyme (Table 2).

Conclusions

Pathogen detection via DNAzyme attracts research interest due to its unique target recognition and signal generation characteristics. However, DNAzyme based sensors developed for pathogenic detection have faced obstacles such as isolation of pathogen-specific DNAzymes, sensitivity, and the detection limit. Different modifications overcome the obstacles, such as the incorporation of fluorophore/quencher (F/Q) systems. The modifications illustrate that incorporating new chemical functionalities into the DNAzyme improves sensitivity and specificity to detect different target pathogens. Fluorescence-based DNAzyme biosensors have mainly incorporated organic molecules such as fluorescein as the fluorophore. However, replacing fluorophores with the Quantum Dots (QDs) can improve the detection sensitivity of the fluorescence sensors due to the unique features of the QDs, such as higher quantum yield, photostability, and chemical stability, cost-effectiveness. The fluorescence emission of the QDs can be regulated by modifying the size. Based on the size, the emission color of the QDs varies and can be excited simultaneously by a single excitation source. Incorporating QDs provides more efficient, and higher sensitivity in the detection than organic fluorophores could be promising [44]. Incorporating nanomaterials such as graphene quenches the fluorescence drastically due to their super quenching ability and can be used to develop “Turn on” and “Turn off” biosensors. The combination of nanotechnology with these biosensors has been carried out, and the studies indicate that it efficiently enhances the biosensor’s performance [27, 28, 45]. However, pathogen detection via DNAzyme based sensors can be further improved by combining different amplification, fluorescent, colorimetric, electrochemical techniques, and nanotechnology. In general, very few studies have focused on detecting microorganisms via

DNAzymes, so further research studies are vital to improve the selectivity, stability, and sensitivity of this fascinating and promising biosensor technology having extensive potential applications in pathogen detection.

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REFERENCES

1. *Palumbo J. D., Borucki M. K., Mandrell R. E., Gorski L.* Serotyping of *Listeria monocytogenes* by enzyme-linked immunosorbent assay and identification of mixed-serotype cultures by colony immunoblotting. *J. Clin. Microbiol.* 2003, 41 (2), 564–571. https://doi.org/10.1128/JCM.41.2.564-571.2003
2. *Järvinen A. K., Laakso S., Piiparinen P., Aittakorpi A., Lindfors M., Huopaniemi L., Mäki M.* Rapid identification of bacterial pathogens using a PCR- and microarray-based assay. *BMC Microbiol.* 2009, 9 (1). 1–16.
3. *Ali M. M., Aguirre S. D., Lazim H., Li Y.* Fluorogenic DNAzyme Probes as Bacterial Indicators. *Angew. Chemie*. 2011, 123 (16), 3835–3838. https://doi.org/10.1002/anie.201100477
4. *Breaker R. R., Joyce G. F.* A DNA enzyme that cleaves RNA. *Chem. Biol.* 1994, 1 (4), 223–229. https://doi.org/10.1016/1074-5521(94)90014-0
5. *Santoro S. W., Joyce G. F.* A general purpose RNA-cleaving DNA enzyme. *Proc. Natl. Acad. Sci. USA.* 1997, 94 (9), 4262–4266. https://doi.org/10.1073/pnas.94.9.4262
6. *Brown A. K., Liu J., He Y., Lu Y.* Biochemical characterization of a uranyl ion-specific DNAzyme. *Chem. BioChem.* 2009, 10 (3), 486–492. https://doi.org/10.1002/cbic.200800632
7. *Huang P. J. J., Liu J.* Rational evolution of Cd^{2+}-specific DNAzymes with phosphorothioate modified cleavage junction and Cd^{2+} sensing. *Nucleic Acids Res.* 2015, 43 (12), 6125–6133. https://doi.org/10.1093/nar/gkv519
8. *Huang P. J. J., Lin J., Cao J., Vazin M., Liu J.* Ultrasensitive DNAzyme beacon for lanthanides and metal speciation. *Anal. Chem.* 2014, 86 (3), 1816–1821. https://doi.org/10.1021/ac403762s
9. *Ma L.* A New Na(+)-specific DNAzyme Mutant from in Vitro Selection. *University of Waterloo.* 2017.
10. *Sambrook J., Russell D. W.* Molecular cloning: a laboratory manual. *Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.* 2001.
11. *Proudnikov D., Mirzabekov A.* Chemical methods of DNA and RNA fluorescent labeling. *Nucleic Acids Res.* 1996, 24 (22), 4535–4542. https://doi.org/10.1093/nar/24.22.4535
12. *Liu J., Cao Z., Lu Y.* Functional nucleic acid sensors. *Chem. Rev.* 2009, 109 (5), 1948–1998. https://doi.org/10.1021/cr030183i
13. *Thomas I. B. K., Gaminda K. A. P., Jayasinghe C. D., Abeysinghe D. T., Senthilnithy R.* DNAzymes, Novel Therapeutic Agents in Cancer Therapy: A Review of Concepts to Applications. Basu A., editor. *J. Nucleic Acids.* 2021, V. 2021, P. 1–21. https://doi.org/10.1155/2021/9365081
14. *Zhou W., Ding J., Liu J.* Theranostic dnazymes. *Theranostics*. 2017, 7 (4), 1010–1025. https://doi.org/10.7150/thno.17736
15. *Zhao W., Ali M. M., Brook M. A., Li Y.* Rolling circle amplification: Applications in nanotechnology and biodetection with functional nucleic acids. V. 47, Angewandte Chemie — International Edition. *Angew. Chem. Int. Ed. Engl.* 2008, P. 6330–6337. https://doi.org/10.1002/anie.200705982
16. *Liu Z., Yao C., Wang Y., Yang C.* A G-quadruplex DNAzyme-based LAMP biosensing platform for a novel colorimetric detection of: Listeria monocytogenes. *Anal. Methods.* 2018, 10 (8), 848–854. https://doi.org/10.1039/C7AY02908J
17. *Li B., Du Y., Li T., Dong S.* Investigation of 3,3′,5,5′-tetramethylbenzidine as colorimetric substrate for a peroxidatic DNAzyme. *Anal. Chim. Acta.* 2009, 651 (2), 234–240. https://doi.org/10.1016/j.aca.2009.09.009
18. *Gu L., Yan W., Wu H., Fan S., Ren W., Wang S., Lyu M., Liu J.* Selection of DNAzymes for Sensing Aquatic Bacteria: Vibrio Anguillarum. *Anal. Chem.* 2019, 91 (12), 7887–7893. https://doi.org/10.1021/acs.analchem.9b01707
19. *Ali M. M., Wolfe M., Tram K., Gu J., Filipe C. D. M., Li Y., Brennan J. D.* A DNAzyme-Based Colorimetric Paper Sensor for *Helicobacter pylori*. *Angew. Chem. — Int. Ed.* 2019, 58 (29), 9907–9911. https://doi.org/10.1002/anie.201901873
20. *Ali M. M., Slepenkin A., Peterson E., Zhao W.* A Simple DNAzyme-Based Fluorescent Assay for Klebsiella pneumoniae. *Chem. BioChem.* 2019, 20 (7), 906–910. https://doi.org/10.1002/cbic.201800701

21. *Rothenbroker M., McConnell E. M., Gu J., Urbanus M. L., Samani S. E., Ensminger A. W.* Selection and Characterization of an RNA — Cleaving DNAzyme Activated by Legionella pneumophila. *Angew. Chem.* 2021, 133 (9), 4832–4838. https://doi.org/10.1002/anie.202012444
22. *Ma X., Wang C., Qin M., Tian X., Fan S., Zu H., Lyu M., Wang S.* Rapid detection of Aeromonas hydrophila with a DNAzyme-based sensor. *Food Control.* 2021, V. 123, P. 107829. https://doi.org/10.1016/j.foodcont.2020.107829
23. *Aguirre S. D., Monsur Ali M., Kanda P., Li Y.* Detection of bacteria using fluorogenic DNAzymes. *J. Vis. Exp.* 2012, V. 63, P. 1–8. https://doi.org/10.3791/3961
24. *Aguirre S. D., Ali M. M., Salena B. J., Li Y.* A sensitive DNA enzyme-based fluorescent assay for bacterial detection. *Biomolecules.* 2013, 3 (3), 563–577. https://doi.org/10.3390/biom3030563
25. *Cao T., Wang Y., Zhao L. L., Wang Y., Tao Y., Heyman J. A.* A simple mix-and-read bacteria detection system based on a DNAzyme and a molecular beacon. *Chem. Commun.* 2019, 55 (51), 7358–7361. https://doi.org/10.1039/C9CC03746B
26. *Tram K., Kanda P., Salena B. J., Huan S., Li Y.* Translating bacterial detection by DNAzymes into a litmus test. *Angew. Chem. — Int. Ed.* 2014, 53 (47), 12799–12802. https://doi.org/10.1002/anie.201407021
27. *Liu M., Zhang Q., Brennan J. D., Li Y.* Graphene-DNAzyme-based fluorescent biosensor for Escherichia coli detection. *MRS Commun.* 2018, 8 (3), 687–694. https://doi.org/10.1557/mrc.2018.97
28. *Zheng L., Qi P., Zhang D.* DNA-templated fluorescent silver nanoclusters for sensitive detection of pathogenic bacteria based on MNP-DNAzyme-AChE complex. *Sensors Actuators, B Chem.* 2018, V. 276, P. 42–47.
29. *Zhou Z., Zhang Y., Guo M., Huang K., Xu W.* Ultrasensitive magnetic DNAzyme-copper nanoclusters fluorescent biosensor with triple amplification for the visual detection of *E. coli* O157:H7. *Biosens Bioelectron.* 2020, 167 (17), 112475. https://doi.org/10.1016/j.bios.2020.112475
30. *Liu M., Zhang Q., Chang D., Gu J., Brennan J. D., Li Y.* A DNAzyme Feedback Amplification Strategy for Biosensing. *Angew. Chem. — Int. Ed.* 2017, 56 (22), 6142–6146. https://doi.org/10.1002/anie.201700054
31. *Sun Y., Chang Y., Zhang Q., Liu M.* An origami paper-based device printed with DNAzyme-containing DNA superstructures for Escherichia Coli detection. *Micromachines.* 2019, 10 (8), 531. https://doi.org/10.3390/mi10080531
32. *Liu M., Zhang Q., Kannan B., Botton G. A., Yang J., Soleymani L., Brennan J. D., Li Y.* Self-Assembled Functional DNA Superstructures as High-Density and Versatile Recognition Elements for Printed Paper Sensors. *Angew. Chem.* 2018, 130 (38), 12620–12623. https://doi.org/10.1002/anie.201806489
33. *Zhou Z., Brennan J. D., Li Y.* A Multi-component All-DNA Biosensing System Controlled by a DNAzyme. *Angew. Chem. — Int. Ed.* 2020, 59 (26), 10401–10405. https://doi.org/10.1002/anie.202002019
34. *Kusters J. G., Van Vliet A. H. M., Kuipers E. J.* Pathogenesis of *Helicobacter pylori* infection. *Clin. Microbiol. Rev.* 2006, 19 (3), 449–490. https://doi.org/10.1128/CMR.00054-05
35. *Suerbaum S., Michetti P. Helicobacter pylori* Infection. *N Engl. J. Med.* 2002, 347 (15), 1175–1186. https://doi.org/10.1056/NEJMra020542
36. *Liu Z., Yuan Y., Wu X., Ning Q., Wu S., Fu L.* A turn-off colorimetric DNAzyme-aptasensor for ultra-high sensitive detection of viable Cronobacter sakazakii. *Sensors Actuators: B Chem.* 2020, V. 322, P. 128646. https://doi.org/10.1016/j.snb.2020.128646Get rights and content
37. *Li S. T., Zhang Y., Tian J. J., Xu W. T.* Luminescent DNAzyme and universal blocking linker Super Polymerase Chain Reaction visual biosensor for the detection of Salmonella. *Food Chem.* 2020, V. 324, P. 126859. https://doi.org/10.1016/j.foodchem.2020.126859
38. *Ding X., Li H., Deng L., Peng Z., Chen H., Wang D.* A novel homogenous detection method based on the self-assembled DNAzyme labeled DNA probes with SWNT conjugates and its application in detecting pathogen. *Biosens Bioelectron.* 2011, 26 (11), 4596–4600. https://doi.org/10.1016/j.bios.2011.04.041
39. *Seok Y., Byun J. Y., Mun H., Kim M. G.* Colorimetric detection of PCR products of DNA from pathogenic bacterial targets based on a simultaneously amplified DNAzyme. *Microchim. Acta.* 2014, 181 (15–16), 1965–1971. https://doi.org/10.1007/s00604-014-1297-3
40. *Ning Y., Li W., Duan Y., Yang M., Deng L.* High Specific DNAzyme-Aptamer Sensor for Salmonella paratyphi A Using Single-Walled Nanotubes–Based Dual Fluorescence-Spectrophotometric Methods. 2014, 19 (7), 1099–1106. https://doi.org/10.1177/1087057114528538
41. *Hui C. Y., Liu M., Li Y., Brennan J. D.* A Paper Sensor Printed with Multifunctional Bio/Nano Materials. *Angew Chem. — Int. Ed.* 2018, 57 (17), 4549–4553. https://doi.org/10.1002/anie.201712903

42. *Sun Y., Duan N., Ma P., Liang Y., Zhu X., Wang Z.* Colorimetric Aptasensor Based on Truncated Aptamer and Trivalent DNAzyme for Vibrio parahemolyticus Determination. *J. Agric. Food Chem.* 2019, 67 (8), 2313–2320. https://doi.org/10.1021/acs.jafc.8b06893
43. *Qin M., Ma X., Fan S., Wu H., Yan W., Tian X., Lu J., Lyu M., Wang S.* Rapid detection of Pseudomonas aeruginosa using a DNAzyme-based sensor. *Food Sci. Nutr.* 2021, 9 (7), 3873–3884. https://doi.org/10.1002/fsn3.2367
44. *Wu C. S., Khaing O. M. K., Fan X.* Highly sensitive multiplexed heavy metal detection using quantum-dot-labeled DNAzymes. *ACS Nano*. 2010, 4 (10), 5897–5904. https://doi.org/10.1021/nn1021988
45. *Zhou Z., Zhang Y., Guo M., Huang K., Xu W.* Ultrasensitive magnetic DNAzyme-copper nanoclusters fluorescent biosensor with triple amplification for the visual detection of *E. coli* O157:H7. *Biosens. Bioelectron.* 2020, V. 167, P. 112475. https://doi.org/10.1016/j.bios.2020.112475

ВИКОРИСТАННЯ ДЕЗОКСИРИБОЗИМІВ В АНАЛІЗІ ПАТОГЕННИХ БАКТЕРІЙ

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Метою огляду було проаналізувати використання біосенсорів DNAzyme для виявлення патогенів. Останнім часом дезоксирибозими (ДНКзими) набувають дедалі більшого значення як біосенсори в різних галузях аналізу: від детекції іонів металів у навколишньому середовищі до досліджень у тераностиці та виявлення мікроорганізмів. Незважаючи на можливість рутинного використання складних інструментальних методів виявлення патогенної мікробної контамінації, такі підходи пов'язані з витратою часу, скрутним попереднім обробленням зразків та наявністю високовартісних приладів. Специфічні щодо патогенів ДНКзими пропонують як альтернативні інструменти, що мають низку переваг: неінвазивний швидкий аналіз із виявленням бактерій *in situ* і в режимі реального часу, висока чутливість і селективність. Широкий спектр тестів на основі патогенспецифічних ДНКзимів було розроблено з використанням колориметричних та флуоресцентних методів, що дають змогу визначати патогенну бактеріальну контамінацію у різних зразках. В огляді узагальнено інформацію про способи підбору патогенспецифічних ДНКзимів *in vitro*, різні стратегії, що їх використовують для конструювання сенсорів, та потенційного застосування в тераностичній практиці.

Ключові слова: патоген, ДНКзим, біосенсори, пероксидазаподібний ДНКзим.

ИСПОЛЬЗОВАНИЕ ДЕЗОКСИРИБОЗИМОВ В АНАЛИЗЕ ПАТОГЕННЫХ БАКТЕРИЙ

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Целью обзора был анализ использования биосенсоров DNAzyme для обнаружения патогенов. В последнее время дезоксирибозимы (ДНКзимы) приобретают все большее значение как биосенсоры в самых разных областях анализа: от детекции ионов металлов в окружающей среде до исследований в тераностике и обнаружения микроорганизмов. Несмотря на возможность рутинного использования сложных инструментальных методов обнаружения патогенной микробной контаминации, такие подходы сопряжены с затратой времени, затруднительной предварительной обработкой образцов и наличием дорогостоящих приборов. Специфические в отношении патогенов ДНКзимы предлагаются в качестве альтернативных инструментов, имеющих ряд преимуществ: неинвазивный быстрый анализ с обнаружением бактерий *in situ* и в режиме реального времени, высокая чувствительность и селективность. Широкий спектр тестов на основе патогенспецифичных ДНКзимов был разработан с использованием колориметрических и флуоресцентных методов, позволяющих определять патогенную бактериальную контаминацию в различных образцах. В обзоре обобщена информация о способах подбора патогенспецифичных ДНКзимов *in vitro*, различных стратегиях, используемых для конструирования сенсоров, и их потенциальном использовании в тераностической практике.

Ключевые слова: патоген, ДНКзим, биосенсоры, пероксидазаподобный ДНКзим.

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CURCUMIN-BASED MULTIFUNCTIONAL NANOSYSTEMS

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The use of multifunctional nanosystems in medicine and research is of contemporary interest.

Aim. The purpose of the work was to summarize publications on the prospects of creating and using nanocontainers based on curcumin (Cur). Cur fluorescence in nanoparticles (NP) makes it possible to investigate the distribution of fluorescent and non-fluorescent components, significantly accelerating the study and implementation of drugs in practice. Particular attention is paid to the use of hydrophobic substances in NP, to penetrate into a living cell.

Understanding the interaction of NP with living cells is extremely important when these particles are used to transport and deliver water-insoluble drugs to cells. Cur is one of the drugs with various and very promising pharmaceutical effects, it is poorly soluble in aqueous media, and the use of nanocarriers is an effective way to significantly increase its bioavailability. Cur has its own fluorescence, which enables to use it in multifunctional fluorescent nanosystems, for example, with Pluronic® micelles.

The use of the fluorescence method makes it possible to trace the stages of interaction of Cur-loaded NP with cultured cells and their localization in cell organelles.

With this approach, nanoscale dynamics of drug distribution and stability is observed over time.

Conclusions. The main conclusion is that for unstable in the aquatic environment drugs such as Cur, it is necessary to use the most hydrophobic nanostructures without traces of water, which include the nuclei of Pluronic® micelles. This method makes it possible to use other poorly water-soluble drugs.

A promising area of nanomedicine is the creation of complex bio-compatible nanomaterials based on several active drugs that reduce the toxicity of preparations to normal cells.

Key words: multifunctional nanosystems, nanocontainers for medical preparations, curcumin.

Modern problems of nanomedicine have been reviewed in the works of Ukrainian scientists [1–9]. Particular attention is drawn to complex nanostructured materials with different functional biological properties [3–6, 10, 11].

Fluorescent nanomaterials, which have medical applications, are used to create fluorescent images of biological objects [7–9, 12–14], and for targeted transportation of drugs [2–6, 8–11, 15, 16]. The creation of complex biocompatible nanomaterials with antitumor activity is developing rapidly. They are used to deliver drugs to the localization of pathological processes. Using several drugs enhances the effect of the main preparation [8, 16, 17]. New combined preparations of nanomaterials, which are characterized by high biocompatibility, low toxicity and high specificity, are used increasingly in practice [5, 6].

The nanonization of drugs and the emergence of new nanomaterials as safe and effective drugs are developments of the recent decades. [18]. Among nanocarriers, synthetic polymer structures are of particular interest due to their universal and unique properties, which can be customized for certain practical needs [19]. The modern concept of drug delivery is based on the localization of drugs in the affected area in humans or animals. Thus, the therapeutic effect is achieved by increasing the drug concentration in the affected areas and simultaneously reducing it in the surrounding tissues. The process of administration of pharmaceutical

compounds occurs at a predetermined rate, and localized action increases the effectiveness of the drug and reduces systemic toxic effects on tissues [20].

Nanoscale multifunctional systems

The combined approach to treatment consists of co-encapsulation of several drug compounds in order to create multifunctional nanostructures for the long-term therapeutic efficacy and reduction of side effects [21, 22].

Modern nanomedicine is increasingly studying nanocarriers that can be loaded with several drugs [22], which have both hydrophobic and hydrophilic properties. The complex formulation enhances drug activity by reducing the required dose of drugs, which makes such systems more attractive and useful as treatments [23]. For example, co-encapsulation in niosomes of gallic acid and Cur, or a mixture of ascorbic acid and quercetin affects their physicochemical properties and has a synergistic antioxidant effect, which is more advantageous than the one-component nanostructures [22]. But the development of multifunctional, more specific and effective carriers, leading to a significant increase in the productivity of therapy, requires precise methods and simple techniques of control and production [8].

Molecular imaging technologies, such as fluorescence techniques, are needed to study nanobio interactions. These methods are sensitive, accurate, fast and relatively inexpensive. In modern research on the creation and determination of the lifetime of multifunctional systems, two fluorescent components are increasingly used [7], which significantly improves the receipt of scientific information about the parameters of nanocomposites [22, 24].

Various types of NP based on organic material, such as Cur complexes [9], flavonoids with ions of both transition and non-transition metals [25], or inorganic material, such as nanodiamonds [7, 8] are distinguished among the promising nanomaterials for visualization of drug delivery and use in scientific works. Most of the existing large arsenal of NP can be modified by applying an additional polymer coating [26].

Vesicles: liposomes and niosomes

Vesicles consist of two-layer amphiphilic molecules that surround the water core. Liposomes and niosomes are vesicular systems [1], which consist of a two-layer membrane that surrounds the aqueous core. The vesicular system is a platform for drug delivery, which provides effective bioavailability by controlled release of therapeutic drugs over a long period [1, 24].

Liposomes are prepared using phospholipids and have been widely used as vehicles to deliver drugs and genes for the past few years. Limitations for their widespread use are associated with phospholipid degradation, low liposome stability, and difficulties in preparation technologies [1, 18, 28]. Another major disadvantage of liposomes is their instability and short half-life in the bloodstream [1].

Niosomes are polymers that in aqueous solutions have the properties of nonionic surfactants and create vesicles [27]. Niosomes, for example, of the polymers Span (20, 40, 60, 80), Tween 20–80, Brij, consist of a hydrophilic nucleus formed from an aqueous solution and a bilayer hydrophobic shell that replaces phospholipids used in liposomes [27]. The unique amphiphilic nature of niosomes contributes to their effectiveness in the encapsulation of lipophilic or hydrophilic drugs. Other additives, such as cholesterol, can be used to maintain the rigidity of the niosome structure [18, 20, 24].

Niosomes versus liposomes

Recently, niosomes have been widely used in drug delivery as an alternative to liposomes due to their better stability, biodestructiveness, biocompatibility and low toxicity [1, 24]. Compared to liposomes, niosomes do not require special conditions for processing and storage [20]. Liposomes with phospholipids are replaced by vesicular structures of non-phospholipids of various amphiphilic molecules, namely surfactants, surfactant ionic liquids, or polymers [18, 28]. Drugs loaded into niosome vesicles showed high stability and good ability to load drug compounds, both individually and in combination. It was found that micelle-based drugs are stable for 24 hours [21], and have a smaller diameter compared to that of classical micelles, which is about 400 nm [19].

Besides, niosomes offer a number of major advantages over liposomes. Niosomes have high stability, ease of preparation and relatively low cost of surfactants [24]. Niosomes are advantageous because of their non-toxicity and biodegradability [28], which makes these systems attractive for many

chemical, biological and industrial applications [24, 28]. Niosomes enable encapsulation of a wide range of drugs [20] and thus they are a more effective tool for drug delivery in a therapeutic mode than liposomes [20].

Pharmaceutical nanocarriers: Span®, Tween®, Brij®, Pluronic®

Pharmaceutically and scientifically valuable nanocarriers include such biocompatible structures as liposomes, niosomes and multicomponent polymers [28].

Among the macromolecular systems used for targeted drug delivery, the most suitable for the creation of multifunctional devices are niosomes based on Span, Tween, Brij and Pluronic® polymers, which have the most studied characteristics. Lately, special attention is paid to amphiphilic triblock polymers Pluronic®, which have become of increased interest in the development of drug delivery systems [21].

Pluronic® polymers are triblock polymers that in aqueous solution have the properties of nonionic surfactants and form small micelles with a hydrophobic nucleus and a hydrophilic outer shell. The polymers are able to accumulate and transfer to cells both hydrophobic and hydrophobic substances. Such polymer micelles are usually less than 100 nm [19, 21].

Pluronic® are triblock copolymers with different molecular weights of polyethylene oxide (PEO), polypropylene oxide (PPO) and polyethylene oxide (PEO), which can self-assemble in an aqueous medium in the form of micelles and has the ability to increase drug solubility [29, 30].

The amphiphilic copolymer Pluronic® F127, which contains a hydrophilic part that ensures the solubility of micelles in water [31], PEO, and a hydrophobic part, PPO, has attracted attention due to its low toxicity and ability encapsulate hydrophobic agents [32]. The micelles of Pluronic® F127 are very stable in the aquatic environment, so that the critical concentration of their micelles (CMC) is 0.023% [33]. The hydrophobic core can accept water-insoluble compounds and serve as a nanocontainer for the inclusion of lipophilic therapeutic drugs. The micellar structure of this copolymer in an aqueous medium can be used to introduce hydrophilic and hydrophobic [34] drugs and release them over time [29, 35]. Micelles with carriers penetrate cells better than the drugs themselves [36–41], and conjugation of drugs with micelles is a successful self-assembly platform for future therapeutic use [42–44].

Pluronic® (PLU) micelles are used to improve Cur delivery. The micelles significantly enhance Cur availability compared to crude Cur due to protection against degradation on the way to target cells. Pluronic® F127 micelles are an effective way to cross the membranes of endothelial cells that form the blood-brain barrier [45–47].

Hydrophobic layers better store Cur in an anhydrous medium. However, due to the small number of water molecules present in the hydrophobic layer of micelles and vesicles [24], this leads to the gradual degradation of Cur. The most hydrophobic nucleus in polymers is present in Pluronic® micelles. The triblock polymer Pluronic® F 127 loaded with Cur retains active Cur in aqueous solution better than niosomes and protects Cur for 24 hr [21, 24]. Thus, hydrophobic substances that decompose rapidly in water are better preserved by Pluronic® micelles.

Multi-dye systems

The use of niosomes and block polymers makes it possible to include and transfer both hydrophobic and hydrophilic molecules and particles, and to create multicomponent nanocarriers based on them.

Fluorescent probes make it possible to remotely study the processes of transfer of NP and substances through the cell wall and their location in the middle of cells. It is especially interesting to use several dyes with different properties.

Fluorescent studies include FRET studies, which have shown significant potential for determining true and reliable information on NP behavior *in vitro* and *in vivo* [48]. In recent decades, FRET has been widely used to characterize various heterogeneous assemblies, including micelles, vesicles, proteins, lipids, DNA, nucleic acids, microemulsions, etc. [28]. In multifunctional NP, dyes can be combined into a system that creates fluorescent resonant energy transfer (FRET). The efficiency of FRET processes depends on the superposition of the fluorescence spectra of donor molecules and the absorption spectra of acceptor molecules [28]. In the micellar system, differences in the microheterogeneous environment of the probe molecule (donor/acceptor) can be understood by solvatochromic shift in their fluorescent spectra [28]. Such a system can be used both in solution to study the accumulation of various substances inside

the micelle, its stability, the rate of release of drug compounds, and to visualize their transfer together with the micelle *in vitro* and *in vivo* [49].

On the example of F127, studies were conducted to control changes in the microheterogeneity of the F127 micelle with the addition of various compositions. The resulting differences in spectra clearly indicate the structural transitions in the micelles, niosomes and polymers of Pluronic® [28]. By studying such effects, it is possible to remotely control the processes occurring in nanomaterials. Thus, FRET can provide a better understanding of the structural characteristics and dynamics of changes in various self-assembling systems and nanocomposites [28].

Curcuma (*Curcuma longa*) is a member of the family *Zingiberaceae* and is widely used in Asia as a traditional medicine [50] and in cooking as a dietary supplement [23, 51–53]. Curcuma is known to have been used in India and China for at least 2500 years to treat [54] infections, stress, depression, and dermatological diseases [55]. The most active component of turmeric is Cur [42, 55–57].

Curcumin (diferuloylmethane) is a yellow compound that is lipophilic, phenolic, and practically insoluble in water [55]. Cur has a molecular weight of 368.37 g/mol and a melting point of 183 °C. It is known that Cur is more stable in cell culture or human blood and unstable in alkaline media [51].

The small Cur molecule is a multi-purpose antioxidant with various functional groups. Phenolic hydroxyl and methoxy groups [55], diketone and double bonds contribute to its antioxidant activity [9, 56, 58]. The b-keto-enol tautomer of Cur has triple chelation sites of metals, including a double phenolic group and keto-enol fragments, which can form chelates with redox metal ions Cu^{2+}, $Fe^{2+}$and other ions [9, 51, 58]. Based on various experimental and theoretical results, it has been shown that the phenolic OH group plays an important role in the antioxidant mechanism of Cur, neutralizing ROS [9, 23, 59]. Cur affects many important enzymatic reactions, which is manifested in various biological properties of Cur [60].

The main obstacle to the therapeutic effects of Cur on the way to its introduction is its rapid metabolism in the body [56, 57, 61], chemical instability and low solubility [61]. The main metabolite of Cur in the body is the Cur glucuronide, and other metabolites include Cur sulfate, hydroxycurcumin, hexahydrocurcuminol and hexahydrocurcumin glucuronide [56].

Curcumin has a long-established safety record

The European Food Safety Authority (EFSA), the US Food and Drug Administration (FDA), the UN Expert Committee on Food Additives and the World Health Organization report that Cur is generally recognized as safe (GRAS) [50, 51, 55, 62], and acceptable and safe doses are from 4 to 8g per day [51, 63].

Dose studies have shown the safety of Cur at doses up to 12 g per day for 3 months [64] without adverse effects on humans. Despite this well-established safety, there are some concerns when consuming Cur in large doses about the possibility of inhibiting certain enzymes, which may lead to the toxic effects of Cur. Some negative side effects of Cur have been reported in the literature. For example, nausea and diarrhea, headache, rash, increased serum alkaline phosphatase and lactate dehydrogenase have been reported with 0.45 to 3.6 g per day of Cur for one to four months [50, 63].

The potential of curcumin against human diseases

Cur has been shown to target many signaling molecules and to show activity at the cellular level [50]. Extensive clinical trials over the last quarter of a century have been concerned with the pharmacokinetics, safety and efficacy of Cur against numerous human diseases [64]. Cur has a wide range of therapeutic effects [9, 17, 57, 61, 65], which includes antioxidant [50, 54, 56, 58, 65–68], anti-inflammatory [23, 50, 54, 56 58, 63, 66], antidiabetic, antiangiogenic, immunomodulatory [56], antitumor [42, 56, 61, 65, 67–69], antiproliferative, antimetastatic, antibacterial [50, 54–56], chemoprophylactic, chemotherapeutic activity [61], antifungal, antiviral, antimalarial and hepatoprotective ability and the ability to alleviate cardiovascular and neurodegenerative disorders [42, 51, 55, 61, 70, 71]. At present, Cur plays an important role in the prevention and treatment of various diseases, including cancer [55, 69], autoimmune [54, 55], neurological, cardiovascular, diabetic and lung diseases [55], and psoriasis [23].

Curcumin has antitumor activity for the colon, cervical, uterine, ovarian, prostate head and neck, breast, pulmonary, stomach and

gastric, pancreatic, bladder oral, oesophageal, and bone cancer [72]. It contains a mixture of strong bioactive molecules known as curcuminoids, which have the ability to reduce cancer in the early stages and progression of tumor development. In particular, these compounds block several enzymes required for tumor growth and may therefore be involved in the treatment of tumors [72].

Curcumin for the treatment of chronic diseases

To date, there are more than 200 clinical trials of Cur, which have shown a pronounced protective role of this compound against cardiovascular disease, metabolic diseases, neurological, skin, liver diseasei, various cancers, etc. [57]. Clinical use of native Cur is limited due to low solubility, physicochemical instability, poor bioavailability, rapid metabolism, and poor pharmacokinetics [50, 54, 63, 73]. However, these problems can be overcome using an efficient delivery system [42]. The therapeutic potential of Cur during clinical trials trumped the myth that poor bioavailability of Cur is a problem because the low bioavailability of Cur is eliminated in the treatment of chronic diseases [57]. In many clinical studies, Cur in nanoforms effectively improves its bioavailability [42, 57], which has been described for the combination of Cur with many natural and synthetic compounds and for various Cur formulations that have shown significant efficacy. Thus, Cur is a safe, inexpensive and effective drug for the treatment of various chronic diseases [51, 57, 61, 74].

Curcumin and pleiotropic effects

Cur is perhaps one of the most diverse therapeutic agents so far isolated from natural sources. The therapeutic benefits of this extraordinary natural compound have been demonstrated in the treatment of various diseases [53, 54], including cancer, inflammation, immunological disorders [54], diabetes and oxidative stress, which are often associated with hyperlipidemia [75]. Cur is effective for the treatment of various inflammatory diseases by inhibiting inflammatory cell proliferation, metastasis and angiogenesis through various molecular targets [55]. Due to its unique molecular chemical structure and functional groups, Cur can bind and subsequently either inhibit or activate various endogenous biomolecules, including enzymes, receptors, signaling molecules, metal ions, transcription factors, and even certain proteins found in cell membranes [75]. To date, many proteins are known as a target for Cur [76, 77].

Cur's pleiotropic activity derives from its ability to modulate numerous signaling molecules, such as proinflammatory cytokines, apoptotic proteins, NF-κB, cyclooxygenase-2, 5-LOX, STAT3, C-reactive protein, prostaglandin E2, prostate-specific antigen, adhesive molecules, phosphorylase kinase, transforming growth factor-β, triglycerides, ET-1, creatinine, HO-1, AST and ALT in human [64]. Cur regulates numerous transcription factors, cytokines, protein kinases, redox status, and enzymes associated with inflammation [74].

Much of the pharmacologically beneficial effects of Cur occur through non-covalent interactions with biomolecules. With so many different biological targets, Cur (polyphenol) causes numerous pleiotropic effects, which is therapeutically beneficial because many pathological conditions, such as COVID-19, involve more than one signaling pathway, receptor, protein/enzyme or gene [75, 78].

Recently, Cur has been found to have beneficial properties for the prevention and treatment of several disorders. The relevant feature here is the structural α-β-unsaturated carbonyl system, which is necessary to establish contacts with critical cysteine residues of several targets. This excellent mechanism of action gives the molecule the ability to affect a large number of targets, given its pleiotropic behavior [79]. Due to its antioxidant and anti-inflammatory properties, Cur plays a significant beneficial and pleiotropic regulatory role through synergistic binding to multiple network targets [61]. In practice, Cur has shown a beneficial effect on the progression of inflammatory diseases through numerous mechanisms of action: antiviral, anti-inflammatory, anticoagulant, antiplatelet and cytoprotective. Such effects of Cur make it promising as a new adjunctive therapy for the treatment of COVID-19 [78].

Bioavailability due to biopolymers

Despite its therapeutic benefits, Cur, as a highly pleiotropic molecule with an excellent safety profile targeting multiple diseases with strong evidence at the molecular level, has not been able to achieve its optimal therapeutic result in past clinical trials mainly due to its poor solubility and poor bioavailability [50,

54, 63, 73]. These problematic properties are primarily associated with poor absorption, rapid metabolism and rapid excretion from the gastrointestinal tract [50, 51, 58, 62].For further research, it is recommended to use the method of increasing the bioavailability of Cur [51] in combination with other substances [54].

Innovative Cur drugs based on the nanotechnology approach will increase both its bioavailability and therapeutic efficacy [79]. In clinical trials, carriers have been developed that improve the release of Cur and enhance its bioavailability. To this end, various Cur formulations have been studied, including capsules, tablets, powders, emulsions, NP, and liposomal encapsulation [64]. Cur was used both alone and in combination with other drugs [64]. Thus, the effect of Cur was significantly enhanced [50]. For example, piperine blocks the metabolic pathway of Cur and has been used to delay its metabolism [50, 51, 80, 81].

There are known methods for efficient delivery of Cur using compositions that include liposomes, niosomes, micelles, conjugates, NP and nanoglobules [27]. Cur encapsulation methods in various self-assembled biologically active systems, such as micelles, vesicles, proteins and cyclodextrins, have shown high efficacy in increasing the solubility, stability and bioavailability of Cur [24]. The increased intensity of Cur fluorescence during encapsulation in hydrophobic micro-media of micelles and niosomes is a consequence of reduced interaction of Cur with water molecules [24].

To fully exhibit the healing properties of Cur (anti-inflammatory, antitumor and antioxidant) [50], a targeted delivery system is required [23]. Polymeric nanoscale drug delivery systems are widely used due to their reduced adverse effects and increased drug bioavailability [23]. Polymeric NP with Cur are already known, in which significant therapeutic activity has been recorded *in vitro* and *in vivo* [23].

It has become known that poly-ε-caprolactone NP together with the stabilizing surfactant Pluronic® F-68 (Cur–PCL) load Cur well and have antioxidant and cytoprotective properties [23]. Pluronic® F-68 triblock copolymer provides steric stabilization of the nanopreparation, which allows the system to function for a long time and increase the transport of drugs across cellular barriers. These NP did not induce cell death of dermal fibroblasts, but they did reduce cell proliferation without affecting cell migration and adhesion [23].

Curcumin as a potential agent in cancer therapy

The broad therapeutic efficacy of Cur is associated with synergistic interactions with different biological targets, as well as the modulation of several signaling pathways. This kind of behavior can be useful in the treatment of multifactorial diseases such as cancer [69, 79]. The combination of Cur with antitumor drugs is a valuable strategy for obtaining an enhanced response with minimized side effects [56, 65, 67, 68, 79, 82].

Mixed ligand-Cur complexes with lanthanum (curcumin-terpyridyl-lanthanum (La^{3+}) and rare earth metals such as Sm^{3+}, Eu^{3+} and Dy^{3+} are toxic to cancer cells [83, 84] and have antibacterial activity [83–86]). Cur complexes have shown enhanced photocytotoxicity in HeLa cells [83, 84].

The antitumor activity of Cur depends on the dose and time. Cur has the ability to target the molecular pathways that are responsible for the growth and survival of cancer. Cur can impair the proliferation and metastasis of cancer cells, causing cell cycle arrest and other effects. The inhibitory effect of Cur on the viability and colony formation of cancer cells is important to increase the sensitivity of cancer cells to chemotherapy [56]. Cur nanoplatforms can lead to enhanced therapeutic efficacy while reducing systemic toxicity [42] of chemotherapeutic agents [56]. For example, Cur reduces the activity and expression of p-glycoprotein (P-gp) and multidrug resistance-1 (MDR1) of MCF-7 tumor to promote the accumulation of paclitaxel in [56, 82]. The complex action of calcitriol with Cur enhanced the response of MCF-7 cancer cells to paclitaxel [56, 82].

Cur is well tolerated by humans. Cur concentration peaks 1–2 hours after oral administration and begins to decline after 12 hours [87]. Oral administration of 8 g of Cur leads to a serum concentration of 0.5 to 1.8 μm, which indicates its poor bioavailability [56, 87].

Cur has antitumor properties, but a number of problems with the drug delivery regime limit its therapeutic use. Chemical complex formation can be considered as a strategy to increase the potency of Cur in the treatment of breast cancer [88]. The study showed the antitumor properties of two Cur complexes – iron-curcumin [Fe (Cur) 3] and boron-curcumin [B (Cur) 2] in the breast cancer cell line MDA-MB-231 [88]. Cell proliferation, migration, and invasion were also analyzed. All three compounds inhibited cell invasion, and

only Cur and B (Cur) 2 inhibited cell migration. Taken together, these results showed that Fe (Cur) 3 and B (Cur) 2 may exhibit similar antitumor properties as Cur and suggests that chemical complexation may be considered as a strategy to increase Cur potential in the treatment of breast cancer [88]. Cellular localization of Cur and B and Fe complexes was determined by fluorescence microscopy. The results showed that the three compounds were localized in the perinuclear and cytoplasmic regions of the cell, and showed cytotoxicity with IC50 values of 25, 35 μM and 8 μM (μM) for Cur, B (Cur) 2 and Fe (Cur) 3, respectively [88]. The use of literature sources makes it possible to compare the desired concentration of Cur to combat some cancers. For example, *in vivo* Cur at a concentration of 20 μm significantly contributes to the antitumor activity of doxorubicin against triple negative breast cancer cells, inhibiting the transition of the epithelium to the mesenchyme and metastasis [56]. However, in practice, oral administration of pure Cur results in the concentration in serum from 0.5 to 1.8 μm [87], which is insufficient in this case.

Many recent studies have focused on the development and synthesis of Cur analogues that improve bioavailability and target selectivity [98]. Synthetic Cur derivatives have been obtained to develop effective therapeutic agents in the treatment of cancer and neurodegenerative diseases [61].

Further *in vitro* and *in vivo* studies are needed to study the effects of Cur, to mitigate the treatment of various cancers [72]. Cur can be developed as a therapeutic drug by improving the properties of the formulation or delivery systems, which ensure its enhanced absorption and cellular assimilation [63]. These features, combined with pharmacological safety and low cost, make Cur an attractive agent for further research [74].

Curcumin as a fluorescent dye

Cur is a dye with a solvent-dependent absorption band that ranges from 408 to 430 nm. The maximum fluorescence is even more dependent on the solvent and occurs between 480 and 560 nm. Cur is very poorly soluble in water, where it emits weak fluorescence, and the quantum yield of Cur fluorescence in most solvents is low and decreases significantly in the presence of water [90].

In a hydrophobic medium, Cur's own fluorescence is an order of magnitude greater than in an aqueous medium, and the intensity of Cur's fluorescence can reveal the change in the hydrophobicity of the medium in which it is located [24].

Cur is highly degradable in aqueous solution. In phosphate buffer solution (pH 7.4), the level of Cur degradation after 1 h is more than 80% [24]. Spectrally, this is determined by the rapid decrease in the intensity of Cur absorption and the decrease in fluorescence. The greater the degree of interaction of Cur with water, the greater the degree of degradation [24].

When Cur is in a non-aqueous medium with high viscosity or in phospholipid membranes, the intensity of its fluorescence increases significantly [24, 91]. It is expected that in cellular images, its radiation can be observed only when it is in a composite with hydrophobic nanocarriers, or contained in membranes or intracellular hydrophobic structures.

Using various Cur nanocomplexes with carriers, it is possible to study the encapsulation of hydrophobic substances in liposomes, niosomes and Pluronic® micelles by the intensity of Cur fluorescence, and to visualize their interaction with cells in microscopic studies. The results show that nonionic surfactants that form micellar compounds significantly reduce the level of Cur degradation from 12 to 24 h by including it in the hydrophobic part of the micelles [21, 24]. This increased fluorescence intensity, along with a significant shift in the maximums of Cur emissions during encapsulation in hydrophobic microenvironments of micelles and niosomes, is a consequence of reduced interaction of Cur with water molecules. A more rigid and limited niosome microenvironment increases the intensity and fluorescence time of Cur against micelles with hydrophilic nucleus [24].

ROS

Free radical oxidation plays an important role in our lives. Large amounts of produced free radicals condition lipid peroxidation, protein denaturation, neurodegenerative, fibrous and other pathological changes. Overproduction of reactive oxygen species (ROS) and reactive nitrogen (RNS), which are potent oxidants, causes DNA damage and lipid oxidation. This leads to oxidative stress and cell damage. Oxidative stress is a major factor leading to the development of various diseases such as neurodegenerative and heart diseases, diabetes, and cancer [92, 93].

The anti-inflammatory and antioxidant properties of Cur, which have a wide range

of therapeutic potentials *in vitro* and *in vivo*, have been studied in order to create drugs that counteract free radical oxidation [51, 54, 94]. In *in vivo* systems, Cur acts by regulating the levels of enzymatic and non-enzymatic antioxidants in target tissues [58]. Experimental results *in vitro* and *in vivo* indicate a protective effect of Cur on experimentally induced inflammation, hepatotoxicity and cardiotoxicity in rats [66]. The studies of rat myocardium under Cur treatment have shown inhibition of free radical distribution and increased levels of SOD (superoxide dismutase), CAT (catalase) and glutathione peroxidase [58, 66].

The Cur molecule may exhibit prooxidant properties, depending on the environment and environmental conditions in which it is located. Cur has many biological functions, and many of these functions are related to the induction of oxidative stress. However, how Cur causes oxidative stress in cells is not fully understood [53]. The antitumor effect of Cur was confirmed *in vitro* on the example of leukemic cells. Curcumin targets several enzymes involved in the metabolic pathway of ROS, as a result of its action inhibits the growth of tumor cells [95].

The effective activity of Cur was studied against the fungus *Botrytis cinerea*, which causes gray rot of plants. It has been shown that the ROS produced by Cur caused apoptosis in hyphae of the fungus *Botrytis cinerea*. Cur has been suggested to cause oxidative stress through a NADPH oxidase-dependent mechanism [96].

The Cur-Cu (II) complex can cause DNA breakdown due to the uncontrolled production of both ROS and RNS, which ultimately leads to oxidation of DNA by the oxidative chain [79]. It should be emphasized that intracellular ROS levels do not necessarily correlate with the strength of Cur's antitumor activity. It is possible that the molecules that actually killed the leukemic cells were obtained during the production of ROS, such as reactive carbonyls and reactive aldehydes [97].

In recent years, a number of derivatives have been developed and patented, aimed both at improving Cur's multifaceted biological profile and at overcoming its undesirable effects [61]. The studies revealed antitumor activity of the allylated monocarbonyl analogue Cur CA6 against gastric cancer cells. The CA6 showed significant cytotoxicity to gastric cancer, which was considered as induction of G2/M cell cycle arrest, apoptosis and induction of endoplasmic reticulum stress. CA6 increased ROS levels by directly binding and inhibiting thioredoxin reductase R1 (TrxR1) [98].

ROS are by-products of biochemical processes in aerobic organisms. The concentration of ROS is regulated by the activity of such antioxidant enzymes as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) [51]. The imbalance between the production and disposal of ROS leads to oxidative stress, which leads to negative consequences for cells, tissues, namely inflammatory reactions and apoptosis [51].

Large-scale studies have shown that inflammation alters signaling pathways and increases the number of inflammatory biomarkers, lipid peroxides and free radicals. Acute and chronic inflammation are an important risk factor for some types of cancer [55]. Membrane lipid peroxidation [58] and free radical-mediated oxidative damage to DNA and proteins are thought to be associated with a variety of chronic pathological complications such as cancer, atherosclerosis, and neurodegenerative diseases [52]. Oxidative damage and inflammation in preclinical studies indicate the root cause of cancer and other chronic diseases, such as diabetes, hypertension, Alzheimer's disease, etc. [63].

The effect of Cur on free radicals is carried out by several different mechanisms. It can absorb various forms of free radicals, such as ROS [9, 50, 52, 58, 66] and remove free radicals of reactive nitrogen (RNS) [50, 51]. The decrease in ROS depends on the dose of Cur and time [23]. The anti-inflammatory effect of Cur, directed against pathological conditions, is most likely mediated by its ability to inhibit the activity of enzymes that generate ROS, such as xanthine hydrogenase/oxidase [50, 51, 55], and the ability to inhibit cyclooxygenase [50–52, 55, 58], lipoxygenase (LOX) [50–52, 55, 58], induced nitric oxide synthase (iNOS) [52, 55, 58]. Cur inhibition of the activity of important enzymes that mediate inflammatory processes [52] is important for the downregulation of oxidative stress [50-52].

Poly-ε-caprolactone NP enhance the mechanism of action of Cur to reduce ROS in dermal fibroblasts [23]. Interestingly, Cur-PCL NP and poly-ε-caprolactone reduce oxidative stress caused by hydrogen peroxide and have a cytoprotective effect, so these NP may have clinical application in disorders associated with the formation of reactive oxygen species [23].

Cur has been shown to improve systemic markers of oxidative stress [50, 99]. There

is evidence that it may enhance the serum activity of antioxidants such as SOD [50–52, 58, 80, 99], catalase [50, 58, 99], glutathione peroxidase (GSH) [50, 51, 58] and at the same time favorably increase the serum activity of antioxidants of lipid peroxides [51]. Curcuminoids in serum increased GSH concentrations [80, 99], significantly reduced lipid peroxides [99] and significantly reduced malonic dialdehyde concentrations [80].

Cur, which has a strong antioxidant and anti-inflammatory effect, can be a prophylactic and chemotherapeutic agent for cancer of the colon, skin, mouth and intestines [58], as well as other chronic diseases [63]. Cur inhibited iron ion-catalyzed lipid peroxidation [58] in *in vitro* experiments in liver homogenates and inhibited heat-induced hemolysis of rat erythrocytes [66]. This may be one of the mechanisms by which Cur exhibits anti-inflammatory and antitumor activity. Cur can reduce the toxicity of iron ions, possibly by chelating iron ions, reducing oxidative stress caused by lipid peroxidation, and improving the antioxidant defense mechanism [100].

Toxicity caused by lead ions in various organ systems occurs due to increased oxidative stress due to the formation of ROS and RNS. Lead poisoning causes numerous clinical consequences for almost all organs, but the main targets are the brain, liver and kidneys. Lead is a multi-organ toxicant that is involved in various types of cancer, damage to reproductive organs in both humans and animals [51]. For the treatment of diseases or poisonings caused by heavy metal ions (Pb, Cu, Fe), standard chelator drugs are used, which often show numerous side effects from mild to severe. In the treatment of lead toxicity, Cur acts as a chelator [51].

Cur can be effective in treating various skin conditions such as dermatitis, psoriasis and scleroderma [55]. A study in mice indicates that Cur can protect the skin, removing free radicals and reducing inflammation by inhibiting nuclear factor NF- B and cytokines such as IL-1b and IL-6 [55].

Neurodegenerative diseases: Alzheimer's, Parkinson's and multiple sclerosis

Aging is a significant risk factor for neurodegenerative diseases [70]. It is believed that Cur can be effective in the mechanisms associated with aging, preventing changes in cellular proteins that occur due to aging, helping to maintain protein homeostasis [55]. Experimental studies have shown that Cur can be used for the prevention and treatment of Alzheimer's disease [58]. Nasal administration enables the drug to be delivered across the blood-brain barrier. Cur can be administered likewise and has anti-inflammatory, antitumor, antioxidant and cytoprotective effects [23]. Cur reduces neurodegenerative damage due to its antioxidant and anti-inflammatory properties [70]. Previous studies have shown that Cur interacts with several targets involved in inflammation of the nervous system, reducing inflammation, relieving neuropathic pain, nerve ischemia, and demyelination [50, 51].

Millions of people worldwide suffer from autoimmune diseases. In recent decades, Cur has been shown to be used by a variety of mechanisms [54] as a therapeutic agent against autoimmune diseases such as multiple sclerosis (MS) or rheumatoid arthritis (RA) [55].

Multiple sclerosis is a chronic inflammatory autoimmune disease characterized by degradation of the myelin sheath [55]. Differentiated effector memory T cells (TEM) have the pathogenic property to quickly infiltrate tissues or organs, which leads to their damage [54]. In recent years, there has been increasing evidence *in vitro* and *in vivo* that Kv1.3 channels in TEM cell control T cell proliferation and activation [54]. In activated TEM cells, the number of channels per cell increases from about 250 to 1500 [54]. Thus, Kv1.3 is a key player in the modulation of autoimmune disorders, and the blockade of Kv1.3 represents a promising approach to immunosuppression for the treatment of autoimmune diseases [54]. Cur is able to inhibit the proliferation and proinflammatory secretion of cytokines [55] in TEM cells by directly blocking the open hKv1.3 channels, depending on time and concentration (5–100 mM) [54]. This leads to an anti-inflammatory effect, which is an important pharmacological mechanism for the treatment of autoimmune diseases [54]. Collectively, Cur has an immunosuppressive effect on various pathogenic subgroups of T lymphocytes, so in the future Cur can be used as a powerful anti-inflammatory agent for the treatment of autoimmune diseases [54].

A neurodegenerative disease such as Alzheimer's is characterized by inflammation, oxidative damage, and abnormal protein production [55]. Neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, are the main results of increased ROS production in the body [51]. Cur having antioxidant and anti-inflammatory properties

can improve cognitive function by reducing b-amyloid plaques [55]. Cur, due to its neuroprotection and antioxidant properties, can attenuate the course of Parkinson's disease, which is characterized by the loss of dopaminergic neurons in the substantia nigra [55]. It protects the neurons of the substantia nigra, improves dopamine levels in the 6-OHDA model of rats from Parkinson's disease (Figure) [55].

Experimental studies have shown that Cur can be used for the prevention and treatment of Alzheimer's disease [58]. In the experiment, Cur was administered peripherally *in vivo* to Tg mice, crossed the blood-brain barrier and bound to amyloid plaques in the brain, significantly reducing amyloid levels by inhibiting b-amyloid peptide aggregation [51, 58, 70].

Cur reduces the generation of ROS, inhibits lipid peroxidation and reduces the level of malonic dialdehyde. Cur has been shown to reduce plaque pathogenesis and inhibit the production of oligomers and fibrils [51, 70] and promote microglia formation, delaying neuronal deterioration in patients [55]. Cur alleviates neuroinflammation [101], thus it is indicated as a potential neuroprotective agent [55].

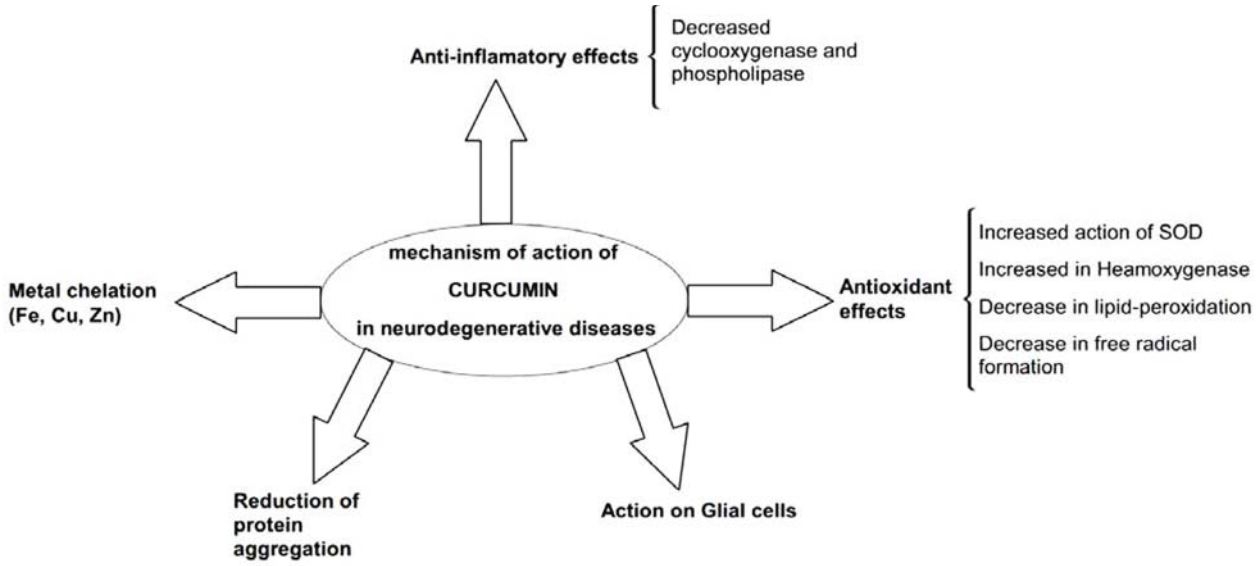
The neuroprotective mechanism of Cur against neurodegenerative disorders of the brain is also due to the ability to bind redox-active metal ions, such as Fe^{2+}, Cu^{2+}, Zn^{2+} [58], Mn^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+} [51]. The formation of Cur chelates with redox metal ions [71] leads to a decrease in ROS generation and a decrease in edema around the neuronal cells of the body [51].

Conclusions

The work summarized the literature data on the prospects of using curcumin in therapy by methods that improve its solubility. Particular attention was paid to the use of Cur as a complex drug together with other drugs in the composition of nanomaterials. Due to fluorescence, Cur makes it possible to trace the processes that occur during the penetration of the nanostructure into the cell and its interaction with the components of cellular organelles.

Cur is non-toxic and has many beneficial effects so it is considered as a drug or pharmaceutical agent. The use of various methods to improve the solubility of hydrophobic Cur in the aquatic environment and reduce the rate of its metabolism in the body are effective for the treatment of chronic diseases. The use of niosomes and block polymers makes it possible to include and transfer both hydrophobic and hydrophilic molecules and particles. it is possible to create multicomponent nanocarriers with scientific and medical applications, based on the niosomes and block polymers.

Multifunctional nanosystems penetrate into cells in a targeted and controlled manner and are used to reduce drug toxicity to normal cells. Effective use of the capabilities of multifunctional nanosystems is possible under



The proposed mechanisms of neuroprotective activity of curcumin and curcumin-like molecules [58] https://doi.org/10.2174/092986732102131206115810.

the condition of scientific substantiation of the features of joint use of components and in-depth study of the properties of these substances. Cur has a wide range of possibilities when used as the main component or part of a combination in hybrid NP, which makes it a promising drug, and such structures are promising nanomaterials. The developed Cur complexes with metal ions are more soluble in water than pure Cur and increase the target effect of the drug. Complexes of Cur-ions of metal show different biochemical activity, which depends on the nature of the metal ion. Based on Cur, double combinations with synergistic reinforcement with Cur have already been developed: Cur-quercetin, Cur-piperine, Cur-silibinin and Cur-doxorubicin. Created multifunctional therapeutic nanosystems will be widely used in medicine in the future.

A number of biologically active polymer conjugates and polymer compositions, such as micelles, hydrogels, and polymer-coated NP that can deliver multiple drugs are currently in clinical development. Nanoscale multifunctional systems have been created, which consist of several substances that have an advantage over one-component due to the synergistic action of components. The results showed that co-encapsulation of preparations affected their physicochemical properties and could produce a synergistic effect. Thus, the combined approach to treatment consists of co-encapsulation of several drug compounds into multifunctional nanostructures, which led to long-term therapeutic efficacy, reducing side effects. The development of multifunctional, more specific and effective carriers for therapy requires precise methods and simple techniques, which are primarily aimed at research to control and study their various characteristics in order to obtain the necessary parameters. The creation of optimal drug delivery systems requires such research systems that would provide the ability to quickly obtain the necessary information, including optical methods.

Developments on purposeful transport of nanocomplexes from several drugs proceed. The current direction is the development of methods for creating hydrophilic structures with a hydrophobic core containing Cur, which are aimed at increasing the amount and duration of action of the drug. Of greatest interest are monodisperse nanocarriers with a hydrophobic core without traces of water with well-known characteristics, biodegradable and with minimal toxic effects, which include Pluronic®. The use of self-assembling structures is a modern strategy for creating nanostructures in order to transfer them across the cell membrane. Particular attention is paid to the non-toxicity of polymers used for self-assembly. Among the macromolecular systems useful for targeted drug delivery are Pluronic® multifunctional amphiphilic triblock polymers, which have studied characteristics. Pluronic® F-127 provides an attractive route for encapsulation and delivery of hydrophobic compounds or ingredients. Its micelles with loaded carriers penetrate cells better than the preparations themselves. Practice-oriented tasks are aimed at creating and in-depth study of various functional properties of such combined nanomaterials to improve the therapeutic effect of drugs. The new tasks are related to multifunctional nanocarriers (nanocontainers), which carry a combination of already known medical or natural preparations. The creation of multifunctional structures makes it possible to add different components, and fluorescence makes it possible to monitor the processes occurring in the cell and track the time and location of drug unload.

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REFERENCES

1. *Priskoka A. O., Checkman I. S.* Nanotechnologies in development of drug delivery systems. *Ukr. Med. J.* 2010, 1 (75), I–II, 14–18.(In Ukrainian).
2. *Chekman* I. *S.* Nanopharmacology. K.: *Zadruga*. 2011, 424 p. (In Ukrainian).
3. *Prylutska S. V., Grynyuk I. I., Grebinyk S. M., Matyshevska O. P., Prylutskyy Yu. I., Ritter U., Siegmund C., Scharff P.* Comparative study of biological action of fullerenes C_{60} and carbon nanotubes in thymus cells. *Mat.-wiss. u. Werkstofftech.* 2009, V. 40, P. 238–241. https://doi.org/10.1002/mawe.200900433
4. *Prylutska S. V., Burlaka A. P., Prylutskyy Yu. I., Ritter U., Scharff P.* Comparative study of antitumor effect of pristine C_{60} fullerenes and doxorubicin. *Biotechnol.* 2011, V. 4, P. 82–87.

5. *Prylutska S. V.* Using of C_{60} fullerene complexes with antitumor drugs in chemotherapy. *Biotechnol. acta*. 2014, 7 (3), 9–20. https://doi.org/10.15407/biotech7.03.009
6. *Prylutska S. V., Didenko G. V., Kichmarenko Yu. M., Kruts O. O., Potebnya G. P., Cherepanov V. V., Prylutskyy Yu. I.* Effect of C_{60} fullerene, doxorubicin and their complex on tumor and normal cells of BALB/c mice. *Biotechnol. acta*. 2014, 7 (1), 60–65. https://doi.org/10.15407/biotech7.01.060 (In Ukrainian)
7. *Kanyuk M. I.* Ultrafine fluorescent diamonds in nanotechnology. *Biotechnol. acta*. 2014, 7 (4), 9–24. https://doi.org/10.15407/biotech7.04.009 (In Ukrainian).
8. *Kanyuk M. I.* Use of nanodiamonds in biomedicine. *Biotechnol. acta*. 2015, 8 (2), 9–25. https://doi.org/10.15407/biotech8.02.009
9. *Kaniuk M. I.* Prospects of Curcumin use in Nanobiotechnology. *Biotechnol. acta*. 2016, 9 (3), P. 23–36. http://dx.doi.org/10.15407/biotech9.03.023.
10. *Prylutska S. V., Remeniak O. V., Honcharenko Yu. V., Prylutskyy Yu. I.* Carbon nanotubes as a new class of materials for nanobiotechnology. *Biotechnol.* 2009, 2 (2), 55–66. (In Ukrainian).
11. *Prylutska S. V., Remenyak O. V., Burlaka A. P., Prylutskyy Yu. I.* Perspective of carbon nanotubes application in cancer therapy. *Oncology*. 2010, 12 (1), 5–9. (In Ukrainian).
12. *Sagnou M., Benaki D., Triantis C., Tsotakos T., Psycharis V., Raptopoulou C. P., Pirmettis I., Papadopoulos M., Pelecanou M.* Curcumin as the OO bidentate ligand in "2+1" complexes with the $[M(CO)_3]^+$ (M = Re, 99m Tc) tricarbonyl core for radiodiagnostic applications. *Inorg. Chem.* 2011, 50 (4), 1295–1303. https://doi.org/10.1021/ic102228u
13. *Asti M., Ferrari E., Groci S., Atti G., Rubagotti S., Lori M., Capponi P. C., Zerbini A., Saladini M., Versari A.* Synthesis and characterization of ^{68}Ga-labeled curcumin and curcuminoid Complexes as potential radiotracers for imaging of cancer and Alzheimer's disease. *Inorg. Chem*. 2014, 53 (10), 4922–4933. https://doi.org/10.1021/ic403113z
14. *Priyadarsini K. I.* The Chemistry of Curcumin: From Extraction to Therapeutic Agent. *Molecules*. 2014, 19 (12), 20091–20112. https://doi.org/10.3390/molecules191220091
15. *Golub A., Matyshevska O., Prylutska S., Sysoyev V., Ped L., Kudrenko V., Radchenko E., Prylutskyy Yu., Scharff P., Braun T.* Fullerenes immobilized at silica surface: topology, structure and bioactivity. *J. Mol. Liq*. 2003, 105 (2–3), 141–147. https://doi.org/10.1016/S0167-7322(03)00044-8
16. *Moorthi C., Kathiresan K.* Curcumin–Piperine/Curcumin–Quercetin/Curcumin — Silibinin dual drug- loaded nanoparticulate combination therapy: A novel approach to target and treat multidrug-resistant cancers. *J. Medical Hypotheses and Ideas*. 2013, 7 (1), 15–20. https://doi.org/10.1016/j.jmhi.2012.10.005
17. *Mullaicharam A. R., Maheswaran A.* Pharmacological effects of curcumin. *Int. J. Nutr. Pharmacol. Neurol. Dis.* 2012, 2 (2), 92–99. https://doi.org/10.4103/2231-0738.95930
18. *Moghassemi S., Hadjizadeh A.* Nanoniosomes as nanoscale drug delivery systems. An illustrated review. *J. Controlled Release*. 2014, V. 185, P. 22–36. https://doi.org/10.1016/j.jconrel.2014.04.015
19. *Pitto-Barry A., Barry N. P. E.* Pluronic® block-copolymers in medicine: from chemical and biological versatility to rationalisation and clinical advances. *Polym. Chem.* 2014, V. 5, P. 3291–3297. https://doi.org/10.1039/c4py00039k
20. *Yeo P. L., Lim C. L., Chye S. M., Ling A. P. K., Koh R. Y.* Niosomes: a review of their structure, properties, methods of preparation, and medical applications. *Asian Biomed. (Res. Rev. News)*. 2017, 11 (4), 301–14. https://doi.org/10.1515/abm-2018-0002
21. *Tavano L., Mauro L., Naimo G. D., Bruno L., Picci N., Andò S., Muzzalupo R.* Further Evolution of Multifunctional Niosomes Based on Pluronic Surfactant: Dual Active Targeting and Drug Combination Properties. *Langmuir*. 2016, 32 (35), 8926–8933. https://doi.org/10.1021/acs.langmuir.6b02063
22. *Tavano L., Muzzalupo R., Picci N., Cindio B.* Co-encapsulation of antioxidants into niosomal carriers: Gastrointestinal release studies for nutraceutical applications. *Colloids and Surfaces B: Biointerfaces*. 2014, V. 114C, P. 82–88. https://doi.org/10.1016/j.colsurfb.2013.09.058
23. *Del Prado-Audelo M. L., Rodríguez-Martínez G., Martínez-López V., Ortega-Sánchez C., Velasquillo-Martínez C., Magaña J. J., González-Torres M., Quintanar-Guerrero D., Sánchez-Sánchez R., Leyva-Gómez G.* Curcumin-loaded poly- ε-caprolactone nanoparticles show antioxidant and cytoprotective effects in the presence of reactive oxygen species. *J. Bioactive and Compatible Polymers*. 2020, 35 (3), 270–285. https://doi.org/10.1177/0883911520921499
24. *Mandal S., Banerjee C., Ghosh S., Kuchlyan J., Sarkar N.* Modulation of the photophysical properties of curcumin in nonionic surfactant (Tween-20) forming micelles and niosomes: a comparative study of different microenvironments. *J. Phys. Chem. B*. 2013, 117 (23), 6957–6968. https://doi.org/10.1021/jp403724g
25. *Pusz J., Wolowiec S.* Solid compounds of Ce(III), Pr(III), Nd(III), and Sm(III) ions with

chrysin. *J. Therm. Anal. Calorim*. 2012, V. 110, P. 813–821. https://doi.org/10.1007/s10973-011-1989-4
26. *Mathew A. P., Uthaman S., Cho K. H., Cho C. S., Park I. K.* Injectable hydrogels for delivering biotherapeutic molecules. *Int. J. Biol. Macromol.* 2018, V. 110, P. 17–29. https://doi.org/10.1016/j.ijbiomac.2017.11.113
27. *Xu Y. Q., Chen W. R., Tsosie J. K., Xie X., Li P., Wan J. B., He C. W., Chen M. W.* Niosome Encapsulation of Curcumin: Characterization and Cytotoxic Effect on Ovarian Cancer Cells. *J. Nanomaterials*. 2016, V. 2016, P. 1–9. https://doi.org/10.1155/2016/6365295
28. *Roy A., Kundu N., Banik D., Sarkar N.* Comparative Fluorescence Resonance Energy-Transfer Study inPluronic Triblock Copolymer Micelle and Niosome Composed ofBiological Component Cholesterol: An Investigation of Effect ofCholesterol and Sucrose on the FRET Parameters. *J. Phys. Chem. B.* 2016, 120 (1), 131–142. https://doi.org/10.1021/acs.jpcb.5b09761
29. *Le T. M. P., Pham V. P., Dang T. M. L., La T. H., Le T. H., Le Q. H.* Preparation of curcumin-loaded pluronic F127/chitosan nanoparticles for cancer therapy. *Adv. Nat. Sci.: Nanosci. Nanotechnol.* 2013, 4 (2), 025001. https://doi.org/10.1088/2043-6262/4/2/025001
30. *Hosniyeh H., Fatemeh A., Rassoul D., Aeyed N. O.* Chitosan–Pluronic nanoparticles as oral delivery of anticancer gemcitabine: preparation and *in vitro* study. *Int. J. Nanomed.* 2012, V. 7, P. 1851–1863. https://doi.org/10.2147/IJN.S26365.
31. *Kozlov M. Y., Melik-Nubarov N. S., Batrakova E. V., Kabanov A. V.* Relationship between pluronic block copolymer structure, critical micellization concentration and partitioning coefficients of low molecular mass solutes. *Macromolecules*. 2000, 33 (9), 3305–3313. https://doi.org/10.1021/ma991634x
32. *Prados J., Melguizo C., Ortiz R., Vélez C., Alvarez P. J., Arias J. L., Ruíz M. A., Gallardo V., Aranega A.* Doxorubicin-loaded nanoparticles: new advances in breast cancer therapy. *Anti-cancer Agents Med. Chem.* 2012, 12 (9), 1058–70. https://doi.org/10.2174/187152012803529646
33. *Ganguly R., Kunwar A., Dutta B., Kumar S., Barick K., Ballal A., Aswal V., Hassan P.* Heat-induced solubilization of curcumin in kinetically stable pluronic P123 micelles and vesicles: An exploit of slow dynamics of the micellar restructuring processes in the aqueous pluronic system. *Colloids and surfaces B: Biointerfaces.* 2017, V. 152, P. 176–182. https://doi.org/10.1016/j.colsurfb.2017.01.023
34. *Chiappetta D. A., Sosnik A.* Poly (ethylene oxide)–poly (propylene oxide) block copolymer micelles as drug delivery agents: improved hydrosolubility, stability and bioavailability of drugs. *Eur. J. Pharmac. Biopharmac.* 2007, 66 (3), 303–317. https://doi.org/10.1016/j.ejpb.2007.03.022
35. *Wenzel J. G. W., Balaji K. S. S., Koushik K., Navarre C., Duran S. H., Rahe C. H., Kompella U. B.* Pluronic F127 gel formulations of deslorelin and GnRH reduce drug release and effect in cattle. *J. Control. Release*. 2002, V. 85, P. 51–59. https://doi.org/10.1016/S0168-3659(02)00271-7
36. *Verma G., Hassan P. A.* Self assembled materials: design strategies and drug delivery perspectives. Cite this: *Phys. Chem. Chem. Phys.* 2013, V. 15, P. 17016–17028. https://doi.org/10.1039/c3cp51207j
37. *Zhang X., Burt H. M., Mangold G., Dexter D., Von Hoff D., Mayer L., Hunter W. L.* Anti-tumor efficacy and biodistribution of intravenous polymeric micellar paclitaxel. *Anticancer Drugs*. 1997, 8 (7), 696–701. https://doi.org/10.1097/00001813-199708000-00008
38. *Shin I. G., Kim S. Y., Lee Y. M., Cho C. S., Sung Y. K.* Methoxy poly (ethylene glycol)/ϵ-caprolactone amphiphilic block copolymeric micelle containing indomethacin.: I. Preparation and characterization. *J. Control. Release*. 1998, 51 (1), 1–11. https://doi.org/10.1016/S0168-3659(97)00164-8
39. *Yu B. G., Okano T., Kataoka K., Sardari S., Kwon G. S.* In vitro dissociation of antifungal efficacy and toxicity for amphotericin B-loaded poly(ethylene oxide)-block-poly(beta benzyl L aspartate) micelles. *J. Control. Release.* 1998. 56 (1–3), 285–291. https://doi.org/10.1016/S0168-3659(98)00095-9
40. *Jeong Y. I., Nah J. W., Lee H. C., Kim S. H., Cho C. S.* Adriamycin release from flower-type polymeric micelle based on star-block copolymer composed of poly(γ-benzyl l-glutamate) as the hydrophobic part and poly(ethylene oxide) as the hydrophilic part. *Int. J. Pharm.* 1999, 188 (1), 49–58. https://doi.org/10.1016/S0378-5173(99)00202-1
41. *Allen C., Han J., Yu Y., Maysinger D., Eisenberg A.* Polycaprolactone–b-poly(ethylene oxide) copolymer micelles as a delivery vehicle for dihydrotestosterone. *J. Control. Release.* 2000, 63 (3), 275–286. https://doi.org/10.1016/S0168-3659(99)00200-X
42. *Yallapu M. M., Bhusetty Nagesh P. K., Jaggi M., Chauhan S. C.* Therapeutic Applications of Curcumin Nanoformulations. *AAPS J.* 2015, 17 (6), 1341–1356. https://doi.org/10.1208/s12248-015-9811-z
43. *Selvam P., El-Sherbiny I. M., Smyth H. D.* Swellable hydrogel particles for controlled release pulmonary administration using propellant-driven metered dose inhalers. *J. Aerosol Med. Pulm. Drug Deliv.* 2011, 24 (1), 25–34. https://doi.org/10.1089/jamp.2010.0830

44. *Ye Y., Li Y., Fang F.* Upconversion nanoparticles conjugated with curcumin as a photosensitizer to inhibit methicillin-resistant Staphylococcus aureus in lung under near infrared light. *Int. J. Nanomedicine.* 2014, V. 9, P. 5157–5165. https://doi.org/10.2147/IJN.S71365
45. *Pardridge W. M.* Blood–brain barrier delivery. *Drug Discovery Today*. 2007, 12 (1), 54–61. https://doi.org/10.1016/j.drudis.2006.10.013
46. *Andrieux K., Couvreur P.* Nanomedicine as a promising approach for the treatment and diagnosis of brain diseases: the example of Alzheimer's disease. *Ann. Pharm. Fr. Elsevier*. 2013, 71 (4), 225–233. https://doi.org/10.1016/j.pharma.2013.04.001
47. *Tsai Y. M., Chien C. F., Lin L. C., Tsai T. H.* Curcumin and its nano-formulation: the kinetics of tissue distribution and blood–brain barrier penetration. *Int. J. Pharmac.* 2011, 416 (1), 331–338. https://doi.org/10.1016/j.ijpharm.2011.06.030
48. *Gravier J., Sancey L., Hirsjärvi S., Rustique E., Passirani C., Benoît J. P., Coll J. L., Texier I.* FRET Imaging Approaches for *in Vitro* and *in Vivo* Characterization of Synthetic Lipid Nanoparticles. *Mol. Pharmac.* 2014, 11 (9), 3133–3144. https://doi.org/10.1021/mp500329z
49. *Charron D. M., Zheng G.* Nanomedicine development guided by FRET imaging. *Nano Today.* 2018, V. 18, P. 124–136. https://doi.org/10.1016/j.nantod.2017.12.006
50. *Hewlings S. J., Kalman D. S.* Curcumin: A Review of Its' Effects on Human Health. *Foods.* 2017, 6 (10), 92, 1–11. https://doi.org/10.3390/foods6100092
51. *Kabeer A., Mailafiya M. M., Danmaigoro A., Rahim E. A., bu Bakar M. Z. A.* Therapeutic potential of curcumin against lead-induced toxicity. A review. *Biomed. Res. Therapy*. 2019, 6 (3), 3053–3066. https://doi.org/10.15419/bmrat.v6i3.528
52. *Menon V. P., Sudheer A. R.* Antioxidant and anti-inflammatory properties of curcumin. *Adv. Exp. Med. Biol.* 2007, V. 595, P. 105–125. https://doi.org/10.1007/978-0-387-46401-5_3
53. *Cai W., Zhang B., Duan D., Wu J., Fang J.* Curcumin targeting the thioredoxin system elevates oxidative stress in HeLa cells. *Toxicol. Appl. Pharmacol*. 2012, 262 (3), 341–348. https://doi.org/10.1016/j.taap.2012.05.012
54. *Lian Y. T., Yang X. F., Wang Z. H., Yang Y., Yang Y., Shu Y. W., Cheng L. X., Liu K.* Curcumin serves as a human kv1.3 blocker to inhibit effector memory T lymphocyte activities. *Phytother. Res.* 2013, 27 (9), 1321–1327. https://doi.org/10.1002/ptr.4863
55. *Kocaadam B., Sanlier N.* Curcumin, an active component of turmeric (Curcuma longa), and its effects onhealth. *Crit. Rev. Food Sci. Nutr.* 2017, 57 (13), 2889–2895. https://doi.org/10.1080/10408398.2015.1077195
56. *Ashrafizadeh M., Zarrabi A., Hashemi F., Zabolian A., Saleki H., Bagherian M., Azami N., Bejandi A. K., Hushmandi K., Ang H. L., Makvandi P., Khan H., Kumar A. P.* Polychemotherapy with Curcumin and Doxorubicinvia Biological Nanoplatforms: EnhancingAntitumor Activity. *Pharmaceutics.* 2020, 12 (11), 1084. https://doi.org/10.3390/pharmaceutics12111084
57. *Kunnumakkara A. B., Harsha C., Banik K., Vikkurthi R., Sailo B. L., Bordoloi D., Gupta S. C., Aggarwal B. B.* Is curcumin bioavailability a problem in humans: lessons from clinical trials. *Expert Opinion on Drug Metabolism & Toxicology*. 2019, 15 (9), 705–733. https://doi.org/10.1080/17425255.2019.1650914
58. *Marchiani A., Rozzo C., Fadda A., Delogu G., Ruzza P.* Curcumin and curcumin-like molecules: Fromspice to drugs. *Curr. Med. Chem.* 2014, 21 (2), 204–222. https://doi.org/10.2174/092986732102131206115810
59. *Priyadarsini K. I., Maity D. K., Naik G. H., Kumar M. S., Unnikrishnan M. K., Satav J. G., Mohan H.* Role of phenolic O-H and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. *Free Radic. Biol. Med.* 2003, 35 (5), 475–484. https://doi.org/10.1016/s0891-5849(03)00325-3
60. *Kunwar A., Barik A., Mishra B., Rathinasamy K., Pandey R., Priyadarsini K.* Quantitative cellular uptake, localization and cytotoxicity of curcumin in normal and tumor cells. *Biochim. Biophys. Acta*. 2008, 1780 (4), 673–679. https://doi.org/10.1016/j.bbagen.2007.11.016
61. *Di Martino R. M. C., Bisi A., Rampa A., Gobbi S., Belluti F.* Recent progress on curcumin-based therapeutics: a patent review (2012-2016). Part II: curcumin derivatives in cancer and neurodegeneration. *Expert Opinion on Therapeutic Patents.* 2017, 27 (8), 953–965. https://doi.org/10.1080/13543776.2017.1339793
62. *Anand P., Kunnumakkara A. B., Newman R. A., Aggarwal B. B.* Bioavailability of curcumin: problems and promises. *Mol. Pharm*. 2007, 4 (6), 807–818. https://doi.org/10.1021/mp700113r
63. *Basnet P., Skalko-Basnet N.* Curcumin: an anti-inflammatory molecule from a curry spice on the path to cancer treatment. *Molecules.* 2011, 16 (6), 4567–4598. https://doi.org/10.3390/molecules16064567
64. *Gupta S. C., Patchva S., Aggarwal B. B.* Therapeutic roles of curcumin: lessons learned from clinical trials. *The AAPS J.* 2013, 15 (1), 195–218. https://doi.org/10.1208/s12248-012-9432-8

65. *Joe B., Vijaykumar M., Lokesh B. R.* Biological properties of curcumin-cellular and molecular mechanisms of action. *Crit. Rev. Food Sci. Nutr.* 2004, 44 (2), 97–111. https://doi.org/10.1080/10408690490424702
66. *Naik S. R., ThakareV. N., Patil S. R.* Protective effect of curcuminon experimentally induced inflammation, hepatotoxicity and cardiotoxicityin rats: Evidence of its antioxidant property. *Exp. Toxicol. Pathol.* 2011, 63 (5), 419–431. https://doi.org/10.1016/j.etp.2010.03.001
67. *Gupta S. C., Patchva S., Koh W., Aggarwal B. B.* Discovery of curcumin, a component of golden spice, and its miraculous biological activities. *Clin. Exp. Pharmacol. Physiol.* 2012, 39 (3), 283–299. https://doi.org/10.1111/j.1440-1681.2011.05648.x
68. *Goel A., Aggarwal B. B.* Curcumin, the golden spice from Indian saffron, is a chemosensitizer and radiosensitizer for tumors and chemoprotector and radioprotector for normal organs. *Nutr. Cancer.* 2010, 62 (7), 919–930. https://doi.org/10.1080/01635581.2010.509835
69. *Fang J., Lu J., Holmgren A.* Thioredoxin reductase is irreversibly modified by curcumin: a novel molecular mechanism for its anticancer activity. *J. Biol. Chem.* 2005, 280 (26), 25284–25290. https://doi.org/10.1074/jbc.M414645200
70. *Shi L. Y., Zhang L., Li H., Liu T. L., Lai J. C., Wu Z. B., Qin J.* Protective effects ofCurcumin on acrolein-induced neurotoxicity in HT22 mousehippocampal cells. *Pharmacol. Reports.* 2018, 70 (5), 1040–1046. https://doi.org/10.1016/j.pharep.2018.05.006
71. *Mary C. P. V., Vijayakumar S., Shankar R.* Metal chelating ability and antioxidant properties of Curcumin-metal complexes – A DFT approach. *J. Mol. Graph. Model.* 2018, V. 79, P. 1–14. https://doi.org/10.1016/j.jmgm.2017.10.022
72. *Imran M., Ullah A., Saeed F., Nadeem M., Arshad M. U., Suleria H. A. R.* Cucurmin, anticancer, and antitumor perspectives: A comprehensive review. *Crit. Rev. Food Sci. Nutr.* 2018, 58 (8), 1271–1293. https://doi.org/10.1080/10408398.2016.1252711
73. *Naksuriya O., Okonogi S., Schiffelers R. M., Hennink W. E.* Curcumin nanoformulations: A review of pharmaceutical properties and preclinical studies and clinical data related to cancer treatment. *Biomaterials.* 2014, 35 (10), 3365–3383. https://doi.org/10.1016/j.biomaterials.2013.12.090
74. *Aggarwal B. B., Harikumar K. B.* Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *Int. J. Biochem. Cell Biol.* 2009, 41 (1), 40–59. https://doi.org/10.1016/j.biocel.2008.06.010
75. *Hatamipour M., Johnston T. P., Sahebkar A.* One molecule, many targetsand numerous effects: the pleiotropy of curcumin lies in its chemicalstructure. *Curr. Pharm. Des.* 2018, 24 (19), 2129–2136. https://doi.org/10.2174/1381612824666180522111036
76. *Perrone D., Ardito F., Giannatempo G., Dioguardi M., Troiano G., Russo L. L., DE Lillo A., Laino L., Muzio L. L.* Biological and therapeutic activities, and anticancer properties of curcumin. *Exp. Ther. Med.* 2015, 10 (5), 1615–1623. https://doi.org/10.3892/etm.2015.2749
77. *Sa G., Das T.* Sa G.,Das T.Anti cancer effects of curcumin: cycle of life and death. *Cell Division.* 2008, 3 (14). https://doi.org/10.1186/1747-1028-3-14
78. *Rattis B. A. C., Ramos S. G., Celes M. R. N.* Curcumin as a Potential Treatment for COVID-19. *Front. Pharmacol.* 2021, P. 12. https://doi.org/10.3389/fphar.2021.675287
79. *Di Martino R. M. C., Luppi B., Bisi A., Gobbi S., Rampa A., Abruzzo A., Belluti F.* Recent progress on curcumin-based therapeutics: a patent review (2012–2016). Part I: Curcumin. *Expert Opinion on Therapeutic Patents.* 2017, 27 (5), 579–590. https://doi.org/10.1080/13543776.2017.1276566
80. *Panahi Y., Alishiri G. H., Parvin S., Sahebkar A.* Mitigation of systemic oxidative stress by curcuminoids in osteoarthritis: Results of a randomized controlled trial.*J. Diet. Suppl.* 2016, 13 (2), 209–220. https://doi.org/10.3109/19390211.2015.1008611
81. *Shoba G., Joy D., Joseph T., Majeed M., Rajendran R., Srinivas P. S.* Influence of piperine on the pharmacokinetics of curcumin inanimals and human volunteers. *Planta Med.* 1998, 64 (4), 353–356. PMID: 9619120. https://doi.org/10.1055/s-2006-957450
82. *Attia Y. M., El-Kersh D. M., Ammar R. A., Adel A., Khalil A., Walid H., Eskander K., Hamdy M., Reda N., Mohsen N. E., Al-Toukhy G. M., Mansour M. T., Elmazar M. M.* Inhibition of aldehyde dehydrogenase-1 and p-glycoprotein-mediated multidrug resistance by curcumin and vitamin D3 increases sensitivity to paclitaxel in breast cancer. *Chemico-Biological Interactions.* 2020, V. 315, P. 108865. https://doi.org/10.1016/j.cbi.2019.108865
83. *Priyadarsini K. I.* The Chemistry of Curcumin: From Extraction to Therapeutic Agent. *Molecules.* 2014, 19 (12), 20091–20112. https://doi.org/10.3390/molecules191220091
84. *Hussain A., Somyajit K., Banik B., Banerjee S., Nagaraju G., Chakravarthy A. R.* Enhancing the photocytotoxic potential of curcumin on terpyridyl-lanthanide(III) complex formation.

Dalton Trans. 2013, 42 (1), 182–195. https://doi.org/10.1039/c2dt32042h

85. *Zhou S. S., Xue X., Wang J. F., Dong Y., Jiang B., Wei D., Wan M. L., Jia Y.* Synthesis, optical properties and biological imaging of the rare earth complexes with curcumin and pyridine. *J. Mater. Chem.* 2012, 22 (42), 22774–22780. https://doi.org/10.1039/c2jm34117d
86. *Song Y. M., Xu J. P., Ding L., Hou Q., Liu J. W., Zhu Z. L.* Syntheses, characterisation and biological activities of rare earth metal complexes with curcumin and 1,10-phenanthroline-5,6-dione. *J. Inorg. Biochem.* 2009, 103 (3), 396–400. https://doi.org/10.1016/j.jinorgbio.2008.12.001
87. *Cheng L., Hsu C. H., Lin J. K., Hsu M. M., Ho Y. F., Shen T. S., Ko J. Y., Lin J. T., Lin B. R., Wu M. S., Yu H. S., Jee S. H., Chen G. S., Chen T. M., Chen C. A., Lai M. K., Pu Y. S., Pan M. H., Wang Y. J., Tsai C. C., Hsieh C. Y.* Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res.* 2001, 21 (4B), 2895–2900.
88. *Mohammed F., Rashid-Doubell F., Taha S., Cassidy S., Fredericks S.* Effects of curcumin complexes on MDA-MB-231 breast cancer cell proliferation. *Int. J. Oncol.* 2020, 57 (2), 445–455. https://doi.org/10.3892/ijo.2020.5065
89. *Mourmoura E., Vial G., Laillet B., Rigaudiere J. P., Hininger-Favier I., Dubouchaud H., Morio B., Demaison L.* Preserved endothelium-dependent dilatation of the coronary microvasculature at the early phase of diabetes mellitus despite the increased oxidative stress and depressed cardiac mechanical function *ex vivo*. *Cardiovasc Diabetology*. 2013, 12 (49), 1169–1186. https://doi.org/10.1186/1475-2840-12-49
90. *Rebillard A., Lefeuvre-Orfila L., Gueritat J., Cillard J.* Prostate cancer and physical activity: Adaptive response to oxidative stress. *Free Radic. Biol. Med.*. 2013, V. 60, P. 115–124. https://doi.org/10.1016/j.freeradbiomed.2013.02.009
91. *Edwards R. L., Luis P. B., Varuzza P. V., Joseph A. I., Presley S. H., Chaturvedi R., Schneider C.* The anti-inflammatory activity of curcumin is mediated by its oxidative metabolites. *J. Biol. Chem.* 2017, 292 (52), 21243–21252. https://doi.org/10.1074/ jbc.RA117.000123
92. *Larasati Y. A., Yoneda-Kato N., Nakamae I., Yokoyama T., Meiyanto E., Kato J.* Curcumin targets multiple enzymes involved in the ROS metabolic pathway to suppress tumor cell growth. *Sci. Rep.* 2018, 8 (2039). https://doi.org/10.1038/s41598-018-20179-6
93. *Hua C., Kai K., Bi W., Shi W., Liu Y., Zhang D.* Curcumin Induces Oxidative Stress in Botrytis cinerea, Resulting in a Reduction in Gray Mold Decay in Kiwifruit. *J. Agric. Food Chem.* 2019, 67 (28), 7968–7976. https://doi.org/10.1021/acs.jafc.9b00539
94. *Ellis E. M.* Reactive carbonyls and oxidative stress: Potential for therapeutic intervention. *Pharmacol. Ther.* 2007, 115 (1), 13–24. https://doi.org/10.1016/j.pharmthera.2007.03.015
95. *Rajamanickam V., Yan T., Wu L., Zhao Y., Xu X., Zhu H., Chen X., Wang M., Liu Z., Liu Z., Liang G., Wang Y.* Allylated Curcumin Analog CA6 Inhibits TrxR1 and Leads to ROS-Dependent Apoptotic Cell Death in Gastric Cancer Through Akt-FoxO3a. *Cancer Manag. Res.* 2020, V. 12, P. 247–263. https://doi.org/10.2147/CMAR.S227415
96. *Priyadarsini K. I.* Photophysics, photochemistry and photobiology of curcumin: Studies from organic solutions, biomimetics and living cells. *J. Photochem. Photobiol. C: Photochem. Rev.* 2009, 10 (2), 81–95. https://doi.org/10.1016/j.jphotochemrev.2009.05.001
97. *Patra D., El Khoury E., Ahmadieh D., Darwish S., Tafech R. M.* Effect of Curcumin on Liposome: Curcumin as a Molecular Probe for Monitoring Interaction of Ionic Liquids with 1, 2-Dipalmitoyl-sn-Glycero-3-Phosphocholine Liposome. *Photochem. Photobiol.* 2012, 88 (2), 317–327. https://doi.org/10.1111/j.1751-1097.2011.01067.x
98. *Chainoglou E., Hadjipavlou-Litina D.* Curcumin analogues and derivatives with anti-proliferative and anti-inflammatory activity: Structural characteristics and molecular targets. *Expert Opin. Drug Discov.* 2019, 14 (8), 821–842. https://doi.org/10.1080/17460441.2019.1614560
99. *Sahebkar A., Serban M. C., Ursoniu S., Banach M.* Effect of curcuminoids on oxidative stress: A systematic review and meta-analysis of randomized controlled trials. *J. Funct. Foods.* 2015, V. 18 B, P. 898–909. https://doi.org/10.1016/j.jff.2015.01.005
100. *Badria F. A., Ibrahim A. S., Badria A. F., Elmarakby A. A.* Curcumin Attenuates Iron Accumulation andOxidative Stress in the Liver and Spleen ofChronic Iron-Overloaded Rats. *PLoS ONE.* 2015, 10 (7), e0134156.1–13. https://doi.org/10.1371/journal.pone.0134156
101. *Yuan J., Liu W., Zhu H., Zhang X., Feng Y., Chen Y., FengH., Lin J.* Curcumin attenuates blood-brain barrier disruption after subarachnoid hemorrhage in mice. *J. Surg. Res.* 2017, 207 (30), 85–91. https://doi.org/10.1016/j.jss.2016.08.090

МУЛЬТИФУНКЦІОНАЛЬНІ НАНОСИСТЕМИ НА ОСНОВІ КУРКУМІНУ

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Використання мультифункціональних наносистем у медицині та наукових дослідженнях є актуальним.

Мета. Узагальнити дані літератури стосовно перспектив створення та використання наноконтейнерів на основі куркуміну (Cur). Флуоресценція Cur у складі наночастинок (НЧ) дає можливість дослідити розподіл флуоресцентних та нефлуоресцентних компонентів, значно пришвидшити вивчення та впровадження препаратів у практику. Особливу увагу спрямовано на використання гідрофобних речовин у НЧ, які можуть проникати всередину живої клітини.

Розуміння взаємодії НЧ з живими клітинами є вкрай важливим у тих випадках, коли ці частинки використовують для транспортування та доставлення до клітин нерозчинних у воді лікарських засобів. Cur є одним із препаратів із різноманітними і дуже перспективними фармацевтичними ефектами, але він малорозчинний у водному середовищі, а використання наноносіїв є ефективним способом для необхідного значного збільшення його біодоступності. Cur має власну флуоресценцію, що дає можливість використати його як мультифункціональну флуоресцентну наносистему, наприклад, із міцелами Pluronic®.

Використання методу флуоресценції дає можливість простежити етапи взаємодії навантажених Cur НЧ із культивованими клітинами та їхню локалізацію в клітинних органелах.

За допомогою такого підходу в часі спостерігається наноразмірна динаміка розподілу та стійкості лікарських засобів.

Висновки. Для нестійких у водному середовищі препаратів, до яких відноситься Cur, слід застосовувати найбільш гідрофобні наноструктури без слідів води, до яких належать ядра міцел Pluronic®. Такий метод дає можливість використовувати інші малорозчинні у воді лікарські препарати.

Перспективним напрямом наномедицини є створення комплексних біосумісних наноматеріалів на основі декількох діючих препаратів, які зменшують токсичність ліків щодо нормальних клітин.

Ключові слова: мультифункціональні наносистеми, наноконтейнери для медичних препаратів, куркумін.

МУЛЬТИФУНКЦИОНАЛЬНЫЕ НАНОСИСТЕМЫ НА ОСНОВЕ КУРКУМИНА

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Использование мультифункциональных наносистем в медицине и научных исследованиях является актуальным.

Цель. Обобщение данных литературы относительно перспектив создания и использования наноконтейнеров на основе куркумина (Cur). Флуоресценция Cur в составе наночастиц (НЧ) дает возможность исследовать распределение флуоресцентных и нефлуоресцентных компонентов, значительно ускорив изучение и внедрение препаратов в практику. Особое внимание направлено на использование гидрофобных веществ в НЧ, которые могут проникать внутрь живой клетки.

Понимание взаимодействия НЧ с живыми клетками чрезвычайно важно в тех случаях, когда эти частицы используются для транспортировки и доставки к клеткам нерастворимых в воде лекарственных средств. Cur является одним из препаратов с различными и очень перспективными фармацевтическими эффектами, однако он малорастворим в водной среде, а использование наноносителей является эффективным способом для необходимого значительного увеличения его биодоступности. Cur имеет собственную флуоресценцию, что дает возможность использовать его в качестве мультифункциональной флуоресцентной наносистемы, например, с мицеллами Pluronic®.

Использование метода флуоресценции дает возможность проследить этапы взаимодействия нагруженных Cur НЧ с культивируемыми клетками и их локализацию в клеточных органеллах.

С помощью такого подхода во времени наблюдается наноразмерная динамика распределения и устойчивости лекарственных средств.

Выводы. Для неустойчивых в водной среде препаратов, к которым относится Cur, необходимо использовать наиболее гидрофобные наноструктуры без следов воды, к которым относятся ядра мицелл Pluronic®. Такой метод дает возможность использовать другие малорастворимые в воде лекарственные препараты. Перспективным направлением наномедицины является создание комплексных биосовместимых наноматериалов на основе нескольких действующих препаратов, которые уменьшают токсичность лекарств относительно нормальных клеток.

Ключевые слова: мультифункциональные наносистемы, наноконтейнеры для медицинских препаратов, куркумин.

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PRACTICAL USE OF GOAT MILK AND COLOSTRUM

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This review presents the protein and amino acid composition of both goat colostrums and milk and describes the properties of goat colostrums and milk components. In addition, the prospects of use of goat milk and colostrum in the food and cosmetics industry and the feasibility of use of goat milk for baby feeding are shown. Functional foods produced from goat milk have antioxidant, anti-inflammatory, cardioprotective, antihypertensive and antiatherogenic activities in the human body. Goat milk cosmetics are very useful for maintaining a healthy skin and are effective in treatment of various skin diseases. Infant formula based on goat milk provides comfortable digestion for babies and are better at absorbing proteins, fats and other nutrients than infant formula based on cow's milk.

Key words: goat colostrum, goat milk, proteins, amino acids, cosmetology, baby feeding.

Goat colostrum is a pure natural product containing a large amount of biologically active substances such as proteins, vitamins, minerals, antimicrobial peptides, immunoglobulins [1]. Therefore it is promising to create therapeutic and cosmetic products based on goat milk and colostrum, which protect the skin from aging, dermatological problems, harmful effects of the environment. Additionally, the nutritional value of goat colostrum and its lower allergenicity in comparison with bovine colostrum provoke interest to goat's milk and colostrum as functional products. Moreover, the creation of infant formula and fermented dairy products from goat's milk supposes significant advantagesduetoitsprobiotic and antioxidant properties.

Protein and amino acid composition of goat's milk and colostrum,the components' properties. Protein plays a fundamental role in the functional and technological value of goat colostrum. The total protein content in goat colostrum ranges from 2.6 to 4.1 g/l.

The protein composition of goat's milk is presented in the Table 1 [2].

Among these proteins, whey proteins have the highest biological activity. Thus, β-lactoglobulin has antitumor functions, participates in the regulation of enzyme activity, and is able to bind hydrophobic molecules such as retinol, fatty acids, steroids, etc. [3]. β-lactoalbumin promotes the growth of bifidobacteria, as well as assimilation of calcium and zinc [4].

Lactoferrin is an iron-binding glycoprotein, which plays an important role in iron homeostasis and cell proliferation; it has antibacterial, antifungal, antiviral, antioxidant, immunomodulatory and anticancer activities [5].

Ig A protects the intestinal mucosa against *E. coli*, it protects also the mucous membranes of the throat, lungs and intestines of infants. Ig M is an immunoglobulin, which is the first to be formed in response to antigen and is highly effective in boosting immunity by the complement fixation, agglutination and opsonic activity [6]. Ig G is involved in the formation of antibacterial immunity [7].

The amino acid composition of goat's milk [7] is presented in the Table. 2.

Table 1. **Protein composition of goat colostrum**

No.	Protein	Concentration,%
1	Total casein	2.33–4.63
2	α_{S1}-Casein	0–28
3	α_{S2}-Casein	10–25
4	β-Casein	0.6–64
5	κ-Casein	15–29
6	Whey proteins	0.37–0.7
7	β-Lactoglobulin	39.2–72.1
8	α-Lactalbumin	17.8–33.3
9	Lactoferrin	5.1–21.5
10	Immunoglobulins	4.6–21.4

Table 2. **Amino acid composition of goat's milk**

No.	Amino acid	Content, mg/100 g of milk
1	Threonine	138.67
2	Isoleucine	160.54
3	Leucine	341.01
4	Lysine	342.86
5	Methionine	77.95
6	Valine	210.23
7	Arginine	135.65
8	Cysteine	30.62
9	Phenylalanine	175.45
10	Tyrosine	162.51
11	Histidine	122.73
12	Asparagine	117.95
13	Alanine	250.15
14	Glutamine	694.58
15	Glycine	55.83
16	Proline	310.61
17	Serine	152.65

From the data presented, the conclusion can be made that leucine, lysine and valine predominate among the essential amino acids in goat's milk proteins, whereas glutamine, alanine and proline are found in the largest amounts among the nonessential amino acids.

Amino acids in goat's milk and colostrum are not only substrates for biosynthetic and energy processes, but also important regulators of physiological functions, including hormone secretion, nerve impulse conduction, and modulation of the immune response.

Glutamic and aspartic acids, tryptophan, lysine and arginine have the highest immuno- and phagocytosis-stimulating activity.

Among amino acids, a special place is occupied by leucine, which regulates the processes of protein breakdown and synthesis *in vivo*. The experiments on peritoneal neutrophils have shown that leucine enhances phagocytosis of staphylococci by neutrophils.

The effect of leucine on the phagocytic activity of neutrophils in the umbilical cord blood of newborns was investigated in the work of Russian scientists [8]. It was found that, depending on the concentration, leucine has a different effect on the ability of neutrophils to phagocytosis. The dose of 0.1 mg/ml is optimal, which stimulates the completion of phagocytosis – the index of phagocytosis completion increases twice, the phagocytic index increases from 53.2 to 63.9%, and the phagocytic number from 7.5 to 9.33, compared with the control.

The branched-chain amino acids valine, leucine and isoleucine are important amino acids in the immune system. In [9] De Simone et al. evaluated the effect of these amino acids on microglia, the main immune cells of the brain. It was found that the interleukin IL-10 expression and phagocytic activity increase under the influence of these amino acids.

Studies by Chinese scientists [10] have shown that valine enhances the phagocytosis of macrophages, thereby inhibiting the growth of infectious agents *Klebsiella pneumoniae*, *Escherichia coli* in infected tissues. Two mechanisms are involved in this acivity: valine activates the PI3K/Akt1 pathway and promotes NO production through inhibition of arginase activity. The valine-arginine combination effectively kills *K. pneumoniae* and other gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus*) bacteria.

L-arginine also plays an important role in the immune system functioning. First of all, nitric oxide, formed from arginine, has a specific immunomodulatory effect and act as one of the main effectors of cellular immunity, causing antimicrobial, antitumor and antiproliferative effects.

Some studies demonstrate that arginine plays an important role in the ability of macrophages to produce NO, which has many physiological functions, including enhancing the cytotoxicity of macrophages.

The absence of L-arginine blocks the re-expression of T-cell receptors in response to

antigenic stimulation, and is accompanied by a decrease in cell proliferative activity [11].

Arginine is also known to be important for the normal proliferation and functioning of T-cells. The maximum proliferation of T-lymphocytes is achieved at the arginine level in the environment of 100 μmol/l, and no further increase in the proliferation rate is observed at higher concentrations. At arginine deficiency, a progressive decrease in the number of T-cell receptors on the cell membrane occurs.

Additionally, arginine is required for the killing of tumor cells by activated macrophages. Besides, arginine in high concentrations increases the cytotoxicity of monocytes *in vitro*.

Thus, arginine stimulates immunity due to the action on lymphocytes, macrophages and dendritic cells [12].

Kurtseva et al. presented the results of studying the reparative and immunotropic action of a combination of amino acids (glycine + histidine + *lysine*) with *lysine* [13]. It was found that the combination of amino acids – lysine and glycine – enhanced the functions of neutrophils.

When studying the immunotropic effects of amino acids separately, it was found that histidine did not influence the neutrophils function whereas glycine and lysine had a stimulating effect. An addition of glycine resulted in an increase of phagocytic index (PhI) by 1.5 times, and phagocytic number (PhN) — by 1.6 times. An increase in the level of stimulated reaction of the NBT-test by 1.3 times was also observed. An addition of lysine was accompanied by an increase in the absorption capacity of neutrophils, as evidenced by an increase in (PhN) by 1.8 times compared with the control.

It can be assumed that an increase in the absorption capacity of phagocytosis under the influence of lysine is associated with its stress-limiting effect.

According to some data, lysine has a stimulating effect on the phagocytic activity of neutrophils whereas no significant changes in specific indicators of the immune response were detected.

Researches of a number of virologists have revealed that L-lysine is able to inhibit the replication of the herpes simplex virus in cells and thus to reduce the disease duration [14].

Threonine is a major component of intestinal mucins and IgA, which are secreted during lipopolysaccharide (LPS)-induced inflammation. The results of the *in vivo* study in chickens [15] showed that threonine deficiency causes a violation of the inflammatory and secretory immune response.

The need for threonine during lymphocyte proliferation and immunoglobulin A production was established *in vitro* and *in vivo*.Therefore, threonine is a key element involved in the production of mucins and IgA to maintain the intestinal secretory immune system.

Thus, among the essential amino acids, leucine, valine, arginine and lysine have the greatest influence on immune responses. These amino acids enhance the phagocytic activity of macrophages and stimulate the formation of NO in the body, which in turn is able to have a specific immunomodulatory effect and act as one of the main effectors of cellular immunity.

The nonessential amino acids perform also many important functions in the body.

Scientists [16] have found that glutamic acid plays an important role in maintaining lymphocyte homeostasis. It is known that in a medium devoid of glutamic acid or glutamine, lymphocytes completely lose the ability to proliferate.

The ability of glutamic acid to modulate the functional state of lymphocytes by receptor mechanisms was also established.

In [17], the scientists studied experimentally the immunocorrective properties of the composition phenotropil + L-glutamic acid in the models of cyclophosphamide immunodepression and LPS-induced immune stress. It was found that this composition restores cellular and humoral parts of immunity as well as lymphoproliferative processes in immunocompetent organs.

It was found that glutamic acid has a stimulating effect on the activity of the immune system due to increasing the number of antibody- and rosette-forming cells in the spleen of laboratory animals with immunosuppression.

There is growing evidence that some amino acids play a role in the regulation of key metabolic pathways required for maintenance, growth, reproduction, and immunity.

The scientists [18] evaluated the modulating functions of several amino acids in protective immunity against herpes simplex virus type 1 (HSV-1). It was found that glutamine and leucine show increased immune protection against HSV-1 with double administration of glutamine and a single injection of leucine per day. Here, an increased formation of interferon IFNγ in the vaginal tract was observed on the 2^{nd} and 4^{th} days

after infection. The activity of *NK*- and *Th1*-type *CD4 + T* cells, which increases under the influence of glutamine and leucine, is thought to play a crucial role in providing effective protective immunity against HSV-1 infections.

Additionally, glutamine and arginine increase the expression of antioxidant genes and decrease the expression of pro-inflammatory genes in the small intestine and adipose tissue.

It has been shown that glutamine affects the growth of immune cells, the functioning of T cells, as well as the immunoglobulin A (IgA) synthesis [19].

Chinese scientists [20] show also that arginine and glutamine affect the immune responses and intestinal morphology of striped perch. Arginine and glutamine tend to improve the production of neutrophil oxidative radicals. They increase the activity of lysozyme as well. Glutamine is the main source of energy of enterocytes and immune cells. Both arginine and glutamine have been shown to modulate the immune functions in pathological conditions in mammals. Additionally, some studies show that glutamine may be a precursor to arginine for the synthesis of nitric oxide (NO) in immune cells.

Glutamine as the main energy substrate for leukocytes and a key modulator of the cytokine and NO production plays a crucial role in the immune response.

Aspartic acid is also important for the immune system. Scientists from Russia have found [19] that the inclusion of 10% L-aspartic acid to the mineral complex in the diets of piglets is sufficient to provide higher protective properties in animals. Under the influence of this amino acid, the highest phagocytic activity was observed.

Since aminoacids are absorbed in large quantities and the body's need for the mishigh, a so-called «deception» of the body's absorption system occurs, and most likely a change in the phagocytic activity of the blood serum of guinea pigs in the experimental groups towards higher values indicates the intensity of the phagocytosis process, which is most pronounced in animals of the group receiving 10% aspartic acid.

Proline is a key regulator of several biochemical and physiological processes in cells. For example, proline is a signaling molecule, a cellular energy sensor, and a source of superoxide-anion, a free radical involved in redox reactions in humans and animals. Additionally, proline takes part in protecting lymphocytes against apoptosis by stimulating cell growth and antibody synthesis. Proline-enriched polypeptide complex can affect not only adaptive immunity, but also innate immunity, thereby regulating the secretion of inflammatory mediators. In general, proline plays an important role in physiological and immunological functions [21].

One more study was performed to determine the immunostimulatory effect of proline on vaccine-inactivated immunized mice. The survival of mice, the diet of which was supplemented with 0.4% L-proline was higher, indicating an immunostimulatory effect.

Thus, nonessential amino acids also significantly affect the immune response in the body. The leading role is played by glutamic acid, aspartic acid, proline and serine, which are present in high concentrations in goat milk and colostrum. The mechanism of the immunostimulating action of these amino acids consists in maintenance of homeostasis of lymphocytes, stimulation of the development of antibody- and rosette-forming cells in the spleen, enhancementof the interferon formation, an increase of the expression of antioxidant genes and a decrease of the expression of pro-inflammatory genes. Amino acids also enhance phagocytic activity, protect lymphocytes against apoptosis by stimulating antibody synthesisand control T-cell proliferation.

The effect of goat's milk on the skin and its use in cosmetology. Recently, variety of cosmetics are produced in the world from goat's milk, including soaps, creams, body lotions, shampoos, hair conditioners and aftershave lotions. Large cosmetic companies show increasing interest in colostrum-based nutraceutical creams, which help to restore and accelerate the recovery of aging skin [22].

Scientists performed a comparative analysis of goat's and cow's milk and found that the substances of goat's milk more easily penetrate into the deep layers of the skin, since they contain oleic or caprylic fatty acid, and more quickly saturate the skin with vitamins, phospholipids, and fatty acids. This property of goat's milk is associated with high content of fatty acids.

Goat milk replenishes the deficiency of fats and linolenic acid (its content in goat milk is 1.5 times higher than in cow's milk) and provides other fatty acids, linoleic and arachidonic, which are necessary for the skin but not synthesized by the body. Thanks to the fatty acids in goat milk, the skin becomes smooth and healthy.

Essential fatty acids are the precursors of pharmacologically active substances involved in cell division and epidermal differentiation. They are able to change the inflammatory and immunological reactions, which modify the functions of leukocytes and accelerate the process of tissue granulation. Goat's milk acts as a good skin regenerator because it contains caprylic acid. Additionally, the fat globules due to their small size penetrate into the middle layer of the skin (dermis), moisturizing and rejuvenating it [23].

Unsaturated fatty acids are involved in the regeneration of epidermal cells. In goat's milk, they are absorbed into the skin faster due to lower melting point. For comparison, fatty acids in cow's milk melt at the temperature 29–42 °C, i.e. higher than the human body' stemperature, which greatly complicates their absorption by the skin. The fatty acids of goat's milk begin to be actively absorbed and work already at a temperature of 26–28 °C.

Addition, unsaturated fatty acids enhance the anti-aging effect of the components of goat milk – vitamins and phospholipids.

With age, the amount of skin phospholipids decreases significantly, which is associated with lower rates of their formation and self-healing processes in the epidermis. Lack of phospholipids leads to metabolic disorders and impaired blood circulation, which resulted in rapid aging of the skin. Goat milk's phospholipids supplement the skin's phospholipid deficiency, improve blood microcirculation, restore the activity of epidermal cell membranes and slow down the aging process [24].

It has been established that milk lactoferrin shows a significant improvement in dermatological symptoms in the treatment of fungal skin infections [25].

Peptides isolated from goat's milk are often used in the manufacture of cosmetics. For example, hydrolyzed milk protein is used in 200 formulations.

Sodium caseinate is most often used in production of bath salts and oils, in concentrations up to 0.1% — in the facial and neck care products.

Whey protein is used in face and neck skin care products.

Some of these peptides can be used in products that may come into contact with mucous membranes and eyes. For example, milk proteins in concentrations up to 0.5% are used in eye makeup.

Additionally, some milk peptides have been reported in deodorants, hair sprays, face powders, face and neck sprays, body and hand sprays, perfumes. For example, casein was used in deodorant. Milk peptides at a concentration of 0.0002% are also used in face powders [26].

Another advantage of goat milk's proteins is a higher content of amino acidsleucine, glutamine and asparagine as compared with cow's milk. These amino acids help to quickly heal wounds, microcracks on the skin, have an anti-inflammatory effect [27].

Goat milk contains also a large amount of zinc. Zinc is an important element for maintaining healthy skin, wound healing and is directly involved in both innate and adaptive immunity. Zinc has antioxidant activity and helps to eliminate oxygen reactive species due to its role as a cofactor of the antioxidant enzyme superoxide dismutase [28].

Goat milk is an essential source of vitamins: vitamin A, thiamine, riboflavin and niacin. However, compared to cow's milk, goat's milk has less vitamin E, folate and vitamin B12 [29].

Vitamin A enhances the processes of collagen and elastin synthesis in the skin. Thanks to vitamin A, the skin becomes smooth and elastic, as this vitamin regulates the growth of epidermal cells [24, 29]. Vitamin B1 (thiamin) has a positive effect at seborrhea, alopecia, dermatitis and other skin problems. Vitamin B2 (riboflavin) promotes wound healing. Niacin improves cellular respiration, gives a healthy tone and smoothness to the skin [24].

Goat's milk also contains a significant number of growth factors. Goat colostrum growth factors help in building and regenerating the skin.

Epidermal growth factors (EGF) help to prevent the destruction of skin cells that can occur at autoimmune disorders. Transforming growth factor (TGF) and insulin-like growth factor (IGF-1) stimulate skin growth and cell growth and restore it by direct action on DNA and RNA, improving wound healing [30]. Transforming growth factor β (TGF-β), acidic and alkaline fibroblast growth factors (FGF), milk growth factor (MGF) have a protective and regenerating effect on the skin.

Considering this, it is advisable to use goat's milk in regenerating cosmetics.

The main task of regenerating cosmetics is to stimulate cell division of the basal layer of the epidermis. Almost all the means, which are combined with the concept of "regenerating", to some extent affect the rate of cell division. However, most often they play secondary role. They only create certain conditions for better

cells division but do not stimulate division themselves.

Goat milk effectively promotes immune defense of the skin and slows down its aging by the influence on cellular immunomodulation. Goat milk is a source of a complex of antioxidants: amino acids of glutathione, vitamins E, C, A, selenium, Q-enzyme, proteins — lactoperoxidase, lactoferrin, alpha-lactoalbumin. These substances strengthen cell membranes, keeping cells healthy [24].

Many cosmetics are made from goat milk — soaps, creams, lotions, facemasks. Goat milk soap does not contain harsh chemicals and is therefore ideal for sensitive skin. This soap prevents irritation and damage to the skin. It is often recommended by dermatologists and is successfully used to heal people with skin conditions such as psoriasis, eczema, acne, xerodermia and itch [31].

Goat milk is used to produce antibacterial soap, which can inhibit the growth of *S. aureus, B. subtilis, Candida albicans, Micrococcus, Enterococcus, Staphylococcus epidermidis*. Therefore it is effective in treating acne and skin infections, as it inhibits the growth of pathogens [32].

A face mask was developed on the basis of fermented goat milk. Its whey peptides and lactic acid were shown to reduce skin pigmentation; besides, lactic acid inhibits the growth of the causative agent of acne *Propionibacterium acnes* [33].

A cosmetic product containng goat milk and sweet orange extract has been also developed. This product has moisturizing and anti-allergic properties and is effective in the prevention and treatment of eczema [34].

So, considering the significant content of various biologically active substances in goat milk and colostrum, they are used to create the cosmetics effective in combating skin diseases such as psoriasis, eczema, acne, etc.

The use of goat's milk in food. Another promising area of use of goat's milk and colostrum is the production of food products.

Goat milk triggers innate and adaptive immune responses in the human body. Consumption of goat's milk increases the production of NO in the body, which has a cardioprotective and antiatherogenic effect. Additionally, the induction of proinflammatory (TNF- and IL-6) and anti-inflammatory (IL-10) cytokines indicates the ability of goat's milk to maintain immune homeostasis in humans, especially aged people, since they have a reduced immune response, and as a result the elderly demonstrate an increase in infections, cancer and autoimmune diseases. That is why dietary products based on goat's milk can be useful in strengthening age-related immunity [35].

Goat milk has a specific "goat" smell, which restrictsits consumption. The specific smell of goat's milk is associated primarily with the composition of fatty acids. The content of capronic, caprylic and capric acids in goat's milk is slightly higher than in cow's milk. During fermentation, goat's milk loses its odor whereas the nutritional value of goat's milk may increase. Therefore, the production of fermented dairy foodstuffs, such as cheese, kefir, yoghurt, is of great impotance.

Recent studies focused on the natural antioxidant components of milk and dairy products. For example, consumption of goat's milk cheese produced with *Lactobacillus fermentum* CRL1446 affects the activity of intestinal feruloyl esterase, which increases by 1.5 folds. Therefore, goat's milk cheese can be used as a functional product with the activity of feruloyl esterase, which is responsible for an increase of intestinal activity and bioavailability of the antioxidant of ferulic acid in the intestine, thereby providing protection against oxidative stress [36].

The antioxidant properties of kefir from goat milk were also investigated for the absorption of radicals, such as 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonicacid). It was found that the antioxidant activity increases during the first 8–12 hours of fermentation, and then gradually decreases. When stored for 21 days, the antioxidant activity of kefir increases also [37].

Fermented goat's milk, influencing antioxidant activity, plays a positive role in the treatment of iron deficiency anemia. After consumption of fermented goat's milk, a significant increase of some antioxidant endogenous enzymes was found, along with an increase in total level of antioxidants. Thereby milk protects the main cellular components (lipids, proteins, DNA) from oxidative damages occuring during anemia [38].

Probiotic lactobacilli are able to release bioactive peptides during milk fermentation. The antihypertensive effect of fermented goat's milk due to inhibiting angiotensin-converting enzyme (ACE) was investigated. Extremely high activity with regard to ACE inhibition was found in the goat's milk fermented with *Lactobacillus reuteri, Lactobacillus bulgaricus, Lactobacillus rhamnosus, Lactobacillus helveticus* [39].

Additionaly, goat's milk and yoghurtmade using a *Lactobacillus acidophilus* culture, have a protective effect against the intestinal lesions, so they can be used in functional foods at the inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, which are consumptive and immunologically mediated diseases, at which the excessive inflammatory reactions of the intestinal mucosa and destruction of the gastrointestinal tracttissues take place.

It has been proven that when goat's milk or yogurt is consumed, the activity of myeloperoxidase, the level of leukotriene B4, interleukin-1β and tumor necrosis factor-α significantly improved. Goat milk intake also contributes to a significant reduction in oxidative stress, which is manifested in a decrease of malodialdehyde and an increase of glutathione. This preserves the colon cytoarchitecture and reduces the expression of cyclooxygenase-2 and nitric oxide synthase [40].

Yoghurt is also made from goat's milk. Quality yogurt is produced by fermentation at a ratio of lactic acid bacteria – *Lactobacillus delbrueckii* ssp. *bulgaricus, Streptococcus thermophilus* and *L. rhamnosus* GG — 1:1:3 [41].

Cottage cheese is one more functional product made from goat milk. In its production, a starter culture containing *Lactobacillus acidophilus, Streptococcusthermophilus, Bifidobacteriumlongum, B. bifidum, B. infantis*is used.The nutritional and biological value of cottage cheese lies in alarge amount of proteins, lactose, calcium, phosphorus, and valuable probiotic cultures [42].

Recently, the production of functional fermented goat milk products supplemented with synbiotics has attracted attention in terms of health promotion. It has been shown that the most effective prebiotics regarding the antioxidant activity of fermented dairy products are inulin and fructo-oligosaccharide [43].

Also, a high-protein dessert for sports nutrition is made on the basis of goat's milk. Whey protein isolate and micellar casein in a ratio of 30/70 are used as protein sources with a complete amino acid composition, which makes it possible to obtain a dessert with a harmonious milk taste and a homogeneous structure [44].

Goat's milk and colostrum are natural protein bases for creating instant food mixtures with immunomodulatory properties and reduced, compared to cow lactation products, allergenicity. Thus, goat's milk and colostrum can be used as components of a healthy diet for the prevention and treatment of chronic diseases of the cardiovascular system — coronary insufficiency, atherosclerosis, hypertension.

To stabilize the biologically active substances of goat's milk and colostrum in the produced mixtures, the technologyis proposed, which includes preliminary removal of moisture by baromembrane methods, freezing with cryogenic liquids (liquid nitrogen),and sublimation drying [45].

Goat's milk is also used to make cheese and sweet cheese products containing caramel, cocoa and walnuts. Goat cheese is a high-quality protein product because it contains a large number of essential amino acids such as methionine, cysteine and tryptophan. Also, sweet cheese samplesare stable against bacteria and other microorganisms during storage. Thus, goat cheese can be considered as a useful product, especially for feeding children, because it is easily digested [46].

Thus, goat's milk and dairy products provide positive effect on the body due to their antioxidant, anti-inflammatory, cardioprotective, antihypertensive, antiatherogenic properties; they are functionalat iron deficiency anemia, inflammatory bowel disease.

Use of goat milk and colostrum for baby feeding. Good quality infant nutrition is essential for normal growth and development. Infant formula is used as supplement during breastfeeding, or if breast milk is insufficient or breastfeeding is not possible. Cow's milk is most commonly used in infant formula, but recent research shows that using goat's milk for infant feeding has many benefits.

First, whey proteins are often added to infant formulas as a source of main essential amino acids. The infant formulas made from goat's milk contain a sufficient amount of all essential and nonessential amino acids without any addition of whey proteins [47].

Second, comparing the breastfed infants and those fed by formulas with cow and goat milk, it was found that babies who consumed goat milk formulas gain better weight and height. This is likely due to higher protein content in goat's milk formulas than in breast milk and cow's milk formulas.

Also, when children are fed with goat milk formulas, there is a significant decrease in the frequency of colic, which indicates an improvement of the process of food digestion. Additionally, an improvement of

the fatty acids absorption in the intestine was found, which once again confirms the easier assimilation of the fatty components of the goat milk formula [48].

Based on the evaluation of clinical efficacy of the adapted goat milk-based formula, it was found that it meets the physiological needs of infants for basic nutrients and energy, and supports their normal physical development. Moreover, the majority of infants (95%) who consumed goat milk-based formulas showed good tolerance to the product and a high degree of the protein assimilation and utilization [49].

Goat's milk proteins in infant formula are absorbed faster in the gastrointestinal tract of infants than cow's milk proteins. The assimilation of essential amino acids in goat milk in infants does not differ in comparison with human milk [50].

The assimilation of goat milk proteins and the kinetics of their digestion are similar to those of breast milk proteins. Casein from goat milk is generally more efficiently absorbed than casein from cow's milk [51].

Additionally, goat milk contains smaller fat globules, which results in a more uniform distribution of fat in the milk and makes the digestion of goat milk easier.

Thus, feeding babies with goat milk-based formulas is close in efficiency to breastfeeding and has a number of advantages regarding the cow's milk-based formulas. Therefore, the use of goat milk-based formulas for feeding children provides comfortable digestion, prevention of functional disorders of the gastrointestinal tract, reduces the risk of inflammation of the intestinal mucosa.

Thus, goat's milk and colostrum are unique products that can be used to create cosmetics for maintaining healthy skin and treating skin diseases, as well as toproduce functional foods with antioxidant and anti-inflammatory properties and infant formulas, which are very useful due to good digestibility and the content of all substances necessary for the babies.

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REFERENCES

1. *Menchetti L., Traina G., Tomasello G., Casagrande-Proietti P., Leonardi L., Barbato O., Brecchia G.* Potential benefits of colostrum in gastrointestinal diseases. *Front Biosci*. 2016, V. 8, P. 331–351. https://doi.org/10.2741/s467
2. *Yadav A. K., Singh J., Yadav S. K.* Composition, nutritional and therapeutic values of goat milk: A review. *Asian J. Dairy Food Res.* 2016, 35 (2), 96–102. https://doi.org/10.18805/ajdfr.v35i2.10719
3. *Loch J. I., Bonarek P., Polit A., Świątek S., Czub M., Ludwikowska M., Lewiński K.* Conformational variability of goat β-lactoglobulin: Crystallographic and thermodynamic studies. *Int. J. Biol. Macromol.* 2015, V. 72, P. 1283–1291. https://doi.org/10.1016/ j.ijbiomac.2014.10.031
4. *Caroprese M., Ciliberti M. G., Albenzio M., Marino R., Santillo A., Sevi A.* Role of antioxidant molecules in milk of sheep. *Small Ruminant Res*. 2019, V. 180, P. 79–85. https://doi.org/10.1016/j.smallrumres.2019.07.011
5. *Medeiros G. K. V. V., Queiroga R. C. R. E., Costa W. K. A., Gadelha C. A. A., Lacerda R. R., Lacerda J. T. J. G., Pinto L. S., Braganhol E., Teixeira F. C., Barbosa P. P. S., Campos M. I. F., Gonçalves G. F., Pessôa H. L. F., Gadelha T. S.* Proteomic of goat milk whey and its bacteriostatic and antitumour potential. *Int. J. Biol. Macromol*. 2018, V. 113, P. 116–123. https://doi.org/10.1016/j.ijbiomac.2018.01.200
6. *Soloshenko K. I., Lych I. V., Voloshyna I. M., Shkotova L. V.* Polyfunctional properties of goat colostrum proteins and their use. *Biopolymers and Cell*. 2020, 36 (3), 197–209. http://dx.doi.org/10.7124/bc.000A2B (In Ukrainian).
7. *Lima M. J. R., Teixeira-Lemos E., Oliveira J., Teixeira-Lemos L. P., Monteiro A. M., Costa J. M.* Nutritional and health profile of goat products: focus on health benefits of goat milk. *Goat Sci*. 2018, V. 10, P. 189–232. https://doi.org/10.5772/intechopen.70321
8. *Sheybak V. M., Tis A. A., Sheybak L. N.* Phagocytic activity of neonatal cord blood neutrophils *in vitro* in the presence of leucine. *Eksperiment. i klinich. Farmakol.* 2005, 68 (1), 48–49. (In Russian).
9. *DeSimone R., Vissicchio F., Mingarelli C., De Nuccio C., Visentin S., Ajmone-Cat M. A., Minghetti L.* Branched-chainaminoacids influence the immune properties ofmicroglial

cells and their responsiveness to pro-inflammatory signals. *Biochim. Biophys. Acta*. 2013, 1832 (5), 650–659. https://doi.org/10.1016/j.bbadis.2013.02.001
10. *Chen X. H., Liu S. R., Peng B., Li D., Cheng Z. X., Zhu J. X., Peng X. X.* Exogenous L-valine promotes phagocytosis to kill multidrug-resistant bacterial pathogens. *Front. Immunol.* 2017, V. 8, P. 207. https://doi.org/10.3389/fimmu.2017.00207
11. *Virarkar M., Alappat L., Bradford P. G., Awad A. B.* L-arginine and nitric oxide in CNS function and neurodegenerative diseases. *Crit. Rev. Food Sci. Nutr.* 2013, 53 (11), 1157–1167. https://doi.org/10.1080/10408398.2011.573885 PMID: 24007420.
12. *Nagahama M., Semba R., Tsuzuki M., Aoki E.* L-arginine immunoreactive enteric glial cells in the enteric nervous system of rat ileum. *Biol. Signals Recept.* 2001, 10 (5), 336–340. https://doi.org/10.1159/000046901
13. *Pickart L., Vasquez-Soltero J. M., Margolina A.* GHK Peptide as a Natural Modulator of Multiple Cellular Pathways in Skin Regeneration. *Biomed. Res. Int.* 2015, P. 648108. https://doi.org/10.1155/2015/648108
14. *Iseri V. J., Klasing K. C.* Changes in the amount of lysine in protective proteins and immune cells after a systemic response to dead *Escherichia coli*: implications for the nutritional costs of immunity. *Integr. Comp. Biol.* 2014, 54 (5), 922–930. https://doi.org/10.1093/icb/icu111
15. *Zhang Q., Chen X., Eicher S. D., Ajuwon K. M., Applegate T. J.* Effect of threonine on secretory immune system using a chicken intestinal *ex vivo* model with lipopolysaccharide challenge. *Poult. Sci.* 2017, 96 (9), 3043–3051. https://doi.org/10.3382/ps/pex111
16. *Fukuda K., Nishi Y., Usui T.* Free amino acid concentrations in plasma, erythrocytes, granulocytes, and lymphocytes in umbilical cord blood, children, and adults. *J. Pediatr. Gastroenterol. Nutr.* 1984, 3 (3), 432–439. https://doi.org/10.1097/00005176-198406000-00022
17. *Tyurenkov I. N., Samotruyeva M. A., Grazhdantseva N. N., Khlebtsova Ye. B., Berestovitskaya V. M., Vasilyeva O. S.* Immunomodulatory properties of the composition of phenotropil and glutamic acid. *Biomeditsina*. 2011, V. 3, P. 63–69. (In Russian).
18. *Uyangaa E., Lee H. K., Eo S. K.* Glutamine and leucine provide enhanced protective immunity against mucosal infection with herpes simplex virus type 1. *Immune Netw.* 2012, 12 (5), 196–206. https://doi.org/10.4110/in.2012.12.5.196
19. *Cruzat V., Macedo Rogero M., Noel Keane K., Curi R., Newsholme P.* Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. *Nutrients*. 2018, 10 (11), 1564. https://doi.org/10.3390/nu10111564
20. *Cheng Z., Gatlin III D. M., Buentello A.* Dietary supplementation of arginine and/or glutamine influences growth performance, immune responses and intestinal morphology of hybrid striped bass (Morone chrysops×Morone saxatilis). *Aquaculture*. 2012, V. 362, P. 39–43. https://doi.org/10.1016/j.aquaculture.2012.07.015
21. *Ren W., Zou L., Ruan Z., Li N., Wang Y., Peng Y., Wu G.* Dietary L-proline supplementation confers immunostimulatory effects on inactivated *Pasteurella multocida* vaccine immunized mice. *Amino Acids*. 2013, 45 (3), 555–561. https://doi.org/10.1007/s00726-013-1490-4
22. *Reddy R. S., Ramachandra C. T., Hiregoudar S., Nidoni U., Ram J., Kammar M.* Influence of processing conditions on functional and reconstitution properties of milk powder made from Osmanabadi goat milk by spray drying. *Small Ruminant Res.* 2014, 119 (1–3), 130–137. https://doi.org/10.1016/j.smallrumres.2014.01.013
23. *Ribeiro A. C., Ribeiro S. D. A.* Specialty products made from goat milk. *Small Ruminant Res.* 2010, V. 89, P. 225–233. https://doi.org/10.1016/j.smallrumres.2009.12.048
24. *Wertz P. W.* Epidermal lipids. *Semin. Dermatol.* 1992, 11 (2), 106–113.
25. *Tomita M., Wakabayashi H., Yamauchi K., Teraguchi S., Hayasawa H.* Bovine lactoferrin and lactoferricin derived from milk: production and applications. *Biochem. Cell Biol.* 2002, 80 (1), 109–112. https://doi.org/10.1139/o01-230
26. *Anaeto M., Adeyeye J. A., Chioma G. O., Olarinmoye A. O., Tayo G. O.* Goat products: Meeting the challenges of human health and nutrition. *Agric. Biol. J. N. Am.* 2010, 1 (6), 1231–1236. https://doi.org/10.5251/abjna.2010.1.6.1231.1236
27. *Dawson B., Favaloro E. J.* High rate of deficiency in the amino acids tryptophan and histidine in people with wounds: implication for nutrient targeting in wound management-a pilot study. *Adv. Skin Wound Care*. 2009, 22 (2), 79–82. https://doi.org/10.1097/01.ASW.0000345280.20779.17
28. *Zenebe T., Ahmed N., Kabeta T., Kebede G.* Review on Medicinal and Nutritional Values of Goat Milk. *J. Nutr.* 2014, 3 (3), 30–39. https://doi.org/10.5829/idosi.ajn.2014.3.3.93210
29. *Khan I. T., Nadeem M., Imran M., Ullah R., Ajmal M., Jaspal M. H.* Antioxidant

properties of Milk and dairy products: a comprehensive review of the current knowledge. *Lipids Health Dis.* 2019, 18 (1), 41. https://doi.org/10.1186/s12944-019-0969-8
30. *Sánchez-Macías D., Moreno-Indias I., Castro N., Morales-Delanuez A., Argüello A.* From goat colostrum to milk: physical, chemical, and immune evolution from partum to 90 days postpartum. *J. Dairy Sci.* 2014, 97 (1), 10–16. https://doi.org/10.3168/jds.2013-6811
31. *Bergfeld W. F., Belsito D. V., Hill R. A., Klaassen C. D., Marks Jr J. G., Shank R. C., Snyder P.W.* Safety Assessment of Milk Proteins and Protein Derivatives as Used in Cosmetics. 2017.
32. *Ashraf Z., Jamil A., Umar S., Nadeem S. G.* Antimicrobial Pattern Associated With Handmade Goat Milk Soap. *J. Biol. Res. Appl. Sci.* 2016, 7 (2), 19–23.
33. *Jaya F., Thohari I., Susilorini T. E., Asmara D. R.* Microbiological properties of preparing facial mask cream from goat milk kefir. *IOP Conf. Ser: Earth Environ. Sci.* 2019, 230 (1), 012105. https://doi.org/10.1088/1755-1315/230/1/012105
34. *Kazimierska K., Kalinowska-Lis U.* Milk Proteins-Their Biological Activities and Use in Cosmetics and Dermatology. *Molecules.* 2021, 26 (11), 3253. https://doi.org/10.3390/molecules26113253
35. *Jirillo F., Martemucci G., D'Alessandro A. G., Panaro M. A., Cianciulli A., Superbo M., Magrone T.* Ability of goat milk to modulate healthy human peripheral blood lymphomonocyte and polymorphonuclear cell function: *in vitro* effects and clinical implications. *Curr. Pharm. Des.* 2010, 16 (7), 870–876. https://doi.org/10.2174/138161210790883534
36. *Abeijón Mukdsi M. C., Haro C., González S. N., Medina R. B.* Functional goat milk cheese with feruloyl esterase activity. *J. Funct. Foods.* 2013, 5 (2), 801–809. https://doi.org/10.1016/j.jff.2013.01.026
37. *Yilmaz-Ersan L., Ozcan T., Akpinar-Bayizit A., Sahin S.* The antioxidative capacity of kefir produced from goat milk. *Int. J. Chem. Eng. Appl.* 2016, 7 (1), 22–26. https://doi.org/10.7763/IJCEA.2016.V7.535
38. *Moreno-Fernandez J., Diaz-Castro J., Alférez M. J., Boesch C., Nestares T., López-Aliaga I.* Fermented goat milk improves antioxidant status and protects from oxidative damage to biomolecules during anemia recovery. *J. Sci. Food Agric.* 2016, 97 (5), 1433–1442. https://doi.org/10.1002/jsfa.7882
39. *Voloshyna* I. M., *Shkotova L. V. Lactobacillus* bacteria: biological and therapeutic properties. *Mikrobiol. Z.* 2019, 81 (6), 131–146. https://doi.org/10.15407/microbiolj81.06.131 (In Ukrainian).
40. *Araújo D. F. S., Guerra G. C. B., Pintado M. M. E., Sousa Y. R. F., Algieri F., Rodriguez-Nogales A., Araújo R. F. Jr., Gálvez J., Queiroga R. C. R. E., Rodriguez-Cabezas M. E.* Intestinal anti-inflammatory effects of goat whey on DNBS-induced colitis in mice. *PLoS One.* 2017, 12 (9), e0185382. https://doi.org/10.1371/journal.pone.0185382
41. *Jia R., Chen H., Chen H., Ding W.* Effects of fermentation with *Lactobacillus rhamnosus* GG on product quality and fatty acids of goat milk yogurt. *J. Dairy Sci.* 2016, 99 (1), 221–227. https://doi.org/10.3168/jds.2015-10114
42. *Aljutaily T., Huarte E., Martinez-Monteagudo S., Gonzalez-Hernandez J. L., Rovai M., Sergeev I. N.* Probiotic-enriched milk and dairy products increase gut microbiota diversity: a comparative study. *Nutr. Res.* 2020, V. 82, P. 25–33. https://doi.org/10.1016/j.nutres.2020.06.017
43. *Shu G., He Y., Wan H., Hui Y., Li H.* Effects of prebiotics on antioxidant activity of goat milk fermented by *Lactobacillus plantarum* L 60. *Acta Univ. Cibiniensis, Ser. E: Food Technol.* 2017, 21 (2), 11–18. https://doi.org/10.1515/aucft-2017-0010
44. *Arsenyeva T. P., Lugova M. V., Yakovchenko N. V.* Development of the composition of a high-protein frozen dessert for sports nutrition based on goat milk. *Polzunovskiy vestnik.* 2019, N 2, P. 26–31. https://doi.org/10.25712/ASTU.2072-8921.2019.02.006 (In Russian).
45. *Costa R. G., Beltrão Filho E. M., de Sousa S., da Cruz G. R., Queiroga R. de C., da Cruz E. N.* Physicochemical and sensory characteristics of yoghurts made from goat and cow milk. *Anim. Sci. J.* 2016, 87 (5), 703–709. https://doi.org/10.1111/asj.12435
46. *Attalla N. R., Mohamed E. F., El-Reffaei W. H. M., Bassyoni N. I.* Production and evaluation of sweet spreadable goat cheese. *Int. J. Nutr. Food Sci.* 2014, V. 3, P. 79–90. https://doi.org/10.11648/j.ijnfs.20140302.20
47. *Zhou S. J., Sullivan T., Gibson R. A., Lönnerdal B., Prosser C. G., Lowry D. J., Makrides M.* Nutritional adequacy of goat milk infant formulas for term infants: a double-blind randomised controlled trial. *Br. J. Nutr.* 2014, 111 (09), 1641–1651. https://doi.org/10.1017/s0007114513004212
48. *Verduci E., D'Elios S., Cerrato L., Comberiati P., Calvani M., Palazzo S., Martelli A., Landi M., Trikamjee T., Peroni D. G.* Cow's Milk Substitutes for Children: Nutritional Aspects of Milk from Different Mammalian Species, Special Formula and Plant-Based Beverages.

Nutrients. 2019, 11 (8), 1739. https://doi.org/10.3390/nu11081739
49. *Gallier S., Tolenaars L., Prosser C.* Whole Goat Milk as a Source of Fat and Milk Fat Globule Membrane in Infant Formula. *Nutrients*. 2020, 12 (11), 3486. https://doi.org/10.3390/nu12113486
50. *Maathuis A., Havenaar R., He T., Bellmann S.* Protein digestion and quality of goat and cow milk infant formula and human milk under simulated infant conditions. *J. Pediatr. Gastroenterol. Nutr.* 2017, 65 (6), 661–666. https://doi.org/10.1097/MPG.0000000000001740
51. *Hodgkinson A. J., Wallace O. A. M., Boggs I., Broadhurst M., Prosser C. G.* Gastric digestion of cow and goat milk: Impact of infant and young child *in vitro* digestion conditions. *Food Chem.* 2018, V. 245, P. 275–281. https://doi.org/10.1016/j.foodchem.2017.10.028

ПРАКТИЧНЕ ВИКОРИСТАННЯ КОЗИНОГО МОЛОКА ТА МОЛОЗИВА

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В огляді наведено інформацію стосовно протеїнового й амінокислотного складу козиного молозива та молока, проаналізовано властивості компонентів молозива та молока кіз. Окрім цього, показано перспективи застосування козиного молока та молозива у харчовій і косметичній промисловості, доцільність використання козиного молока для дитячого вигодовування. Функціональні харчові продукти з козиного молока виявляють антиоксидантні, протизапальні, кардіопротекторні, антигіпертензивні та антиатерогенні властивості в організмі людини. Косметичні засоби на основі козиного молока є дуже корисними для підтримання здорового вигляду шкіри і ефективними у лікуванні різних захворювань шкіри. Дитячі суміші на основі козиного молока забезпечують комфортне травлення у немовлят і є кращими у засвоюванні протеїнів, жирів та інших поживних речовин порівняно із сумішами з коров'ячого молока.

Ключові слова: козине молозиво, козине молоко, протеїни, амінокислоти, косметологія, дитяче харчування.

ПРАКТИЧЕСКОЕ ИСПОЛЬЗОВАНИЕ КОЗЬЕГО МОЛОКА И МОЛОЗИВА

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В обзоре представлена информация о протеиновом и аминокислотном составе козьего молозива, проанализированы свойства компонентов молозива коз. Кроме того, показаны перспективы применения козьего молока и молозива в пищевой и косметической промышленности, целесообразность использования козьего молока для детского вскармливания. Функциональные пищевые продукты из козьего молока оказывают антиоксидантный, противовоспалительный, кардиопротекторный, антигипертензивный и антиатерогенный эффект в организме человека. Косметические средства на основе козьего молока очень полезны для поддержания здорового вида кожи и эффективности в лечении различных заболеваний кожи. Детские смеси на основе козьего молока обеспечивают комфортное пищеварение младенцев и являются лучшими в усвоении протеинов, жиров и других питательных веществ по сравнению со смесями из коровьего молока.

Ключевые слова: козье молозиво, козье молоко, протеины, аминокислоты, косметология, детское питание.

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ULTRASONIC DISINTEGRATION OF LIGNOCELLULOSE RAW MATERIALS AS A PRE-TREATMENT OF A SUBSTRATE FOR MICROBIOLOGICAL PRODUCTION OF BIOBUTANOL

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Aim. The purpose of the study was to investigate the effect of ultrasonic disintegration on the lignocellulosic raw materials (biomass of the non-cereal part of rape) with its subsequent use as a substrate for the production of biobutanol.

Methods. Butanol-producing strains and the biomass of the non-cereal part of rape *Brassica napus* were used in the present study. Ultrasonic disintegration of lignocellulosic raw materials was performed on the specially designed equipment.

Results. The effect of ultrasonic disintegration on lignocellulosic raw materials was investigated for further application in biofuel production based on microbiological conversion. The possibility of using the obtained components after the pre-treatment of lignocellulose by ultrasonic disintegration as a substrate for the microbiological synthesis of butanol was shown. The highest accumulation of butanol (2.4 g/l) was obtained with the use of 5% dry matter content in the medium, 5 min treatment and the specific power of ultrasonic disintegration of 0.72 W/ml.

Conclusions. The possibility of producer strains of the genus *Clostridium* to use cellulose in the fermentation process has been shown. When using ultrasonic disintegration for pretreatment of the non-cereal part of the biomass of rape, the accumulation of butanol increased by 3 folds.

Key words: ultrasonic disintegration, biobutanol, lignocellulosic raw materials, biofuel.

Modern biotechnology offers great opportunities for the use of lignocellulosic plant biomass, such as agricultural and woodworking plant waste. In Ukraine, more than 50 million tons of grain are harvested annually. The main grain crops in Ukraine in terms of production are corn (38.50%), wheat (28.34%), sunflower (16.43%), barley (6.74%), soybeans (4.19%) and rape (4.08%). Other crops in total make up 1.72% of the gross harvest (peas 0.78%, rye 0.23%, sorghum 0.21%, oats 0.17%, panicgrass 0.16%, rice 0.07%, triticale 0.05%, buckwheat 0.04%, etc.) [1]. Renewable energy sources of raw materials such as straw and crop residues account for about 14% [2].

Lignocellulose is the main building material of plant cell walls. The main macrocomponents of lignocellulose are cellulose, hemicellulose and lignin. The production of biofuels from biomass generally requires three successive processes, namely destruction and hydrolysis, cultivation and isolation of the final product. Numerous studies on the use of biomass have shown that the rate of hydrolysis is limited by the rigidity of cell walls, which inhibits or delays the further process of biodegradation. There are many methods of destroying cell

walls. The most common are grinding, various types of hydrolysis, ultrasonic and microwave (at 100 °C) disintegration, osmotic shock and autoclaving (at 121 °C), which have their advantages and disadvantages and, thus, different end results [3, 4].

Ultrasonic disintegration (USD) of the cell wall occurs at a relatively low temperature, compared with microwave processing and autoclaving. It does not require the pretreatment with chemical destructors, which reduces the cost of the preparation process. USD is commonly used for cell lysis and homogenization; however, it can also be an effective method of breaking hard cell membranes [5].

Biobutanol production is the next important stage in the development of various types of biofuels. The use of biobutanol should meet the growing need for environmentally friendly motor fuel. Production of second-generation biobutanol from renewable non-food sources of raw materials such as cellulose-containing waste should solve the problem of using agricultural waste [6]. One of the methods of complex preliminary preparation of lignocellulosic raw materials is USD. This method provides significant destruction of biomass and makes it possible to identify the components of lignocellulosic raw materials [7]. Subsequently, the components of lignocellulosic biomass can serve as a substrate for microbiological conversion by microorganisms of the genus *Clostridium* and the production of biobutanol [8].

The aim of the work was to study the ultrasound disintegration of lignocellulosic raw material (the biomass of non-cereal part of rape) to subsequentially use it as a substrate for biobutanol production.

Materials and Methods

The objects of the study were the strain *Clostridium* sp. IMB B-7570 from the "Collection of strains of microorganisms and plant lines for food and agricultural biotechnology" of the Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine (hereinafter the Collection); green biomass of *Brassica napus* of the "Chempion Ukrayiny" variety harvested in 2021 at the Olenivske Experimental Farm of the National Research Center "Institute of Mechanization and Electrification of Agriculture" of the National Academy of Agrarian Sciences of Ukraine.

The stages of research included the preparation of plant raw materials with the preparation of a suspension based on crushed raw materials, treatment of the resulting suspension with ultrasound and its subsequent cultivation to obtain biobutanol. Preliminary preparation of plant raw materials consisted of two-stage grinding of raw materials to a given weighted average size of crushed particles (200 mesh), mixing the entire mass of crushed raw materials, preparation of suspensions with a given dry matter content and subsequent sonication of the suspension. The crusher "Elikor-5" (PJSC "Electromotor", Ukraine) was used for preliminary grinding, and the laboratory mill "LZM-1" (LLC "LIS", Ukraine) was applied for the final grinding. The weighted average size of the crushed particles of raw materials was determined by laboratory sift "RLU-3" (LLC "Status", Ukraine) with a set of laboratory sieves. Plant raw materials were ground to a weighted average particle size of 0.78 mm (passing the laboratory sieve No. 64 and retained on the sieve No. 67). The crushed raw materials were mixed for 5 minutes using a laboratory batch drum mixer [9]. For the preparation of the suspension, purified tap water was used with the corresponding mass fraction of crushed plant raw materials, taking into account its humidity. Laboratory scales TVE-1 (LLC "NVP "Technovagy"", Ukraine) were used for weighing of raw materials.

The USD of the suspension was performed with a laboratory ultrasonic bath, consisting of a stainless-steel gastronomic container of standard size "GN ¼" ("TorhOborud", Ukraine) with a depth of 65 mm, at the bottom of which were attached piezoceramic Langevin ultrasonic transducers (Fig. 1) with an operating frequency of 28 kHz and an ultrasonic power of 60 W (PE "Voron", Ukraine). The laboratory unit was powered by a 1.5 kW ultrasonic generator UCE-NT 1500 ("UCE Ultrasonic", China), which provided the set operating time and automatic tuning of the resonant frequency of the ultrasonic transducers in the range of 20–40 kHz. After ultrasound, the raw materials were immediately sent for cultivation.

The yield of butanol per unit volume of suspension and weight of dry matter (g/l) was investigated depending on the duration of ultrasonic treatment of the suspension (t, min.), the dry matter content in the suspension (s, %) and the specific power of ultrasound (μ, W/l). The specific power of the ultrasound (μ) was changed by changing the volume of the suspension at a constant power of the transducers. The duration of ultrasonic treatment of the suspension was 5 and 25 min,

the dry matter content was 50 and 100 g/l, the specific power of ultrasound was 0.18 and 0.72 W/l.

As a control, mash of rape mass of 50 and 100 g per liter of water was used and sterilized for 2 h and a pressure of 2 atm. The moisture content of the raw material was determined using a weighing moisture analyzer "RADWAG MA 50/C/1" (Poland).

Cultivation of microorganisms was performed on solid media in Petri dishes in anaerostat "AE 01" (RF) with a nitrogen atmosphere to obtain single colonies. To obtain the inoculum, single colonies were selected and placed in a liquid medium. Glycerol medium was used as the inoculum medium with the following composition (g/l): glycerol p. a. — 20, yeast extract — 1.0; $(NH_4)_2SO_4$ — 0.6; $(NH_4)_2HPO_4$ — 1.6; pH 6.5. The medium was sterilized for 30 min at a pressure of 1 atm. and was used to accumulate and add to the fermentation medium the same concentration of bacteria in the active phase. The inoculum was fermented for 24 h and the accumulation of bacteria was evaluated according to the turbidity standard [9]. The anaerostat was placed in a thermostat at a temperature of 35 ± 1 °C. Cultivation was performed in 500 ml flasks using 250 ml of medium, the flasks were covered with hydroacid seals with concentrated sulfuric acid. The flasks were weighed and thermostated at 35 ± 1 °C. After 72 h of culture, the cells were pelleted using a "Labofuge 400R" ultracentrifuge (Germany) at 13,000 rpm for 10 min. After cultivation, fermentation products were distilled off from the culture fluid. The presence of ethanol and butanol in the culture fluid was determined using a gas chromatograph with a flame ionization detector. A 3 m long packed column was used with Carbowax 1500 on chromaton N-A-W-DMSC (0.20–0.25 mm). The temperature of the column was 60 ± 2 °C, that of the evaporator was 160 ± 5 °C. The ratio of nitrogen-hydrogen-air flows was 1: 1: 10.

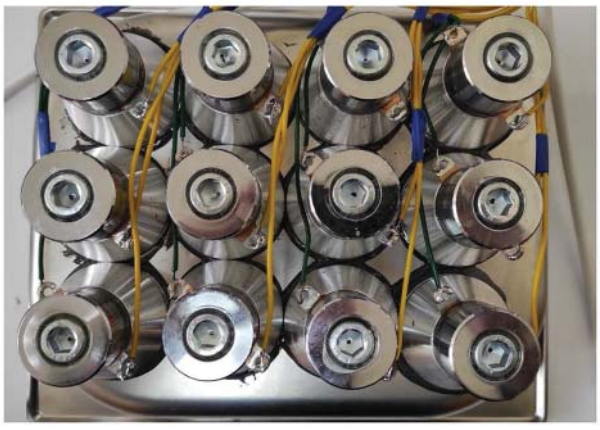

Fig. 1. **Laboratory ultrasonic bath with transducers (bottom view)**

All experiments were performed in triplicates. Statistical processing of experimental data was done using Microsoft Excel. The difference between the two means was considered significant at $P < 0.05$.

Research of ultrasound decomposition of plant raw materials to obtain biobutanol was conducted at the Department of Labor Protection and Biotechnical Systems in Animal Husbandry in the National University of Life and Environmental Sciences of Ukraine, and the State Institution "Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine" (laboratory of industrial and food biotechnology).

Results and Discussion

The main idea of the research was to establish the parameters of pre-treatment of plant raw materials, in particular, grinding and USD, at which the accumulation of biobutanol in the cultivation process would be maximal. The results of the study showed the possibility of using USD of rape biomass and its use as a substrate for biobutanol (Table). The technological parameters of USD and solvent accumulation are given in Table. Experiments 1–8 were performed with biomass after USD, and experiments 9 and 10 were conducted without USD and served as a control. The accumulation of biobutanol after USD of rapeseed biomass was greater than that of untreated rapeseed biomass and untreated rape biomass of other plants [10]. The duration of ultrasonic treatment of the suspension t did not have a significant effect on the final result, which indicates the need to adjust the limits of change of this parameter and set the upper limit at 5 minutes.

The USD parameters indirectly affected the accumulation of the target product. In some samples there was an increased accumulation of ethanol instead of butanol. In our opinion, this change is due to the change in the carbon-nitrogen ratio of the medium after USD. The largest accumulation of biobutanol (2.44 g/l) was for the following values of parameters: s = 50 g/l, μ = 0.72 W/ml, which corresponds to the upper limit of variation of these parameters. The lowest accumulation of biobutanol (1.16 g/l) was observed in the case of s = 100 g/l and μ = 0.18 W/ml, which corresponds to the lower limit of variation.

Technological parameters of USD and solvent accumulation

Experiment	t, min	s, g/l	μ, W/l	Solvent accumulation, g/l			Plant biomass residue, g/l
				ethanol	acetone	butanol	
1	25	100	0.72	0.34 ± 0.07	0.09 ± 0.01	1.57 ± 0.09	73.55 ± 0.02
2	25	100	0.18	0.30 ± 0.07	0.03 ± 0.01	1.16 ± 0.07	34.26 ± 0.07
3	**25**	**50**	**0.72**	**0.32 ± 0.07**	**0.20 ± 0.03**	**2.44 ± 0.09**	**18.46 ± 0.06**
4	25	50	0.18	0.31 ± 0.07	0.07 ± 0.01	1.63 ± 0.06	34.55 ± 0.03
5	5	100	0.72	0.33 ± 0.07	0.06 ± 0.01	1.22 ± 0.04	85.56 ± 0.04
6	5	100	0.18	0.24 ± 0.04	0.06 ± 0.01	1.24 ± 0.03	83.29 ± 0.05
7	**5**	**50**	**0.72**	**0.33 ± 0.07**	**0.20 ± 0.04**	**2.37 ± 0.09**	**18.55 ± 0.01**
8	**5**	**50**	**0.18**	**0.52 ± 0.07**	**0.10 ± 0.02**	**2.16 ± 0.09**	**19.76 ± 0.02**
9	–	50	–	0.13 ± 0.04	0.02 ± 0.01	0.24 ± 0.02	42.55 ± 0.03
10	–	100	–	0.05 ± 0.01	0.02 ± 0.01	0.73 ± 0.04	96.14 ± 0.07

Note: the largest accumulation of butanol is highlighted in fat.
Hereinafter: $P < 0.05$ compared to control, native medium used as control.

In control samples that did not undergo USD, the accumulation of biobutanol was 0.24 and 0.73 g/l, depending on the biomass concentration of 50 and 100 g/l, respectively.

The dependences of biobutanol accumulation on the parameters s and μ are obtained in the form of power functions for the specific accumulation per unit volume of suspension Bv, g/l (1)

$$Bv = 7.84563 \cdot s^{-0.721369} \cdot \mu^{0.142132}, \quad (1)$$

where s is the content of dry matter in the suspension, %; μ is the specific power of ultrasound, W/ml;
and for the specific accumulation per unit mass of dry matter Bm, g/kg (2)

$$Bm = 784.563 \cdot s^{-1.72137} \cdot \mu^{0.142132}. \quad (2)$$

For dependence (1), which is adequate for 95% confidence interval, the coefficient of multiple determination D = 0.882368, the coefficient of multiple correlation R = 0.939344. For Fisher's test, F = 18.7527; the probability F of the criterion P = 0.996669. All model coefficients are significant at a confidence interval of at least 94%. For dependence (2), which is also adequate at 95% confidence interval, the coefficient of multiple determination D = 0.974346, the coefficient of multiple correlation R = 0.98709. Fisher's test F = 94.9496; the probability F of the criterion P = 0.999808. All model coefficients are significant at a confidence interval of at least 94%. Graphical view of dependences (1) and (2) is shown in Fig. 2 and Fig. 3, respectively.

As can be seen from Fig. 2 and Fig. 3, the increase in dry matter content in the suspension leads to a decrease in the accumulation of biobutanol. This can be explained by the increase in acoustic resistance of the treated suspension and the corresponding decrease in the effective action of ultrasound. The effect of the specific power of ultrasound on the accumulation of biobutanol within the experiment is less intense, but leads to an increase in the yield of butanol. The effectiveness of this factor increases in the case of reducing the dry matter content in the suspension.

The accumulation of butanol increased due to the higher bioavailability of raw materials. The increase in the bioavailability of the substrate may be due to the fact that during USD, the number of crystalline zones of cellulose decreases and the number of amorphous zones, which are easily broken down by enzymes, rises. Ultrasonic pretreatment of the substrate can change the surface morphology of lignocellulosic materials, partially disrupt the cell wall, which leads to increased availability of cellulose fibers to enzymes (cellulase) and enhances the yield of sugars during hydrolysis [11–13]. In bacteria of the genus *Clostridium*, such enzymes that break down cellulose are part of the extracellular multiprotein complex, the cellulosome.

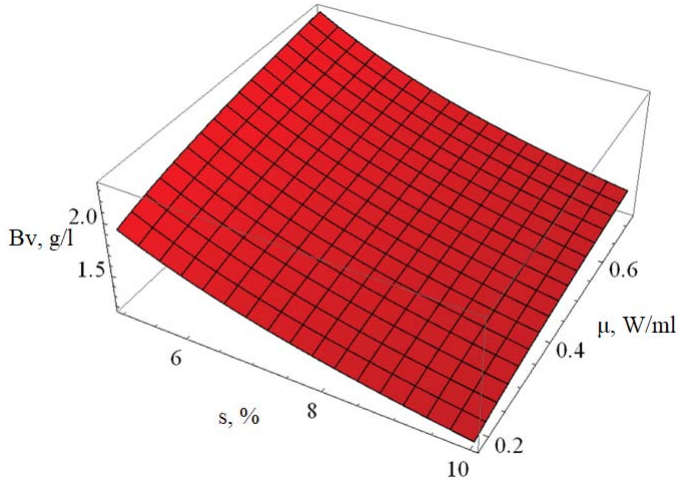

Fig. 2. **The dependence of the specific accumulation of biobutanol per unit volume (Bv) of the suspension on the dry matter content of the suspension (s) and the specific power of ultrasound (μ)**

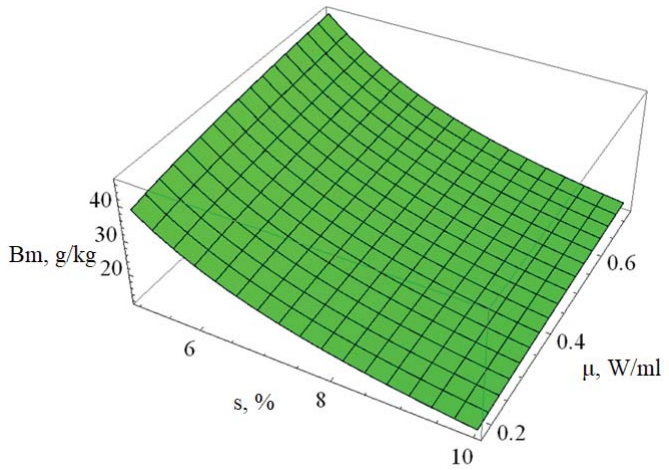

Fig. 3. **The dependence of the specific accumulation of biobutanol per unit mass of dry matter (Bm) on the dry matter content of the suspension (s) and the specific power of ultrasound (μ)**

The increase in the content of available carbohydrates in suspensions depends on the degree of destruction of lignocellulose, so it is important to find the optimal percentage of its destruction in suspension, for further use in biotechnology of liquid biofuels.

Ultrasound treatment of wheat straw [12] and sugar cane stalks [13] with ultrasound frequency of 20 and 24 kHz for 35 and 47 minutes in media of potassium hydroxide and sodium hydroxide, respectively, led to the destruction of 50 to 75 % of lignocellulose. This duration of USD, obviously, is beyond the economic feasibility of use in biofuel technologies.

USD of lignocellulosic biomass has both advantages and disadvantages compared to other pretreatment methods [14, 15]. It should be emphasized that it is not always possible to transfer a certain method of pretreatment of the substrate from one type of plant biomass to another. The choice of the method of pretreatment of lignocellulosic biomass depends on its composition and by-products formed as a result of processing. These factors significantly affect the material and financial costs in biofuel technology, which are associated with the method of pretreatment of lignocellulosic biomass.

Conclusions

Producer strains of the genus *Clostridium* can use plant lignocellulosic raw materials as a substrate in the cultivation process. The use

of ultrasound disintegration for pretreatment of the non-grain part of rape biomass increases the accumulation of butanol. The optimal values of technological parameters of USD as a pretreatment of plant biomass are established. These results indicate the effectiveness of ultrasound as an agent of pretreatment of plant raw materials in liquid biofuel technologies.

This study was conducted within the framework of the Ministry of Education and Science of Ukraine "Development of technology for ultrasonic disintegration of plant biomass of the non-grain part of the crop", State registration No. 0120U102083.

REFERENCES

1. *Tursi A.* A review on biomass: importance, chemistry, classification, and conversion. *Biofuel Res. J*. 2019, 6 (2), 962–979. https://doi.org/10.18331/BRJ2019.6.2.3
2. *Karimi M., Jenkins B., Stroeve P*. Ultrasound irradiation in the production of ethanol from biomass. *Renewable and Sustainable Energy Rev*. 2014, V. 40, P. 400–421. https://doi.org/10.1016/j.rser.2014.07.151
3. *Kumar P., Barrett D. M., Delwiche M. J., Stroeve P*. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res.* 2009, 48 (8), 3713–3729. https://doi.org/10.1021/ie801542g
4. *Alvira P., Tomas-Pejo E., Ballesteros M., Negro M. J*. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresour. Technol*. 2010, V. 101, P. 4851–4861. https://doi.org/10.1016/j.biortech.2009.11.093
5. *Shulga S. M., Tigunova O. A., Blume Y. B*. Lignocellulose as an alternative source for obtaining of biobutanol. *Biotechnol. acta*. 2013, 6 (2), 10–20 (In Ukrainian). https://doi.org/10.15407/biotech6.02.009
6. *Jaismal N., Agarwal A., Tripathi A. D*. Application of microorganisms for biofuel production. In book: Bioenergy Research: Basic andadvanced concepts. Clean Energy Production Technologies. *Springer, Singapore*. 2021, P. 35–72. https://doi.org/10.1007/978-981-33-4611-6_2
7. *Konovalov S., Patrylak L., Zubenko S., Okhrimenko M., Yakovenko A., Levterov A., Avramenko A.* Bench motor testing of blended fuels on their basis. *Chemistry and Chemical Technol*. 2021, 15 (1), 105–177. https://doi.org/10.23939/chcht15.01.105
8. *Pinko T., Flores-Alicha X., Gernaey K. V., Junicke H*. Alone or together? A review on pure and mixed microbial cultures for butanol production. *Renewable and Sustainable Energy Rev*. 2021, V. 147, P. 111244 https://doi.org/10.1016/j.rser.2021.111244
9. *Achkevych O. M*. Substantiation of parameters of the drum mixer of feed additives. Abstract of the dissertation of Cand. tech. Science: 05.05.11. *National University of Life and Environmental science of Ukraine. Kyiv*. 2015, 24 p.
10. *Tigunova O. O., Kamenskyh D. S., Tkachenko T. V., Yevdokymenko V. A., Kashkovskiy V. I., Rakhmetov D. B., Blume Ya. B., Shulga S. M*. Biobutanol production from plant biomass *The Open Agriculture J*. 2020, V. 14, P. 187–197. https://doi.org/10.2174/1874331502014010187
11. *Bundhoo Z. M. A., Mohee R.* Ultrasound-assisted biological conversion of biomass and waste materials to biofuels: A review. *Ultrason. Sonochem*. 2017. http://dx.doi.org/10.1016/j.ultsonch.2017.07.025
12. *Sun R. C., Tomkinson J*. Comparative study of lignins isolated by alkali and ultrasound-assisted alkali extractions from wheat straw. *Ultrason. Sonochem*. 2002, V. 9, P. 85–93. https://doi.org/10.1016/S1350-4177(01)00106-7
13. *Velmurugan R., Muthukumar K*. Utilization of sugarcane bagasse for bioethanol production: Sono-assisted acid hydrolysis approach. *Bioresour. Technol.* 2011, V. 102, P. 7119–7123. https://doi.org/10.1016/j.biortech.2011.04.045
14. *Raita S., Spalvins K., Blumberga D*. Prospect on agro-industrial residues usage for biobutanol production. *Agronomy Res.* 2021, 19 (s1), 877–895. https://doi.org/10.115159/AR.21.084
15. *Anukam A., Berghel J*. Biomass pretreatment and characterization: A review. In book: *Biotechnology application of biomass*. 2020. Open access peer-reviewed chapter. https://doi.org/10.5772/intechopen.93607

УЛЬТРАЗВУКОВА ДЕЗІНТЕГРАЦІЯ ЛІГНОЦЕЛЮЛОЗНОЇ СИРОВИНИ ЯК ПОПЕРЕДНЯ ПІДГОТОВКА СУБСТРАТУ ДЛЯ МІКРОБІОЛОГІЧНОГО ОТРИМАННЯ БІОБУТАНОЛУ

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Мета. Дослідження впливу ультразвукової дезінтеграції на лігноцелюлозну сировину (біомасу незернової частини ріпаку) з подальшим використанням її як субстрату для отримання біобутанолу.

Методи. Для досліджень використовували штами-продуценти біобутанолу; біомасу незернової частини ріпаку *Brassica napus*. Ультразвукову дезінтеграцію лігноцелюлозної сировини виконували на спеціально створеному обладнанні.

Результати. Досліджено вплив ультразвукової дезінтеграції на лігноцелюлозну сировину з подальшим її використанням для отримання біопалива за допомогою мікробіологічної конверсії. Показано можливість застосування отриманих компонентів лігноцелюлози як субстрату після ультразвукової дезінтеграції для мікробіологічного синтезу бутанолу. Встановлено, що найбільше накопичення бутанолу (2,4 г/л) отримано за використання вмісту 50 г/л сухої речовини у середовищі та 5 хв оброблення. Зміна питомої потужності ультразвукової дезінтеграції практично не впливала на накопичення спиртів.

Висновки. Показано, що штами-продуценти роду *Clostridium* можуть використовувати рослинну лігноцелюлозну сировину як субстрат у процесі культивування. Встановлено, що за використання ультразвукової дезінтеграції для попередньої обробки незернової частини біомаси ріпаку накопичення бутанолу збільшилось утричі.

Ключові слова: ультразвукова дезінтеграція, біобутанол, лігноцелюлозна сировина, біопаливо.

УЛЬТРАЗВУКВАЯ ДЕЗИНТЕГРАЦИЯ ЛИГНОЦЕЛЛЮЛОЗНОГО СЫРЬЯ КАК ПРЕДВАРИТЕЛЬНАЯ ПОДГОТОВКА СУБСТРАТА ДЛЯ МИКРОБИОЛОГИЧЕСКОГО ПОЛУЧЕНИЯ БИОБУТАНОЛА

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Цель. Исследование влияния ультразвуковой дезинтеграции на лигноцеллюлозное сырье (биомасса незерновой части рапса) с последующим использованием её как субстрата для получения биобутанола.

Методы. Для исследования использовали штаммы-продуценты бутанола; биомассу незерновой части рапса *Brassica napus*. Ультразвуковую дезинтеграцию лигноцеллюлозного сырья выполняли на специально созданном оборудовании.

Результаты. Исследовано влияние ультразвуковой дезинтеграции на лигноцеллюлозное сырье с дальнейшим использованием для получения биотоплива с помощью микробиологической конверсии. Показана возможность использования после обработки лигноцеллюлозы ультразвуковой дезинтеграцией полученных компонентов как субстрата для микробиологического синтеза бутанола. Установлено, что наибольшее накопление бутанола (2,4 г/л) получено с использованием 5% содержания сухого вещества в среде, 5 мин обработке и удельной мощности ультразвуковой дезинтеграции 0,72 Вт/мл.

Выводы. Показана возможность штаммов-продуцентов рода *Clostridium* применять целлюлозу в процессе ферментации. Установлено, что при использовании звуковой дезинтеграции для предварительной обработки незерновой части биомассы рапса накопление бутанола увеличилось в 3 раза.

Ключевые слова: ультразвуковая дезинтеграция, биобутанол, лигноцеллюлозное сырье, биотопливо.

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BIOAVAILABILITY STUDY OF MAGNESIUM AND PHOSPHORUS COMBINED MEDICATION BASED ON CASEIN

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Aim. The Department of Biochemistry and Physiology of Animals, named after Academician Guly NUBIP of Ukraine, developed magnesium and phosphorus combined medication based on casein. Our aim was to test its bioavailability based on the ability to be hydrolyzed by a mixture of pancreatic digestive enzymes trypsin and chymotrypsin, also check the absence of cytotoxic effects on cell cultures.

Methods. To assess bioavailability, we used hydrolysis of the medication with a mixture of trypsin and chymotrypsin, followed by detection of hydrolysis products by polyacrylamide gel electrophoresis. A standard MTT-test performed on both MT-4 and Namalva cell lines was used to assess cytotoxic effects.

Results. Based on electrophoresis data, it was found that despite chemical modifications of the natural casein, the medication based on it is characterized by a high ability to hydrolyze by digestive enzymes under the same conditions as casein. Also, an MTT-test demonstrates that the medication has no cytotoxic properties against cell lines MT-4 and Namalva.

Conclusions. Since the negative effects of the drug associated with its digestibility and toxicity have not been observed, it is recommended to continue the study of its effects on living organisms.

Key words: magnesium, phosphorus, casein, chelate, *in vitro*, hydrolysis, cell culture, cytotoxicity, MTT reagent, NADH (nicotinamide adenine dinucleotide).

Magnesium is the fourth most common cation in the body, so it is indispensable for the organism, and its deficiency causes severe disorders [1]. The biological action of magnesium is mainly due to the formation of complexes with intracellular ligands and antagonism with calcium for binding with proteins and membrane structures. As mentioned above, properties determine the participation of magnesium in the synthesis of macromolecules such as nucleic acids and proteins. Over 300 enzymes require magnesium as a cofactor for their activity [2]. The participation of magnesium in the antioxidant defense of the organism is explained by the magnesium-dependent synthesis of glutathione [3, 4].

Medications containing phosphorus combined with organic radicals are well-known compounds. These compounds are used as a source of organic phosphorus to enhance mineral, carbohydrate, fat, and protein metabolism. Also, they are characterized by a high rate of metabolism and low toxicity [5, 6].

Macro- and microelements enter the body of animals and humans mainly orally. The lack of certain elements is corrected by the mineral and vitamin-mineral supplements in the diet and in case of acute deficiency — parenterally [7, 8]. The lack of certain elements is caused in humans by the impoverishment of the diet due to deep food processing, and in animals — the intensification of productivity compared to natural conditions [9]. A common problem for humans and animals is the lack or imbalance of some elements in geochemical zones [10].

Assimilation of magnesium in the body is carried out through the digestive system. Metal ions chelated by amino acids are absorbed in the small intestine similarly to dipeptides. The intensity of ion chelation directly correlates with the degree of their

assimilation [11]. After assimilation, chelates are hydrolyzed to release two amino acids. Then they are used for peptide synthesis and magnesium ion, which acts as a cofactor for apoenzymes [12–14].

Studies demonstrate numerous advantages of chelates of microelements and macroelements compared to inorganic and organic salts of these elements. The main advantage of chelates is higher bioavailability, which allows to decrease the dose and thus improves organoleptic parameters, and reduces the possibility of toxic or irritant effects due to overdose [15, 16]. The Department of Biochemistry and Physiology of Animals, named after Academician Guly NUBIP of Ukraine, developed magnesium and phosphorus combined medication based on casein [17].

The task of this study was to investigate the bioavailability of the developed medication, namely the possibility and rate of its hydrolysis by digestive enzymes, as well as its effect on cell viability, and to prove the absence of cytotoxic effects.

The important factor in the construction of the study scheme of the medication is the expected path of its metabolism with decomposition to peptides and individual amino acids. Due to the clarity of the metabolic pathway of the medication, the determination of the biotransformation of the drug in the classical sense is not required [18]. The study of the ability to hydrolyze peptide medication for oral administration is the main criteria for assessing the bioavailability of the latter because the rate of hydrolysis limits the rate of absorption [19]. To assess the bioavailability of the medication, the model hydrolysis with a mixture of digestive enzymes trypsin and chymotrypsin was used, followed by electrophoretic analysis of the obtained hydrolysis products. The comparison was carried out with the starting material for the synthesis of the medication, i. e. casein.

Possible cytotoxic and antiproliferative effects were investigated by standard methods for assessing cell viability and proliferation — the MTT-test and trypan blue staining [20, 21]. MT-4 and Namalva cell lines were used for the assay.

Materials and Methods

For the study, the magnesium and phosphorus combined medication based on casein was used, developed at the Department of Biochemistry and Animal Physiology named after Academician Guly NUBIP of Ukraine (patent 139705 UA dated January 10, 2020). The medication is a homogeneous powder, and chemically it is artificially phosphorylated casein from bovine milk as a ligand that chelates magnesium ions. The magnesium content is 10%, phosphorus — 12–15%, and the rest is protein [22]. For the experiment, the medication was synthesized in 2 identical parallels (medication-1 and medication-2).

Hydrolysis. For the hydrolysis of the medication, a digestive enzyme of the pancreas trypsin with chymotrypsin trace (≈3%) (FERAK, Germany) was used. The efficiency of the hydrolysis of the medication relative to casein (#C3400, Sigma, USA) was compared. The reaction mixture contained 50 mg/ml of the medication or 25 mg/ml of casein dissolved in 0.05 M Tris buffer solution, pH 7.4,containing 0.5 mg/ml of a mixture of trypsin and chymotrypsin [23]. The reaction was carried out for 90 min at a temperature of 37 °C in a water bath. Samples for electrophoretic analysis were taken after 0 min, 45 min, and 90 min from the beginning of the reaction. Samples were dissolved in the Laemmli buffer in a ratio of 1:1. They were added to the wells of the polyacrylamide gel approximately 100 µg of starting material per track [24]. The reaction products were analyzed with the analytical electrophoresis, in 12% of separative and 4% concentrating polyacrylamide gel to prove the hydrolysis process [25]. The analytical electrophoresis procedure was performed in a vertical electrophoresis chamber (Helicon); the molecular weight standard was estimated with a use of a pre-stained protein markers Page Ruler 26619 (10–180 kDa). The process of hydrolytic cleavage of the medication was determined densitometrically by the color intensity of tracks stained with Coomassie brilliant blue R-250. Interpretation of results based on the fact that the color intensity of fractions of the medication after trypsin treatment is inversely correlated with the number of peptide bonds in molecules subjected to hydrolytic cleavage [26]. Densitometric analysis was performed using Image J software.

Cytotoxicity. The studies were performed using cell lines MT-4 (culture of T-cell leukemia) and Namalva (B-cell line obtained with Burkitt's lymphoma). The following equipment was used, such as laminar (LS, laminar systems), CO_2 incubator (Medcenter Einrichtungen GmbH MMM-Group), centrifuge (K-26), multiwell spectrometer

(Labsystems Multiscan MS). The calculation and cell population visualization were performed using an inverted microscope AxioVert (Carl Zeiss) with Axio Vision software. Cells used in the studies were cultured in RPMI 1640 medium (Sigma, USA) containing 10% FBS (Sigma, USA).

The cultivation was performed in a humidified atmosphere with 5% CO_2 at a temperature of 37 °C. Cytotoxic activity was determined by the conventional method of determining mitochondrial activity by MTT-test, pre-determining the number of living cells by trypan blue staining and counting in the Goryaev's cytometer [20].

Cell suspensions were added to 96-well plates in the amount of 100 µl/well, at a concentration of $1 \cdot 10^5$/ml. The medication was applied to a final concentration of 0.031, 0.0625, 0.125, 0.25, 0.5, and 1 mg/ml in a culture medium in three independent parallel each. Four hours before the end of incubation of cells, 20 µl of MTT reagent (Sigma, USA) dissolved in PBS were added to a final concentration of 0.5 mg/ml. After four hours, the plates were centrifuged at 400 *g* for 10 minutes, and the supernatant was removed. 100 µl of DMSO (Serva, Czech Republic) was added to each well, after which the plates were placed on a shaker until the formazan crystals were dissolved. The optical density was determined at a wavelength of 540 nm.

Statistical analysis. Statistical processing of the results was performed by the conventional method of variation statistics using MS Excel software. It determined the mean (*M*), deviations of each measured value from M (a), quadratic deviations for each group (σ), and mean error (*m*). Statistic significance of the results was determined by the Student's *t*-test and the degree of probability of the difference between the values of *P*. The results with $P < 0.05$ were reliable.

Results and Discussion

The electrophoregram (Fig. 1, *A*) shows the comparability of the molecular weights of the casein fraction, the starting material for synthesis, and the final medication (*1, 2, 4, 7*).

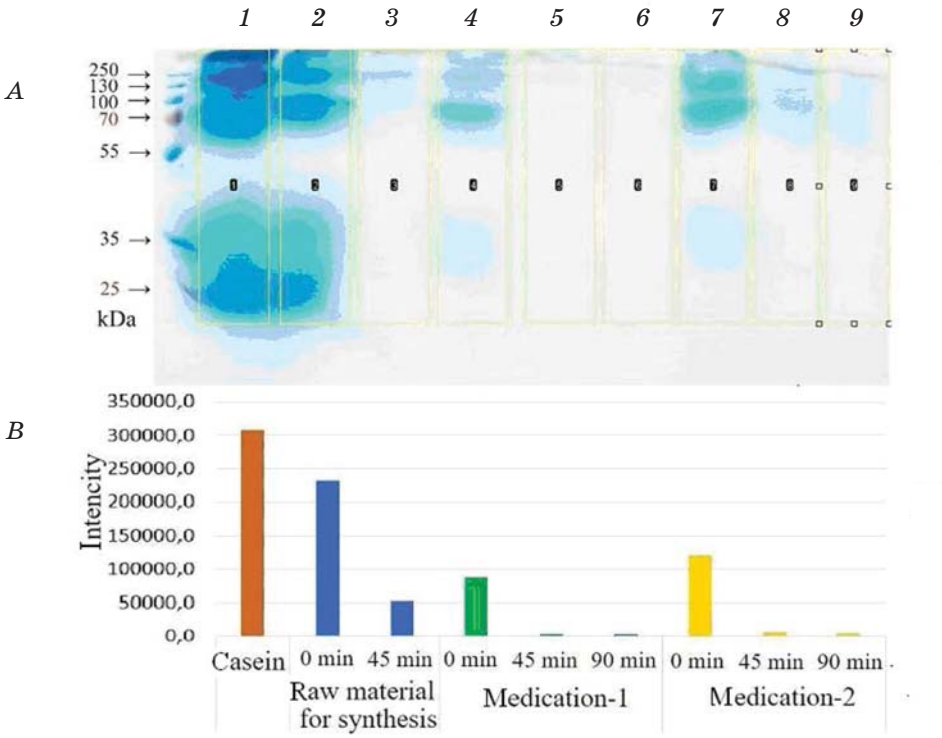

Fig. 1. A — **electrophoregram of the hydrolysate products of the magnesium and phosphorus combined medication based on casein:**

1 — casein, *2* — starting material for synthesis, *3* — starting material after treatment with trypsin 45 min, *4* — medication-1 before treatment with trypsin, *5* — medication- 1 after trypsin treatment 45 min, *6* — medication-1 after trypsin treatment 90 min, *7* — medication-2 before trypsin treatment, *8* — medication-2 after trypsin treatment 45 min, *9* — medication-2 after trypsin treatment 90 min;

***B* — the results of densitometric analysis of electropherogram *A*:**

the intensity of the color of the tracks in conventional units

Reducing the color intensity of tracks *3, 5, 6, 8, 9* obviouslyindicates that the medication has no worse ability to hydrolyze trypsin than the starting material for synthesis.

Hydrolysis followed by the detection of protein fractions in the polyacrylamide gel demonstrated that despite modification of the peptide bonds of the casein molecule, the medications retained the ability to be efficiently hydrolyzed by trypsin and chymotrypsin. The presence of high molecular weight zones (more than 30 kDa) can be explained by the ability of κ-casein to form stable complexes. The formation of these complexes depends on exposure to high temperatures. In the literature, chemical complexes between milk proteins are known as milk protein coaggregates [27, 28]. Due to the formation of stable coaggregates of casein, hydrolysis followed by visualization by electrophoresis demonstrates a particularly good visualization of the process.

Both medications showed identical results in the test for hydrolysis, so further there was used a homogenized mixture of them (hereinafter the medication).

The data obtained by vital staining of cells line MT-4 with trypan blue did not show a significant increase or decrease in proliferation compared to control (Table). Since the MTT-test did not show a significant difference in the effect of the drug on the MT-4 and Namalva cell lines, vital staining of the Namalva line with trypan blue was not performed.

Similar results were demonstrated by the MTT-test (Fig. 2).

The studies of the cytotoxic properties of the studied protein preparation showed the absence of obvious toxic influence against the cell lines MT-4 and Namalva in the all above-mentioned concentrations, so the data are presented for a concentration of 1 mg/ml as potentially the most toxic one (Fig. 2). The choice of MT-4 and Namalva cell lines as models for the evaluation of cytotoxic properties is explained by the subsequent test plan of the drug as an immunostimulant. The proliferative activity of the MT-4 line in the presence of the medication was slightly higher than in control, and the Namalva line was lower. However, a significant difference between the proliferative effect of the

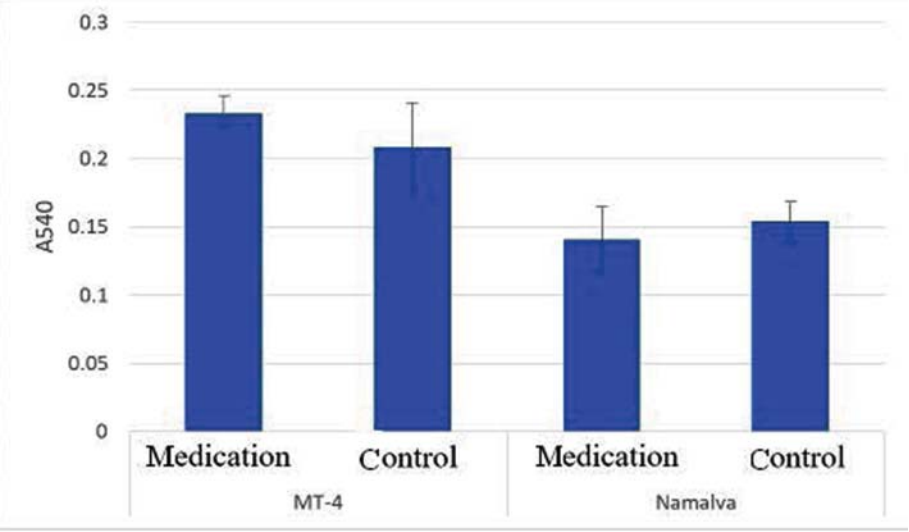

Fig. 2. **The results of indicating no cytotoxic effects of the studied protein preparation in MT-4 and Namalva cell lines ($n = 3$)**

The results of determining the number of living MT-4 cells by trypan blue staining

	Control	Medication
Concentration of living cells MT-4	$34.4 \cdot 10^3 \pm 1.2 \cdot 10^3$	$37.4 \cdot 10^3 \pm 5.6 \cdot 10^3$
Concentration of dead cells MT-4	$2.6 \cdot 10^3 \pm 0.7 \cdot 10^3$	$3.8 \cdot 10^3 \pm 2.2 \cdot 10^3$
% of dead cells MT-4	7.02*	9.22*

Note: * — $P < 0.05$ compared to control.

medication on cell cultures, compared with controls, was not observed. Therefore, it was found that the magnesium and phosphorus combined medication based on casein does not inhibit cellular respiration and does not have a marked cytotoxic effect on cells.

The electrophoregram of the medication fractions before and after hydrolysis shows a significant decrease in the staining intensity of the fractions inherent in the starting material after hydrolysis. It indicates the ability to hydrolyze the medication by the digestive enzymes under the same conditions as bovine milk casein. Casein has high bioavailability, so the medication with the similar properties can be characterized as bioavailable [29].

An important factor indicating the high bioavailability of the medication is that natural casein is used for synthesis, not a mixture of artificial amino acids. The presence of D-amino acids in mixtures of enantiomers can reduce bioavailability, in contrast to raw materials of natural origin containing only L-amino acids [30].

The increase in cell proliferation under the action of the medication was not expected from the beginning, because the cultural medium RPMI 1640 is balanced by the concentration of magnesium and phosphorus. Thus, the absence of antiproliferative effects is a promising result for future application of the studied preparation. Cytotoxicity studies can be used to predict toxic effects on the whole organism [31].

Conclusions

As a result of a set of studies, it was found that the magnesium and phosphorus combined medication based on casein is hydrolyzed by a mixture of trypsin and chymotrypsin and does not show an obvious cytotoxic effect on cell lines MT-4 and Namalva. Studies of possible cytotoxic properties did not show a statistically significant difference in the proliferation of cells under the action of the medication. Thus, the medication can potentially be used for animals after *in vivo* studies.

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Conflicts of Interest. The authors declare no conflicts of interest.

REFERENCES

1. *Pasternak K., Kocot J., Horecka A.* Biochemistry of Magnesium. *J. Elem.* 2010, 15 (3), 601–616. https://doi.org/10.5601/jelem.2010.15.3.601-616
2. *Fawcett W. J., Haxby E. J., Male D. A.* Magnesium: Physiology and Pharmacology. *Brit. J. Anesthesia.* 1999, 83 (2), 302–320. https://doi.org/10.1093/bja/83.2.302
3. *Elin R. J.* Magnesium Metabolism in Health and Disease. *Disease-a-Month.* 1988, 34 (4), 161–218. https://doi.org/10.1016/0011-5029(88)90013-2
4. *Swaminathan R.* Magnesium Metabolism and its Disorders. *Clin. Biochem. Rev.* 2003, 24 (2), 47–66. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1855626/
5. Butafosfan: Committee for Veterinary Medicinal Products, Summary Report 1. *The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines, and Information Technology Unit.* 1999, 630 (99), 1–3. https://www.ema.europa.eu/en/documents/mrl-report/butafosfan-summary-report-1-committee-veterinary-medicinal-products_en.pdf
6. *Kreipe L., Deniz A., Bruckmaier R. M., van Dorland H. A.* First Report about the Mode of Action of Combined Butafosfan and Cyanocobalamin on Hepatic Metabolism in Nonketotic Early Lactating Cows. *J. Dairy Sci.* 2011, 94 (10), 4904–4914. https://doi.org/10.3168/jds.2010-4080
7. *Breymann C., Honegger C., Hösli I., Surbek D.* Diagnosis and Treatment of Iron-Deficiency Anaemia in Pregnancy and Postpartum. *Arch. Gynecol. Obstetrics.* 2017, 296 (6), 1229–1234. https://doi.org/10.1007/s00404-017-4526-2
8. *Fong J., Khan A.* Hypocalcemia: Updates in Diagnosis and Management for Primary Care. *Canadian Family Physician Medecin de Famille Canadien.* 2012, 58 (2), 158–162. https://www.researchgate.net/publication/221968045_Hypocalcemia_Updates_in_diagnosis_and_management_for_primary_care
9. *Shenkin A.* Micronutrients in Health and Disease. *Postgraduate Med. J.* 2006, 82 (971), 559–567. https://doi.org/10.1136/pgmj.2006.047670
10. *Robberecht H., Verlaet A., Breynaert A., De Bruyne T., Hermans N.* Magnesium, Iron, Zinc, Copper, and Selenium Status in Attention-Deficit/Hyperactivity Disorder (ADHD). *Molecules.* 2020, 25 (19), 4440. https://doi.org/10.3390/molecules25194440

11. *Hertrampf E., Olivares M.* Iron Amino Acid Chelates. *Int. J. Vitamin and Nutrition Res.* 2004, 74 (6), 435–443. https://doi.org/10.1024/0300-9831.74.6.435
12. *Chaudhary D. P., Boparai R. K., Bansal D. D.* Implications of Oxidative Stress in High Sucrose Low Magnesium Diet-Fed Rats. *Eur. J. Nutrition.* 2007, 46 (7), 383–390. https://doi.org/10.1007/s00394-007-0677-4
13. *Jeppsen R. B.* Toxicology and Safety of Ferrochel and Other Iron Amino Acid Chelates. *Archivos Latinoamericanos de Nutrición*. 2001, 51 (1), 26–34. http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0004-06222001000500006
14. *Pineda O., Ashmead H. D.* Effectiveness of Treatment of Iron-Deficiency Anemia in Infants and Young Children with Ferrous Bis-Glycinate Chelate. *Nutrition*. 2001, 17 (5), 381–384. https://doi.org/10.1016/s0899-9007(01)00519-6
15. *Walker A. F., Marakis G., Christie S., Byng M.* Mg Citrate Found More Bioavailable than Other Mg Preparations in a Randomized, Double-Blind Study. *Magnesium Res.* 2003, 16 (3), 183–191. https://pubmed.ncbi.nlm.nih.gov/14596323/
16. *José A. J., Vasconcelos A. R., Valzachi Rocha Maluf M. C.* Iron Bisglycinate Chelate and Polymaltose Iron for the Treatment of Iron Deficiency Anemia: A Pilot Randomized Trial. *Current Pediatric Rev.* 2018, 14 (4), 261–268. https://doi.org/10.2174/1573396314666181002170040
17. *Kalachnyuk L. H., Arnauta A. V., Veryovka V. M., Palonko R. I.,* Veterinary Drug Biophosphomag. *UA Patent 139705.* January 10, 2020.
18. Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. Note for Guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. In *ICH Topic S6 (R1) Document*. https://www.ema.europa.eu/en/ich-s6-r1-preclinical-safety-evaluation-biotechnology-derived-pharmaceuticals
19. *Alarcón F., Moyano F., Díaz M.* Use of SDS-Page in the Assessment of Protein Hydrolysis by Fish Digestive Enzymes. *Aquaculture Int*. 2001, V. 9, P. 255–267. https://doi.org/10.1023/A:1016809014922
20. *Fotakis G., Timbrell J. A. In Vitro* Cytotoxicity Assays: Comparison of LDH, Neutral Red, MTT and Protein Assay in Hepatoma Cell Lines Following Exposure to Cadmium Chloride. *Toxicol. Letters.* 2006, 160 (2), 171–177. https://doi.org/10.1016/j.toxlet.2005.07.001
21. *Mosmann T.* Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol. Methods.* 1983, 65 (1–2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4
22. *Palonko R., Arnauta O., Prys-Kadenko V., Smirnov O., Kalachniuk L.* Combined Preparation Based on Chelating Magnesium by Phosphorylated Casein: Characteristics of its Synthesis. *ScienceRise: Biol. Sci*. 2021, 1 (26), 27–31. https://doi.org/10.15587/2519-8025.2021.228758
23. *Deng Yuxi, Gruppen H., Wierenga P. A.* Comparison of Protein Hydrolysis Catalyzed by Bovine, Porcine, and Human Trypsins. *J. Agric. Food Chem.* 2018, 66 (16), 4219–4232. https://doi:10.1021/acs.jafc.8b00679
24. A Guide to Polyacrylamide Gel Electrophoresis and Detection. Bulletin 6040. Bio-Rad Laboratories, Inc. https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6040.pdf
25. Electrophoresis (2.2.31.). *European Pharmacopoeia. Council of Europe*. 2019, 10 (1), 51–57.
26. *Ramos Y., Gutierrez E., Machado Y., Sánchez A., Castellanos-Serra L., González L. J., Fernández-de-Cossio J., Pérez-Riverol Y., Betancourt L., Gil J., Padrón G., Besada V.* Proteomics Based on Peptide Fractionation by SDS. *J. Proteome Res.* 2008, 7 (6), 2427–2434. https://doi.org/10.1021/pr700840y
27. *Macej D. O., Jovanovic T. S., Djurdjevic D. J.* The Influence of High Temperature on Milk Proteins. *Chem. Industry.* 2002, V. 56, P. 123–132. http://aspace.agrif.bg.ac.rs/bitstream/handle/123456789/423/420.pdf;jsessionid=3E24EDCC5DEA1DAB0F3599B7BB60FCD3?sequence=1
28. *Jovanovic S.* SDS-PAGE Analysis of Soluble Proteins in Reconstituted Milk Exposed to Different Heat Treatments. *Sensors.* 2007, 7 (3), 371–383. https://doi.org/10.3390/s7030371
29. *Miralles B., Sanchón J., Sánchez-Rivera L., Martínez-Maqueda D., Le Gouar Y., Dupont D., Amigo L., Recio I.* Digestion of Micellar Casein in Duodenum Cannulated Pigs: Correlation Between *in Vitro* Simulated Gastric Digestion and *in Vivo* Data. *Food Chem.* 2021, V. 343, P. 128424. https://doi.org/10.1016/j.foodchem.2020.128424
30. *Oguri S., Kumazaki M., Kitou R., Nonoyama H., Tooda N.* Elucidation of Intestinal Absorption of D, L-Amino Acid Enantiomers and Aging in Rats. *Biochim. Biophys. Acta.* 1999, 1472 (1–2), 107–114. https://doi.org/10.1016/s0304-4165(99)00110-5
31. *Scheers E. M., Ekwall B., Dierickx P. J. In Vitro* Long-Term Cytotoxicity Testing of 27 MEIC Chemicals on Hep G2 Cells and Comparison with Acute Human Toxicity Data. *Toxicology in Vitro: An Int. J. Published in Association with BIBRA.* 2001, 15 (2), 153–161. https://doi.org/10.1016/s0887-2333(00)00062-x

ДОСЛІДЖЕННЯ КОМБІНОВАНОГО ПРЕПАРАТУ МАГНІЮ І ФОСФОРУ НА ОСНОВІ КАЗЕЇНУ

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Мета. Перевірити біодоступність розробленого комбінованого препарату магнію і фосфору на основі казеїну, ґрунтуючись на здатності гідролізуватися сумішшю травних ензимів підшлункової залози — трипсину і хімотрипсину, та можливість цитотоксичного впливу на культури клітин.

Методи. Для досягнення поставленої мети використовували гідроліз препарату сумішшю трипсину і хімотрипсину з подальшою детекцією продуктів гідролізу методом електрофорезу в поліакриламідному гелі. Для оцінювання цитотоксичних ефектів застосовували стандартний МТТ-тест на культурах клітин МТ-4 і Namalva.

Результати. Досліджено вплив ультразвукової дезінтеграції на лігноцелюлозну сировину з подальшим її використанням для отримання біопалива за допомогою мікробіологічної конверсії. Показано можливість використання отриманих компонентів лігноцелюлози як субстрату після ультразвукової дезінтеграції для мікробіологічного синтезу бутанолу. Встановлено, що найбільше накопичення бутанолу (2,4 г/л) отримано за 5% вмісту сухої речовини у середовищі, 5 хв оброблення та питомій потужності ультразвукової дезінтеграції 0,72 Вт/мл.

Висновки. Оскільки негативних ефектів препарату, пов'язаних з його перетравністю і токсичністю, не виявлено, рекомендовано продовжити вивчення його впливу на моделі *in vivo*.

Ключові слова: магній, фосфор, казеїн, хелат, гідроліз, культура клітин, цитотоксичність, МТТ-реагент, NADH (нікотинамідаденіндинуклеотид).

ИССЛЕДОВАНИЕ КОМБИНИРОВАННОГО ПРЕПАРАТА МАГНИЯ И ФОСФОРА НА ОСНОВЕ КАЗЕИНА

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Цель. Проверить биодоступность разработанного комбинированного препарата магния и фосфора на основе казеина, основываясь на способности гидролизоваться смесью пищеварительных энзимов поджелудочной железы трипсином и химотрипсином, и возможность цитотоксического воздействия на культуры клеток.

Методы. Для достижения поставленных целей был использован гидролиз исследуемого препарата смесью трипсина и химотрипсина с последующей детекцией продуктов гидролиза методом электрофореза в полиакриламидном геле. Для оценки цитотоксических эффектов использовали стандартный МТТ-тест на культурах клеток МТ-4 и Namalva.

Результаты. Исследовано влияние ультразвуковой дезинтеграции на лигноцеллюлозное сырье с дальнейшим использованием для получения биотоплива с помощью микробиологической конверсии. Показана возможность применения полученных компонентов лигноцеллюлозы после обработки ультразвуковой дезинтеграцией как субстрата для микробиологического синтеза бутанола. Установлено, что наибольшее накопление бутанола (2,4 г/л) получено при 5% содержании сухого вещества в среде, 5 мин обработке и удельной мощности ультразвуковой дезинтеграции 0,72 Вт/мл.

Выводы. Поскольку отрицательных эффектов препарата, связанных с его переваримостью и токсичностью, не выявлено, рекомендуется продолжить изучение его влияния на модели *in vivo*.

Ключевые слова: магний, фосфор, казеин, хелат, гидролиз, культура клеток, цитотоксичность, МТТ реагент, NADH (никотинамидадениндинуклеотид).

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ANTIBIOTIC RESISTANCE OF LACTIC ACID BACTERY LEAVEN "VIVO PROBIOYOGURT"

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Lactic acid bacteria play a key role in human microecology and biotechnology — form organoleptic characteristics of products, increase the nutritional, including biological value of functional foods. Natural resistance to antibiotics is one of the important factors that determine the probiotic properties of lacto- and bifidobacteria.

Aim. Study of the antibiotic resistance of functionally active probiotic cultures of «VIVO probioyogurt» leaven to determine the possibility of using a fermented milk product, which is prepared on its basis, during antibiotic therapy to maintain and restore normal intestinal microflora.

Methods. Pure cultures of lactic acid bacteria (LAB) were selected for the study: (*Lactobacillus delbrueckii ssp., L. acidophilus, L.casei, L. rhamnosus, L.paracasei, Streptococcus thermophilus, Bifidobacterium lactis* (2 strains), *B. infantis*), which are part of leaven „VIVO probioyogurt" the quality of which is confirmed by certificates of the International Organization for Standardization ISO 9001: 2008, as well as ISO 22000: 2005. The method of the experiment consisted of the following stages: preparation of nutrient media („Lactobacagar", „Bifidoagar", glucose-peptone medium), working solutions of antibiotics; working suspension of LAB; suspensions of cultures (lacto- and bifidobacteria), cultivation LAB on elective nutrient media with the addition of antibiotics and evaluation of research results. Determination of antibiotic resistance of LAB was performed by the method of double dilutions.

Results. The use of this technique enabled to establish the minimum inhibitory concentration (MIC) of antibiotics of different groups relative to the LAB. The results of the research were processed using a licensed computer program Microsoft Excel.

Conclusions. Evaluation of the results of studies to determine the MIC of antibiotics — benzylpenicillin, azithromycin, lincomycin, gentamicin sulfate, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin in relation to IBD; fermented milk product, which was prepared on the basis of this starter culture, it was advisable to use during antibiotic therapy to restore and maintain normal intestinal microflora.

Key words: antibiotic resistance, lactic acid bacteria, minimum inhibitory concentration, yeast, probiotics.

In recent years, with the global environmental problems exacerbated by pollution and chemical preparations in wide use, human microecology has been strongly disrupted. This causes pathological changes in the digestive and immune organ systems. The problem became ever more urgent during the COVID-19 pandemics, as the doctors assign antibiotics to treat pneumonia. Antibiotics are the choice treatment for a suspected bacterial infection; to protect the gut microbiome during such therapy, it is recommended to use probiotics or high-quality fermented dairy products, which help the normal gut flora of the patients recover [1–5].

Currently, the functional nutrition is widely accepted, e. g., the systematic consumption of foodstuffs exhibiting regulatory effects on the human organism or its specific systems and organs.

Functional nutrition foods include products with governable properties depending on the aim they are supposed to serve. The foods to be regularly consumed by

all population subgroups should support and improve health. These foods should also lower the risk of nutrition-related diseases through constituent functional components that efficiently moderate physiological functions and metabolic reactions in the human organism [6–10].

In 1984, Japan started the first State project whose main goal was to create a system of functional nutrition. In 1991, the system was officially recognized by law as the Food for specified health use (FOSHU). That was when the concept of foodstuffs usable to support the population's health was developed [11–13].

The USA market for functional foods is the largest, amounting to 35–50% by different estimates. The high level of functional foods consumption in the country is due to the liberal nutritional law, market freedom, and the public attention to the innovations in nutrition and health protection.

In 1992, Ukraine and 159 other countries of the world approved the World Declaration and Plan of Action on Nutrition, committing to prevent the chronic shortage of the necessary vitamins, microelements, and other substances in the people's nourishment [11].

Nowadays, an unresolved problem is introducing dairy products containing probiotics apathogenic for the human body but antagonistic to the relatively pathogenic microbes and opportunistic infections. Such properties help preserve and recover normal gut microbiota [7–8]. The most common dairy products are kefir, yogurts, bioyogurts, ryazhenka, etc.

In 2020, Ukraine initiated the reform of the school catering system. Several Ministries (of Science and Education, of Economy, and of Health) joined the task beginning with comprehensively studying how the service was provided. As a result, the Plan for Measures to Reform School Catering (Cabinet of Ministers Decree on August 5, 2020) was developed and approved as part of the abovementioned reforms; the schoolchildren's daily menu now should include 150 mL of a dairy product (kefir, bioyogurt, or ryazhenka) [14].

The main requirements to be met by the probiotic preparations are bile tolerance, temperature tolerance, antimicrobial activity, and resistance to the most common antibiotics. The list suggests that antibiotic resistance is a major requirement for the microbial cultures selected for the probiotics or the fermented foods on their basis.

Many authors [14–22] have researched lactic acid bacteria (LAB) strains and probiotic preparations. For example, Kitaevskaya [16] established by the disk diffusion test that pure LAB cultures (*L. casei*, *L. fermentum*, *L. acidophilum*, *L. brevis*, etc.), except for *L. fermentum,* are highly susceptible to benzylpenicillin (for *L. casei*, the inhibition zone was 24 mm). Also, three strains, of *L. casei*, *L. plantarum*, and *L. brevis,* are sensitive to lincomycin (for *L. casei*, the inhibition zone is 3 mm). LAB cultures are *L. casei*, *L. fermentum* and *L. bavaricus*.

Studying the genetics of the antibiotic resistance of LAB, the authors of [16–18] proved that LAB have chromosomal resistance to many antibiotic substances depending on the species and the strain. The genetic basis of the phenomenon was confirmed to be a result of either own DNA mutations or incorporation of DNA of other microbes. The heredity of LAB resistance to numerous antibiotics is a consequence of mutations or determined by the acquired plasmids [15].

The author of [17] used serial dilutions to find out the MIC of antibiotics for bifidobacteria. The bacteria were resistant to third-generation antibiotics. The MIC for ceftazidime and cefepime was approximately 4 μg/mL.

With this in mind, studying antibiotic resistance of functionally active strains of LAB, which promise a lot for producing a wide range of fermented drinks, is an urgent task.

We aimed to study the resistance to antibiotics of the functionally active probiotic cultures of the "VIVO probioyogurt" starter to find out whether the product based on it is a valid choice to consume to support and recover the gut microbiome during antibiotic therapy.

Materials and Methods

For this research, we chose pure cultures of lactic acid bacteria (LAB) (*Lactobacillus delbrueckii* ssp., *L. acidophilus, L. casei, L. rhamnosus, L. paracasei, Streptococcus thermophiles, Bifidobacterium lactis,* and *B. infantis*), of which the "VIVO probioyogurt" starter is composed. The starter (produced by the Food Resources Institute of the National Academy of Agrarian Sciences of Ukraine) was selected as a source of the pure cultures of lactobacilli and bifidobacteria because it meets the European standards of quality, certified by the International Organization for Standardization (ISO 9001:2008 and ISO 22000:2005).

LAB were cultured on the Lactobacagar selective culture medium, g/L: fermentative

peptone — 15.0, glucose — 5.0, microbiological agar — 10.0, sodium acetate — 4.0, yeast extract — 2.8, potassium dihydrogen phosphate — 1.5, ammonium citrate — 1.5, magnesium chloride — 0.1, ascorbic acid — 0.04, manganese sulfate — 0.04, and distilled water — 1.0 L, pH of 6.8–7.0. Bifidobacteria were cultured on the Bifidoagar selective medium, g/L: fermentative peptone — 2.3, glucose — 7.5, yeast extract — 5.25, sodium chloride — 5.0, lactose — 2.5, bacteriological agar — 0.75, sodium acetate — 0.5, cysteine hydrochloride — 0.5, ascorbic acid — 0.5, magnesium chloride — 0.5, and distilled water — 1.0 L, with pH of 6.8–7.0. The cultures were kept for 48 hr at 37 °C. The working bacterial suspension of the LAB was prepared on the glucose-peptone accumulation medium, g/L: peptone — 5, glucose — 10, sodium chloride — 5, and distilled water — 1 L. The culture media were sterilized at 0.5 atm for 30 min.

We tested such antibiotics: benzylpenicillin, azithromycin, lincomycin, gentamicin sulfate, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin.

The working dilution of the antibiotics was 200 μg/mL:

- Sample No. 1 — benzylpenicillin (Pen) (produced at PJSC Kyivmedpreparat, Ukraine, Kyiv);
- Sample No. 2 — azithromycin (Az) (PJSC Chervona Zirka, Chemical & Pharmaceutical Plant, Ukraine, Kharkiv);
- Sample No. 3 — lincomycin (Lin) (PJSC Kyivmedpreparat, Ukraine, Kyiv);
- Sample No. 4 — gentamicin sulfate (Gen) (Galychfarm, Ukraine, Lviv);
- Sample No. 5 — ceftriaxone (Cef) (PJSC Kyivmedpreparat, Ukraine, Kyiv);
- Sample No. 6 — norfloxacin (Nor) (Zdorovye LLC, Ukraine, Kharkiv);
- Sample No. 7 — amoxil (Am) (PJSC Kyivmedpreparat, Ukraine, Kyiv);
- Sample No. 8 — streptomycin (Str) (PJSC Kyivmedpreparat, Ukraine, Kyiv);
- Sample No. 9 — tetracycline (Tetr) (JSC Vitaminy, Ukraine, Uman);
- Sample No. 10 — erythromycin (Er) (PharmaLife LTD, Ukraine, Lviv).

The antibiotics were diluted aseptically, adding sterile distilled water. To determine MIC, the following concentrations were prepared and tested by adding to the Lactobacagar and Bifidoagar culture media, μg/mL: 100; 50; 25; 12.5; 6.25; 3.13; 1.56; 0.78. The range was chosen based on data in the literature [15, 16,18].

In the bacteriological practice, two methods to determine MIC of antibiotics are the disk diffusion test and the serial dilution one [19, 23]. The latter yields more accurate quantitative data. Testing the LAB resistance to antibiotics was done by the double dilution method [19, 23]. The resistance to antibiotics in this way is characterized by the substance's activity relative to the LAB. The method allows establishing the MIC of the antibiotic in the semi-liquid (Bifidoagar) and solid (Lactobacagar) culture media.

To establish the LAB antibiotic resistance, we prepared a homogeneous working suspension based on the saline solution and the "VIVO probioyogurt" starter: the contents of the vial (0.5 g) were aseptically re-suspended in 10 mL 0.85% NaCl. The suspension was added to the two selective culture media to obtain the lactobacilli and bifidobacteria from the starter's consortium, which is the common practice [18]. For this, the biomaterial from the selective culture media (Lactobacagar and Bifidoagar) was transferred by a loop to test tubes with the glucose-peptone medium and cultured for 4–5 hr to obtain a $1 \cdot 10^5$ cell/mL suspension. Cell density was standardized by diluting the suspension with the medium or the saline solution. The optical density was measured using the DEN-1 densitometer, for which the operating range is 0.0–6.0 McFarland Units [24].

The morphology of the isolated LAB was studied by microscopy using the Microscope Digital Eyepiece DCM-800 (8.0M pixels, CMOS) at 1000× magnification after staining the samples with methylene blue.

The results were treated using Microsoft Excel software. The relative error meets the $P < 0.05$ condition. The bacteriological parameters (LAB titers depending on the antibiotic concentration) are presented on the graphs logarithmically.

Results and Discussion

The probiotics market in Ukraine boasts numerous starters for fermented dairy products recommended for daily use. We chose one, the "VIVO" starter (manufactured in Ukraine) to determine whether the probiotic yogurt is worthwhile in augmenting antibiotic treatment.

To study the antibiotic resistance of the probiotic cultures of the "VIVO probioyogurt" starter, we tested not the individual strains but the antibiotic sensitivity for the *Lactobacillus* and *Bifidobacterium* genera since fermented

dairy products are made from starters using pure LAB cultures in symbiosis.

The obtained bacterial suspension was identified and studied morphologically. The microscopy results are presented in Fig. 1.

According to the studied morphological features of the lactobacilli and bifidobacteria, the species composition of the "VIVO probioyogurt" was as stated: the rod-shaped lactobacilli formed short chains either single or paired, the bifidobacteria were Y- or V-shaped rods. By using the standard double solution method [19, 23], we found the MIC for such antibiotics: benzylpenicillin, azithromycin, lincomycin, gentamicin sulfate, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin, for the lactobacilli and bifidobacteria of the "VIVO probioyogurt" starter (Table 1). The least antibiotic concentration inhibiting LAB growth (visually identified as no colony growing on the Petri dishes and the test tubes containing the culture medium remaining transparent) was taken for the substance's minimal inhibiting concentration (MIC) for the studied cultures. It fully inhibited LAB growth.

By their susceptibility to antibiotics, LAB can be graded susceptible (MIC does not exceed 8 μg/mL), conditionally resistant, and resistant. As for the antibiotic MIC of the lactobacilli and bifidobacteria of the starter (Table 1), the test tubes filled with the culture medium were transparent, without visible signs of opalescence. On the Petri dishes, the antibiotics used in noted concentrations (Table 1) inhibited colony growth, characterizing the MIC for these antibiotic groups. The results show that the bacteria were most resistant to

Table 1. **LAB sensitivity to various antibiotics, P < 0.05**

Sample No.	Antibiotic	Antibiotic MIC for the LAB, μg/mL	
		Lactobacilli	*Bifidobacteria*
1	Pen	12.5	6.25
2	Az	6.25	25
3	Lin	3.13	6.25
4	Gen	6.25	25
5	Cef	50	50
6	Nor	50	25
7	Am	25	50
8	Str	100	100
9	Tetr	100	50
10	Er	25	25

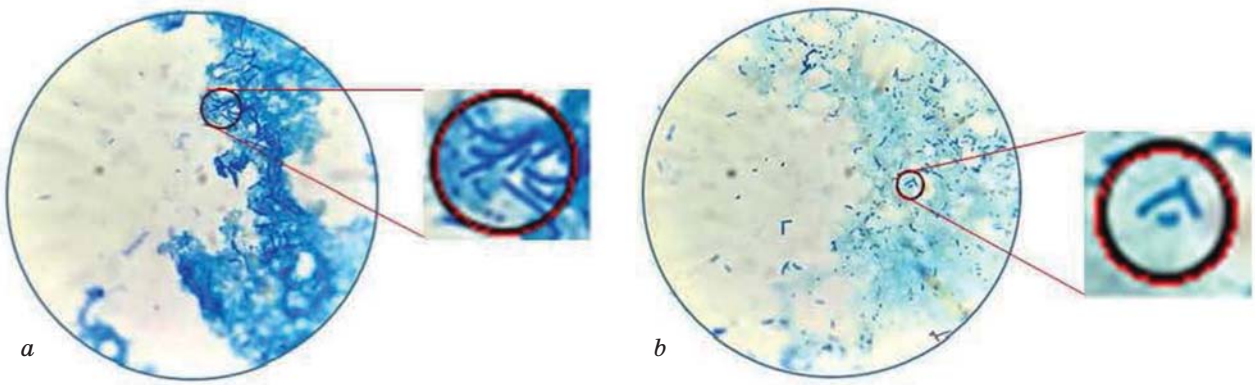

Fig. 1. **Microscopy of the studied suspension samples, at 1 000× magnification:**
a — lactobacilli; *b* — bifidobacteria

streptomycin, tetracycline, erythromycin, and ceftriaxone; the MIC for these antibiotics were 25, 50 and, 100 μg/mL. The bacteria were the least resistant to lincomycin, gentamicin sulfate, and benzylpenicillin (MIC range 3.13–12.5 μg/mL). The bifidobacteria were the most susceptible to benzylpenicillin and lincomycin. The LAB titer in the control samples (without antibiotics) was $1.5 \cdot 10^7$ CFU/mL (lg = 7.17) for the lactobacilli and $2 \cdot 10^7$ cells/mL (lg = 7.3) for bifidobacteria.

To compare and evaluate the results, we studied the susceptibility of reference strains of the lactobacilli and bifidobacteria (*L. lactis* 4/1, *L. casei* 5/4, *L. delbrueckii* 39/2, *L. acidophilus* 31/2, *L. plantarum* 17/2, *B. longum* PXN 30, *B. breve* PXN 25, *B. bifidum* PXN 23)) to the same antibiotics (Table 2).

Table 2 shows the susceptibility of reference LAB strains (*L. lactis* 4/1, *L. casei* 5/4, *L. delbrueckii* 39/2, *L. acidophilus* 31/2, *L. plantarum* 17/2, *B. longum* PXN 30, *B. breve* PXN 25, *B. bifidum* PXN 23) to the following antibiotics: benzylpenicillin, azithromycin, lincomycin, gentamicin sulfate, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin. The reference strains have the bacteria's typical physiological, morphological, and biochemical properties. The studied reference strains of the lactobacilli and bifidobacteria were the most susceptible to benzylpenicillin (No.1) except for *L. casei* 5/4. All the studied reference strains of the lactobacilli were highly susceptible to azithromycin (MIC 3.13; 6.25 μg/mL). The most resistant to this antibiotic were the reference strains of bifidobacteria *B. longum* PXN 30, *B. breve* PXN 25, and *B. bifidum* PXN 23, which to our thought, is caused by antibiotic resistance genes. Many authors [17, 18] consider the genus *Bifidobacterium* to be naturally resistant to some antibiotics, which is also confirmed by our results (Tables 1, 2). Among the lactobacilli reference strains, the most resistant to the tested antibiotics was *L. casei* 5/4. *L. lactis* 4/1 was the most sensitive among the lactobacilli reference strains to the preparations (benzylpenicillin (η 1), azithromycin (η 2), lincomycin (η 3), ceftriaxone (η 5), and amoxil (η 7)). Among the representatives of the *Bifidobacterium* genus, the most resistant to the tested antibiotics was *B. lactis* PXN 30, sensitive to benzylpenicillin (No. 1).

A comparison of MIC values for the lactobacilli and bifidobacteria of the "VIVO probioyogurt" starter and the reference LAB strains (*L. lactis* 4/1, *L. casei* 5/4, *L. delbrueckii* 39/2, *L. acidophilus* 31/2, *L. plantarum* 17/2, *B. longum* PXN 30, *B. breve* PXN 25, and *B. bifidum* PXN 23) shows that the LAB consortium of the starter is sufficiently resistant to several antibiotic preparations (Table 1). The MIC for the starter is comparable to the MIC for the reference strains (Table 2).

We determined the numbers of LAB cultured on Lactobacagar (Fig. 2) and Bifidoagar (Fig. 3) at antibiotics concentrations below the MIC (0.78 to 50 μg/mL). The results are statistically significant at $P < 0.05$.

The study results (Fig. 2) show that adding antibiotics No. 5–10 (ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin) at 0.78–1.56 μg/mL do not significantly inhibit the lactobacilli compared

Table 2. **Susceptibility of the LAB reference strains to various antibiotics, $P < 0.05$**

Strain	MIC of the antibiotic (1–10) for the reference strains of lactobacilli and bifidobacteria, μg/mL									
	1	2	3	4	5	6	7	8	9	10
L. lactis 4/1	6.25	3.13	1.6	12.5	3.13	12.5	6.25	25	50	12.5
L. casei 5/4	25	6.,25	6.25	6.25	50	50	25	100	100	25
L. delbrueckii 39/2	12.5	6.25	3.13	12.5	25	25	12.5	100	100	25
L. acidophilus 31/2	12.5	3.13	1.56	3.13	50	100	12.5	100	100	12.5
L. plantarum 17/2	6.25	6.25	6.25	3.13	12.5	12.5	12.5	50	50	12.5
B. lactis PXN 30	6.25	25	6.25	50	50	25	50	100	25	25
B. breve PXN 25	3.13	12.5	3.13	25	50	12.5	25	100	12.5	12.5
B. bifidum PXN 23	3.13	12.5	6.25	50	50	25	25	100	50	12.5

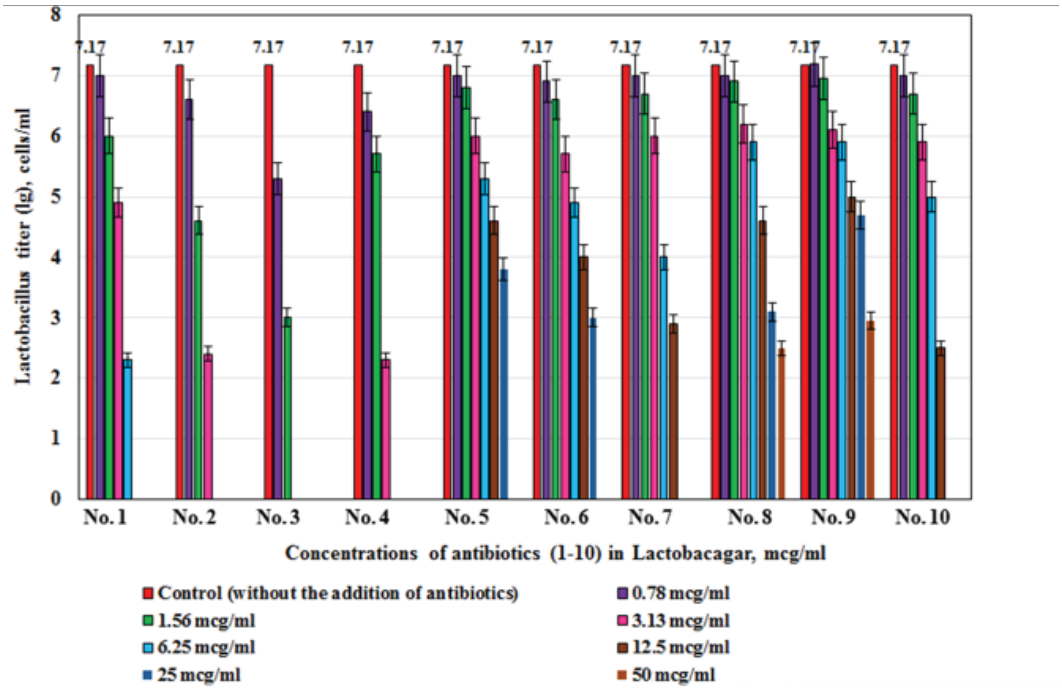

Fig. 2. **Lactobacilli numbers on Lactobacagar with antibiotics:**
1 — benzylpenicillin; 2 — azithromycin; 3 — lincomycin; 4 — gentamicin sulfate;
5 — ceftriaxone; 6 — norfloxacin; 7 — amoxil; 8 — streptomycin; 9 — tetracycline; 10 — erythromycin

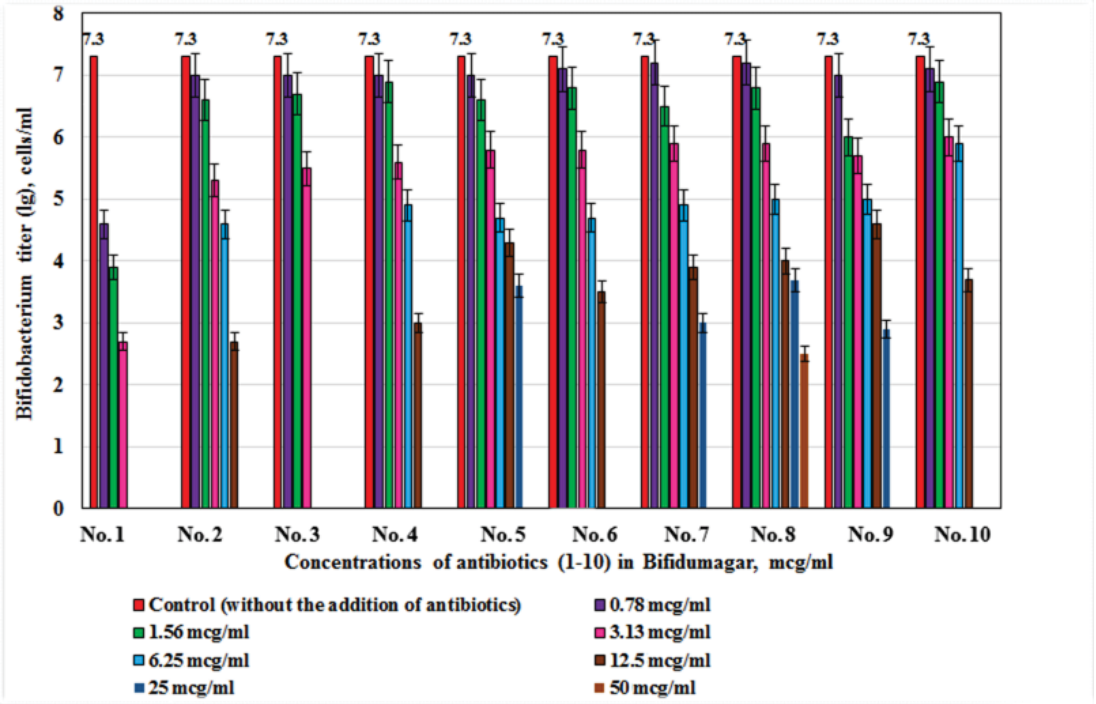

Fig. 3. **The number of bifidobacteria on the Bifidoagar with antibiotics:**
1 — benzylpenicillin; 2 — azithromycin; 3 — lincomycin; 4 — gentamicin sulfate;
5 — ceftriaxone; 6 — norfloxacin; 7 — amoxil; 8 — streptomycin; 9 — tetracycline; 10 — erythromycin

to the control (the change is within 8%). A significantly lower lactobacilli titer was seen at adding antibiotics No. 1, 5–10 (benzylpenicillin, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin) at 12.5 μg/mL and above.

The lactobacilli were most susceptible to such antibiotics as lincomycin, gentamicin sulfate, and azithromycin (MIC of 3.13–6.25 μg/mL).

The bifidobacteria were most susceptible to benzylpenicillin (No. 1), with MIC of 6.25 μg/mL. At 0.78 μg/mL benzylpenicillin, the bifidobacteria titer fell by 37% compared to the control, while for all other antibiotics, the titer decreased by 8% or less. Experimentally, bifidobacteria are the most resistant to streptomycin and tetracycline (MIC 100 μg/mL), while for ceftriaxone, norfloxacin, and amoxil, the MIC was 50 μgmL. Bifidobacteria were the most sensitive to benzylpenicillin (No. 1) and lincomycin (No. 3). If we compare the antibiotic MIC for the lactobacilli and bifidobacteria, the latter appear more resistant for the following antibiotics: azithromycin (No. 2), lincomycin (No. 3), gentamicin sulfate (No. 4), amoxil (No. 7). Notably, both bacteria groups are sensitive to all tested antibiotics, although the MIC are different.

The antibiotics' effect (No. 1–10) on the titer of the lactobacilli and bifidobacteria compared to the control is shown in Fig. 4 and 5.

According to the presented results (Fig. 4), adding benzylpenicillin (No. 1), azithromycin (No. 2), ceftriaxone (No. 5), norfloxacin (No. 6), amoxil (No. 7), streptomycin (No. 8), tetracycline (No. 9) and erythromycin (No. 10) to Lactobacagar at 0.78 μg/mL decreases the bacteria titer by 2–8%. For gentamicin sulfate (No. 4), it was 11%, and for lincomycin (No. 3) 28%.

At concentration of 1.56 μg/mL, the decrease in titer below 8% was seen for antibiotics No. 5–10. The lowest lactobacilli titer was found for the antibiotic No. 3 — lincomycin (58.3% compared to control). For the media with antibiotics No. 5–10 at 313 μg/mL, the lactobacilli decreased by 17–20%.

The concentration of 6.25 μg/mL was found to be the MIC for antibiotics No. 2–4 (azithromycin, lincomycin, gentamicin sulfate). In other samples with various antibiotics the viable lactobacilli decreased by 18–68%.

The concentration of 12.5 μg/mL was the MIC for benzylpenicillin, while for antibiotics No. 5–10 the lactobacilli decreased by 36–65%.

At 25 μg/mL, the number of viable lactobacilli colonies decreased compared to control by 40–50% for the following antibiotics: No. 9 — tetracycline, No. 5 — ceftriaxone, No. 8 — streptomycin, No. 6 — norfloxacin.

Adding antibiotics to Bifidoagar (Fig. 5) at 0.78 μg/mL decreased bifidobacteria titer by 2–4% for samples No. 2–10, and for benzylpenicillin (No. 1), the bifidobacteria titer decreased by 37%. At twice the concentration (1.56 μg/mL), antibiotics No. 2–8 inhibited the viable cell titer by 5.5–11%, and benzylpenicillin did that by 47%.

At 3.13 μg/mL benzylpenicillin, the sample titer decreased by 63% compared to control. For other tested samples (antibiotics No. 2–10), the titer decreased by 19–27%.

The concentration of 6.25 μg/mL was the MIC for benzylpenicillin. For other antibiotics, the bifidobacteria titer decreased by 31–49.3%.

12.5 μg/mL was the MIC for sample No. 10 (erythromycin); for samples No. 2–9, the bacteria titer decreased by 37–63%.

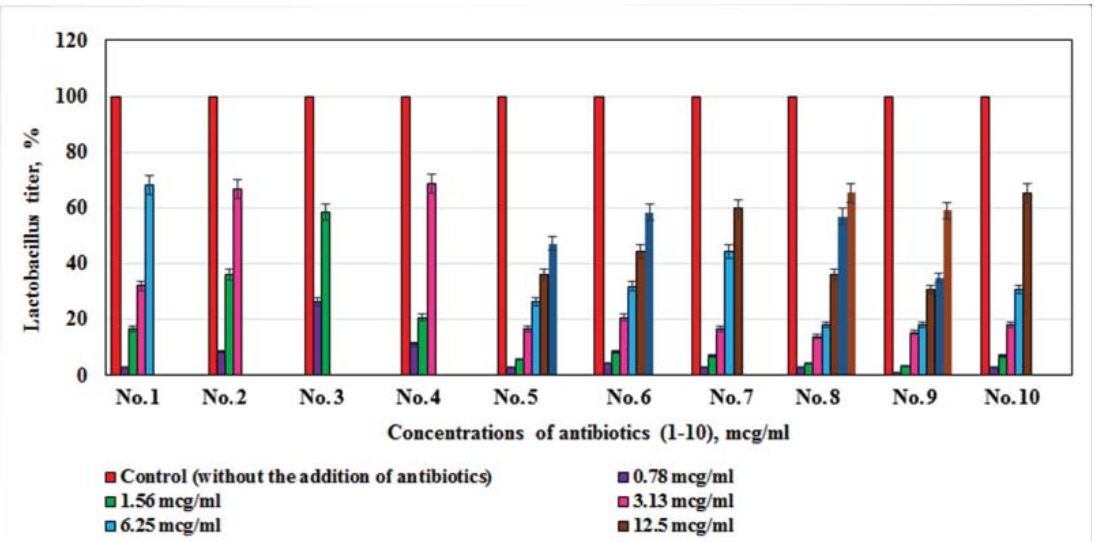

Fig. 4. **A comparison of lactobacilli titer (%) of the control sample (medium without antibiotics) o antibiotic-augmented medium samples:**
1 — benzylpenicillin; 2 — azithromycin; 3 — lincomycin; 4 — gentamicin sulfate; 5 — ceftriaxone; 6 — norfloxacin; 7 — amoxil; 8 — streptomycin; 9 — tetracycline; 10 — erythromycin

25 μg/mL was the MIC for samples No. 2–4 (azithromycin, lincomycin, and gentamicin sulfate); for samples No. 5–9 the titer decreased by 49.3–60.3%.

At 50 μg/mL streptomycin, the bifidobacteria titer decreased by 65.8% compared to control. This decrease was 65.3% for the lactobacilli. The antibiotic's MIC for both groups was 100 μg/mL.

Natural resistance to antibiotics is an essential factor determining the probiotic properties of lactobacilli and bifidobacteria, especially during antibiotic therapy. This is a most urgent problem, as the lactobacilli and bifidobacteria are included in the fermented dairy products and preparations they are based on. Thus, using the probiotic microbes for starters to obtain fermented dairy products for daily use or recommended during/after antibiotic therapy would prevent the concomitant intestine dysbacteriosis.

Comparing the MIC for antibiotics for the two groups of microbes showed that the bifidobacteria were more resistant than the lactobacilli against the following antibiotics: azithromycin, lincomycin, gentamicin sulfate, amoxil. Notably, both the lactobacilli and the bifidobacteria were susceptible to all studied antibiotics but at different levels. The results suggest that overall, the tested LAB cultures' antibiotics susceptibility is either intermediate (MIC above 8 μg/mL) for almost all tested substances or slight (MIC not below 25 μg/mL) for some of them (norfloxacin, ceftriaxone, erythromycin, tetracycline, streptomycin), and so using the functional fermented milk drinks on their basis to support the gut microbiome is a reasonable measure during antibiotic therapy.

Adding antibiotics at 0.78 and 1.56 μg/mL to the culture medium somewhat lowered the LAB titer (within 8%), while the near-MIC levels caused sharp declines in the lactobacilli and bifidobacteria.

The results are well-correlated with the literature [15–18]. According to the papers [20, 21], a consortium of bifidobacteria and lactobacilli was resistant to norfloxacin but somewhat sensitive to azithromycin. The high resistance to the tested antibiotic groups was explained by LAB consortia as probiotics as the more stable alternative to probiotic monocultures.

The previous results [25, 26] for the TM "VIVO" starter for api-products-enriched fermented milk drinks also proved the possibility of obtaining concentrated fermented milk starter for bread making, particularly, for yeast-free bread products based on the fermentation microflora of the starter enriched with pure LAB cultures.

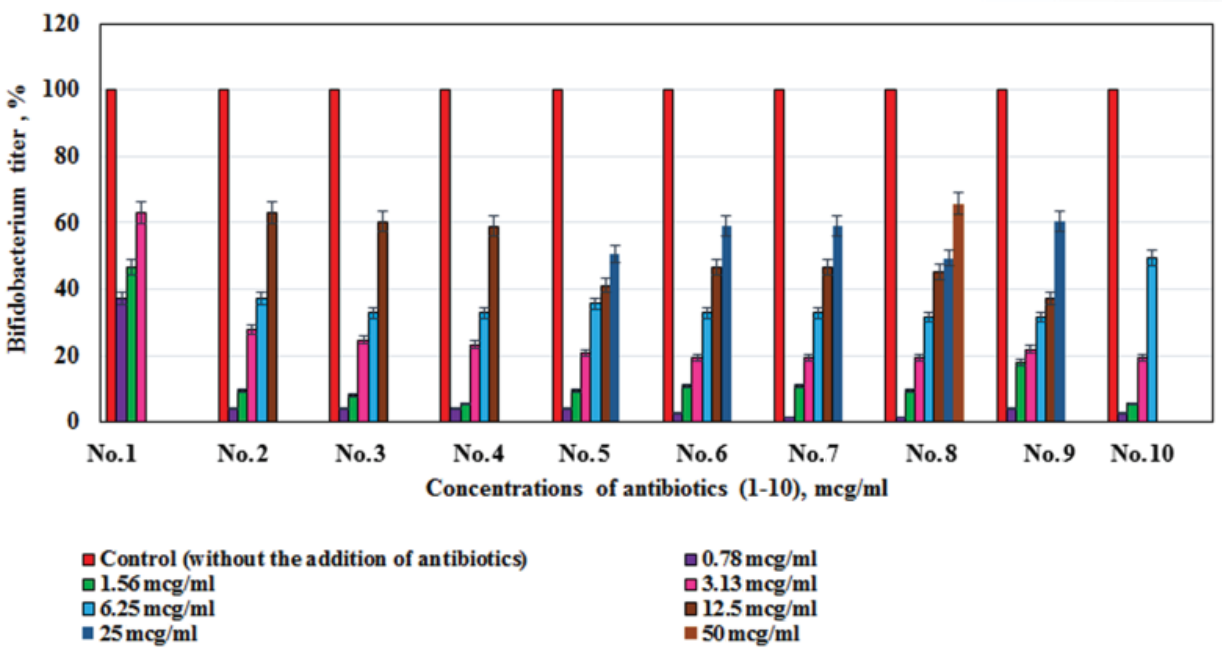

Fig. 5. **Bifidobacteria titer (%) of the control sample (without antibiotics) and of the antibiotic-augmented samples:**
1 — benzylpenicillin; 2 — azithromycin; 3 — lincomycin; 4 — gentamicin sulfate; 5 — ceftriaxone; 6 — norfloxacin; 7 — amoxil; 8 — streptomycin; 9 — tetracycline; 10 — erythromycin

Conclusions

Today, popular functional products of everyday consumption include fermented milk drinks with live probiotics beneficial to humans and antagonistic to the opportunistic and pathogenic microbes, which restore the gut microbiome during antibiotic therapy and protect the normal microbial flora.

One of the main components of probiotic cultures of the LAB is the resistance to various antibiotic compounds.

We employed the double dilution test. The bacteria were cultured on selective media, Lactobacagar and Bifidoagar; the tested antibiotics were common — benzylpenicillin, azithromycin, lincomycin, gentamicin sulfate, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, and erythromycin.

According to the results, the range of the minimal inhibiting concentrations of these substances for the probiotic cultures of the "VIVO probioyogurt" starter is 3.13–100 μg/mL.

The experiment showed that the bifidobacteria are the most susceptible to benzylpenicillin (MIC 6.25 μg/mL). The most resistant the lactobacilli and bifidobacteria were to streptomycin, erythromycin, amoxil, and ceftriaxone (MIC 50–100 μg/mL). The lactobacilli were the most susceptible to such antibiotics as lincomycin, gentamicin sulfate, and benzylpenicillin (MIC 3.13–12.5 μg/mL).

According to the antibiotic resistance results for the LAB of the "VIVO probioyogurt" starter advertised for preparation of fermented milk products, it can be recommended for milk fermentation to obtain high-quality fermented dairy products to support gut microbiota, in particular during the antibiotic therapy, as these LAB exhibit sufficient resistance to the range of antibiotic substances.

The results of the bioactivity assay of the probiotic cultures of this starter regarding the titrated acidity parameters, fermentation time, and the organoleptic evaluation of the obtained drink are evidence that the preparation can be recommended as a base for fermented dairy products enriched by plant-derived functional components or for other new products' fermentation.

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REFERENCES

1. *Neposhyvaylenko N., Kornienko I.* Current problems of individual health of adolescents and the use of modern food biotechnology to solve them. *Collective Monograph: Actual problems of natural sciences: modern scientific discussions. Lublin: Universiti of life sciences in Lublin*. 2020, P. 391–409.
2. *Cherniak L., Mikhyeyev O., Madzhd S., Lapan O., Dmytrukha T., Kornienko I.* The Usage of Plant Test Systems for the Determination of Phytotoxicity of Contaminated with Petroleum Products Soil. *J. Ecol. Eng.* 2021, 22 (6), 66–71. https://doi.org/10.12911/22998993/137363
3. *Omelych I., Neposhyvailenko N., Zberovskyi O., Kornienko I.* Improvement of the methodology for the assessment of soil biogenig pollution through the use of geological approaches and the use of information technologies. *Eastern-European J. Enterpise Technol.* 2021, 3 (10 (111)), 42–56. https://doi.org/10.15587/1729-4061.2021.235845
5. *Shenderov B. A.* Medical microbial ecology and functional nutrition. *Probiotics and functional nutrition. Moskva: Grant*. 2001, 287 p.
6. *Ganina V. I.* Probiotics. *Purpose, properties and bases of biotechnology. Moskva: MGUPB*. 2001, 169 p.
7. *Novik G. I., Samartsev A. A., Astapovich N. I., Kavrus M. A., Mikhaliuk A. N.* Biological activity of probiotic microorganisms. *Appl. Biochem. Microbiol*. 2006, 42 (2), 166–172. https://doi.org/10.1134/S0003683806020098
8. *Samoilov V. A., Nesterenko P. G., Suyunchev O. A.* Probiotic lactic acid products. *Dairy industry*. 2007, V. 7, P. 45–47.
9. *Shenderov B. A.* Probiotics and functional nutrition. *Moskva: Grant*. 2001, 288 p.
10. *Vinogradskaya S. E.* Study of the sensitivity of lactic acid cultures and microflora of fermented milk products to antibiotics. *Collection of scientific works of SevKavGTU, Series "Food"*. 2005, V. 1, P. 19–23.
11. Biofortification and functional products based on the Russian Empire for 2012–2016: the concept of state scientific and technical programs. URL: https://cutt.ly/pn1XW4n (date of the blast: 06/10/2021).

12. Concept of functional food. StudFiles: student file archive. URL: https://cutt.ly/Kn1MWcU (date of the blast: 06/10/2021).
13. State and development prospects of the functional food products market. Studbooks.net: Online student library. URL: https://cutt.ly/3n1XDah (date of the blast: 06/10/2021).
14. KMU. Decree from 24 March 2021 p. No. 305 "About the consolidated norms and the Order of organizing food for the pledges of education and child pledges of health improvement and recovery" [Electronic resource]. gov.ua. 2021. Mode of access to the resource:https://www.kmu.gov.ua/npas/pro-zatverdzhennya-norm-ta-poryadk-a305fbclid=IwAR15lfLHVBJLe-1PSPkXbCFxryfLeXjWG9LUP25xGkj39H
15. *Karapetyan K. J.* Comparative evaluation of a number of properties of new strains of 93 lactic acid bacteria. *Biol. J. Armenia*. 2009, 4 (61), 36–42.
16. *Kitaevskaya S. V.* Resistance of probiotic strains of lactic acid bacteria to antibiotics. *Bulletin of Kazan Technol. Un-ty*. 2012, 21 (15), 108–110.
17. *Shchetko V. A., Golovneva N. A.* Sensitivity of bifidobacteria to antibiotics of various classes. *News of the National Academy of Sciences of Belarus*. 2014, V. 2, 103–107.
18. *Sukhorukova M. V.* Antibiotic susceptibility of bacterial strains included in the linex probiotic. *Clin. Microbiol. Antimicrobial Chemother*. 2012, 3 (14), 245–251.
19. Determination of the sensitivity of microorganisms to antibacterial drugs: guidelines. Approved. G. G. Onishchenko. *Moskva: Federal Center for State Sanitary and Epidemiological Supervision of the Ministry of Health of Russia*. 2004, 91 p.
20. *Savitskaya I. S., Zhubanova A. A., Kistaubaeva A. S., Bolekbaeva A. B.* Antibiotic resistance of lactobacilli — probiotics. *KazNU Bulletin. Biol. series*. 2017, 56 (4), 222–227.
21. *Bagdasaryan A. S., Tokaev E. S., Nekrasov E. A., Oleynik E. A.* Antibiotic resistance of probiotic cultures included in the synbiotic. Proceedings of higher educational institutions. *Food Technol.* 2011, V. 2–3, P. 102–104.
22. *Plotnikova D. T., Sidorenko A. V., Novik G. I.* Study of antibiotic resistance of bacteria of the genera Lactococcus, Enterococcus, Leuconostoc. *Vesti National Academy of Sciences of Belarus*. 2016, V. 3, P. 94–100.
23. *Lysak V. A, Zheldakova R. A., Fomina O. V.* Microbiology. *Workshop: manual. Minsk: BSU*. 2015, 115 p.
24. DEN-1 Densitometer [Electronic resource]. *Biosan Medical-Diological Research & Technologies.* Access mode to the resource: https://biosan.lv/ru/products/den-1
25. *Korniienko I. M.,. Filimonenko O. Y., Kriukovska O. A., Hedzun E. O., Hlushkov A. S.* Research of efficiency of application of api-products in practice of preparation of biologically active functional lactic acid products. *Collection of scientific works of the Dnieper State Technical University.* 2019, 1 (34), 108–112.
26. *Korniienko I., Lutsenko O., Isaienko V., Baranovskyi M., Anatskyi A., Laricheva L.* Optimization of technological parameters of nutrition mixture fermentation process with the use of spline interpolation. *Chem. Technol.*. 2021, 29 (1), 118–136.

АНТИБІОТИКОРЕЗИСТЕНТНІСТЬ МОЛОЧНОКИСЛИХ БАКТЕРІЙ ЗАКВАСКИ «VIVO ПРОБІОЙОГУРТ»

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Молочнокислі бактерії відіграють ключову роль в мікроекології людини та біотехнологіях — формують органолептичні показники продуктів; підвищують харчову і біологічну цінність функціональних продуктів харчування. Природна стійкість до антибіотиків — один

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Молочнокислые бактерии играют ключевую роль в микроэкологии и биотехнологиях — формируют органолептические показатели продуктов; повышают пищевую и биологическую ценность функциональных продуктов питания. Естественная устойчивость к анти-

з важливих чинників, що визначають пробіотичні властивості лакто- та біфідобактерій.

Мета. З'ясувати антибіотикорезистентність функціонально-активних пробіотичних культур закваски «VIVO пробіойогурт» задля встановлення доцільності вживання кисломолочного продукту, виготовленого на її основі, під час антибіотикотерапії для підтримки та відновлення нормальної мікрофлори кишковика.

Методи. Для дослідження було обрано чисті культури молочнокислих бактерій (МКБ): (*Lactobacillus delbrueckii* ssp., *L. acidophilus, L. casei, L. rhamnosus, L. paracasei, Streptococcus thermophiles, Bifidobacterium lactis, B. infantis*), які входять до складу закваски «VIVO пробіойогурт», якість якої підтверджено сертифікатами Міжнародної організації зі стандартизації ISO 9001:2008, а також ISO 22000:2005. Методика експерименту складалась із таких етапів: приготування живильних середовищ («Лактобакагар», «Біфідоагар», глюкозо-пептонне середовище), робочих розчинів антибіотиків, робочої суспензії МКБ, суспензії культур (лакто- та біфідобактерій), культивування МКБ на елективних живильних середовищах із додаванням антибіотиків та оцінювання результатів досліджень. Визначення антибіотикорезистентності МКБ проводили методом подвійних розведень.

Результати. Використання такої методики дало змогу встановити мінімальну інгібувальну концентрацію (МІК) антибіотиків різних груп стосовно МКБ. Результати досліджень оброблено за допомогою ліцензованої комп'ютерної програми Microsoft Excel.

Висновки. За результатами досліджень з визначення МІК антибіотиків — бензилпеніцилін, азитроміцин, лінкоміцин, гентаміцину сульфат, цефтріаксон, норфлоксацин, амоксил, стрептоміцин, тетрациклін, еритроміцин щодо МКБ було встановлено, що лакто- та біфідобактерії закваски «VIVO пробіойогурт» можна віднести до умовно резистентних пробіотичних культур; кисломолочний продукт, виготовлений на основі цієї закваски, доцільно використовувати під час анибіотикотерапії задля відновлення мікрофлори кишковика.

Ключові слова: антибіотикорезистентність, молочнокислі бактерії, мінімальна інгібувальна концентрація, закваска, пробіотики.

биотикам — один из важных факторов, определяющих пробиотические свойства лакто- и бифидобактерий.

Цель. Выяснить антибиотикорезистентность функционально-активных пробиотических культур закваски «VIVO» пробиойогурт для установления целесообразности употребления кисломолочного продукта, изготовленного на ее основе, во время антибиотикотерапии для поддержания и восстановления нормальной микрофлоры кишечника.

Методы. Для исследования были выбраны чистые культуры молочнокислых бактерий (МКБ): (*Lactobacillus delbrueckii* ssp., *L. acidophilus*, *L. casei, L. rhamnosus, L. paracasei, Streptococcus thermophiles, Bifidobacterium lactis, B. infantis*), которые входят в состав закваски «VIVO пробиойогурт», качество которой подтверждено сертификатами Международной организации по стандартизации ISO 9001:2008, а также ISO 22000:2005. Методика эксперимента состояла из следующих этапов: приготовление питательных сред («Лактобакагар», «Бифидоагар», глюкозо-пептонная среда), рабочих растворов антибиотиков, рабочей суспензии МКБ, суспензии культур (лакто- и бифидобактерий), культивировании антибиотиков и оценке результатов исследований. Определение антибиотикорезистентности МКБ проводили методом двойных разбавлений.

Результаты. Использование такой методики позволило установить минимальную ингибирующую концентрацию (МИК) антибиотиков разных групп в отношении МКБ. Результаты исследований обработаны с помощью лицензированной компьютерной программы Microsoft Excel.

Выводы. По результатам исследований на предмет определения МИК антибиотиков – бензилпенициллин, азитромицин, линкомицин, гентамицина сульфат, цефтриаксон, норфлоксацин, амоксил, стрептомицин, тетрациклин, эритромицин относительно МКБ установлено, что лакто- и бифидобактерии закваски «VIVO пробиойогурт» можно отнести к условно резистентным пробиотичным культурам. Кисломолочный продукт, изготовленный на основе этой закваски, целесообразно использовать при анибиотикотерапии для восстановления микрофлоры кишечника.

Ключевые слова: антибиотикорезистентность, молочнокислые бактерии, минимальная ингибирующая концентрация, закваска, пробиотики.

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DETERMINING PROBABILITY OF CANCER CELL TRANSFOMATION AT HUMAN PAPILLOMAVIRUS INFECTION

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Aim. The purpose of the work was to assess the probability of cancerous transformation of cells for viruses of high and low oncogenic risk.

Method. It was used the statistical ensembles to determine the probability of cancer cell transformation.

For analysis there were selected oncoproteins E6 that directly influence the process of cancer cell transformation.

Results. Using normalized squared error (NSE) for viruses of high (20 strains) and low (153 strains) oncogenic risk, rank statistic of 2-exponential type was build. For productive papillomavirus infection, NSE function was determined as the growing accurate 2-exponent of a cell layer basal to the epithelial surface. Logarithm of NSE numerical values is proportional to the cell entropy that is connected with the availability of virus DNA. To calculate entropy, generalized Hartley formula was used with the informational cell of dimension d: $H = N^d LOG(NSE)$, where N is the generalized cell coordinate.

Conclusions. Using a statistical ensemble of E6 proteins separately for viruses of high and low oncogenic risk made it possible to assess the probability of cancerous transformation of cells, which was proportional to the ratio of the area of entropy of cancer transformation to the area of the productive entropy region papillomavirus infection.

Key words: human papillomavirus infection, carcinogenesis, cumulative Hartley entropy.

Human papillomaviruses are a large group of DNA viruses that, besides different forms of infections, may lead to the cancer degeneration of epithelial cells. At present, it is shown [1–4] that the presence of human papillomavirus is the necessary condition for development of cervical carcinoma. However, the papillomavirus infection alone is insufficient for neoplastic cell transformation; additional factors should take part [5–12]: high sexual activity, HIV infection, smoking, alcohol, virus loading, etc. Penetration of the virus takes place in the basal layer of epithelial cells and is accompanied with the cell proliferation. The assembly of papillomaviruses may occur only in mature cells of surface epithelial layers. Malignant neoformation occurs after rather long persistence of viruses in the basal layer of epithelial cells — the virus may be in the inactive, latent state for years. This is because the basal cells are under constant impact of the mature multilayer epithelium and incapable of cell division without the influence of external factors.

The human papillomavirus has an icosahedral protein coat 55 nm in diameter, which contains a minichromosome with double-helical DNA having ~ 8 000 bp [13, 14]. The virus replication takes place in the cell nucleus. As differentiation of epithelial cells is going on, DNA replication and expression of virus early proteins (E1, E2) take place. Late proteins (L1, L2) are produced at the final stage of virus assembly. So complicated scheme of the virus particle maturation ensures almost complete invulnerability of the virus for the immune system.

In the human organism, the papillomavirus may exist in the episome form (circular DNA) [15], which is typical for the productive infection [16], when oncoproteins E6 and E7 are not produced, but intact virus particles

are produced that may be integrated into the cell chromosome (integrated form) [17, 18]. Virus integrated form is capable of inducing malignant transformation wherein oncoproteins E6 and E7 are synthesized; interaction of the last with cell regulatory proteins p53 and pRb leads to deregulation of the cell cycle, which results in cancer cell regeneration [19, 20].

Virus infection course has several forms. The latent course has been determined as the virus persistence in basal epithelial layer [21, 22]. The virus is in the episome form and cannot lead to any pathology. Productive infection is accompanied by clinical infectious presentations (papillomas, warts, condylomas) and intensified cell growth in basal epithelial layer. At that, virus DNA is replicating in the infected cells.

Neoplasia occurs when DNA virus integrates into cell genome. At that, changes may happen in the epithelial cell structure of surface layers. Cell nucleus takes irregular shape; vacuoles appear in the cytoplasm. Most often, the injuries are localized in the transitional zone of cervix uteri at the interface of multilayer pavement and cylindrical epithelium.

In most cases of invasive tumor carcinoma, the virus exists in cells in the integrated form. Typical for malignancy atypical cells appear.

By their transforming activity towards the epithelial cells, all human papillomaviruses may be divided into two groups: papillomaviruses of high carcinogenic risk (HPV 16, 18, 26, 31, 33, 35, 39, 45, 51–53, 56, 58, 59, 66, 68, 70, 73, 82, 85); remainder (more than 150 strains) are papillomaviruses of low carcinogenic risk (HPV 6, 11, 40, 42–44, 54, 61, 62, 71, 72, 81, 83, 84 et al.) [23–25].

Papillomaviruses of low carcinogenic risk show themselves as the productive form of infection. Papillomaviruses of high carcinogenic risk (especially HPV16, 18) may lead to cancer of cervix uteri. There have been observed cases of virus infection spontaneous elimination, which causes are unknown [26, 27].

Separate types of HPV have less than 90% nucleotide identity in gene sequences of E6, E7, L1 [28]. Structural features of E6 and E7 oncoproteins are not so marked to be used for determining their oncogenic potential. Infection with viruses of high carcinogenic risk does not lead by itself to cancer of cervix uteri and disappears in most cases within 1–2 years. However, long virus persistence is essential (as main risk factor) for development of cancer cell transformation [29, 30]. Currently, development of cervix uteri cancer is described as the multistage process [31].

Accurate description the infectious process leading to cancer of cervix uteri is a rather complex problem. Therefore, different mathematical models of papillomavirus infection as a biological phenomenon have been proposed. Collaboration of mathematicians and biologists gives the possibility to evaluate conceptions of carcinogenesis and develop the generalized approach to treatment of the infectious processes.

Several deterministic models, based on differential equations [32–38], were proposed to describe different aspects of papillomavirus infection, including the potential effect of vaccination against HPV infection [39–42]. These models had only numerical solutions and could make it possible to analyze parameters leading to global equilibrium in spread of the infection.

To estimate the impact of additional factors on the infection, models with large numbers of intermediate states were suggested. Markov chains are at the basis of these models — both continuous and discrete [43–47]. In semi-Markov models, the probability to transfer into other state depends on the holding time in the initial state that is more realistic.

Papillomaviruses demonstrate strict species-specificity. Nevertheless, the investigation of papillomavirus infection on animals, especially on mice [48–51], may give more accurate information about the infectious process, which is necessary for developing new treatment methods and effective vaccines against HPV.

HPV of both high and low oncogenous risk have the same life cycle, but they differ greatly in the extent of operating cell cycle. This fact should be taken into account when using generalized approach to the structure of virus oncoproteins, since oncogenicity of the viruses cannot be determined by their real structures without implication of experimental data.

Proposed here method of statistical ensembles may significantly strengthen structural differences between oncoproteins of viruses with high and low oncogenous risk.

Material and Methods

To model processes connected with entropy changes of cells infected with papillomavirus, a statistical ensemble of E6 proteins was build separately for viruses of high and low oncogenic risk. The quantity of different amino acid residues in E6 proteins was

ranked according to their decreasing and approximated with exponential function. For each E6 protein, normalized squared error (NSE) of approximation, which is one of the main characteristic of the protein, was determined. Information on proteins of human papillomaviruses taken from the NCBI database (http://www.ncbi.nlm.nih.gov/genome/).

Results and Discussion

Statistical ensembles of oncoproteins E6

For analysis there were selected oncoproteins E6 that directly influence the process of cancer cell transformation. Protein E7 has short amino acid sequence insufficient for the structure analysis. Structural differences between proteins E6 in different papillomaviruses are not much obvious; therefore, statistical approach should be applies to the whole oncoproteins of two different papillomaviruses groups — with high and low oncogenic risk. Statistical approach gives the summarized characteristic of papillomavirus infection: function of virus spreading, function of spontaneous regression of viral infection, possibility of calculating the probability of cancer cell transformation.

Behavior of proteins E6 for different papillomaviruses may be generalized on basis of the statistical ensemble ("population") formed with using structural features of the proteins. Thereto, quantitative composition of amino acids may be arranged in alphabetical single-letter system of amino acid conventional signs. Obtained at that density of amino acid distribution has a rather complex polynomial approximation, typical for each oncoprotein E6. Such presentation reflects incidental differences of amino acid sequences in proteins E6, but it has no clear physical interpretation.

Let suppose that quantitative content of amino acids in the protein E6 for different viruses is arranged in decreasing order with reiterations. This will make it possible to select a maximally simple ordering function, which will exactly be the function of statistical ensemble. Analysis shows that three functions are relevant for oncoproteins E6: simple exponent, 2-exponent and linear function. To construct the ensemble, only one of them should be selected. For example, if simple exponent is chosen as the main function of ensemble, then the set of exponents will determine Gibbs statistical ensemble.

For each oncoprotein E6, the amino acid content was ranged in decreasing order. Then, using the method of least-squares, three functions were determined that best approximated the dependence of amino acid quantitative composition Q(Am) on the number of ordering (rank) N:

$$\text{simple exponent } Q(Am) = Q_{max}\,EXP\{-\alpha(N-1)\};$$

$$\text{2-exponent } Q(Am) = Q_1\,EXP\{-\alpha_1(N-1)\} + (Q_{max} - Q_1)\,EXP\{-\alpha_2(N-1)\};$$

$$\text{linear function } Q(Am) = Q_{max} - \alpha(N-1).$$

Hyperbolic function $Q(Am) = p/(N + m)^{\beta}$, proposed in [52], gives Q(Am) values close to the ones determined with the use of 2-exponent.

Papillomaviruses having E6 protein with linear Q(Am) characteristic: 7, 21, 25, 34, 36, 55, 67, 72, 73, 76, 80, 179.

Papillomaviruses having E6 protein with exponential Q(Am) characteristic: 16, 6, 35, 73, 43, 97, 40, 49, 5, 75, 76, 43, 41, 89, 102, 15, 20, 29, 110, 118, 132, 135, 139, 143, 144, 156, 172, 174.

E6 protein in the rest of papillomaviruses has 2-exponential characteristic.

To build the ensemble, for all proteins we have chosen the simple exponential dependence Q(Am) on the number of ordering, in spite of the fact that for some proteins such function gives no the best result with the use of least-squares approximation.

The Fig. 1 gives characteristic dependences Q (Am) on the number of ordering.

In the case of simple exponent, for each E6 oncoprotein, normalized squared error (NSE) of Q(Am) approximation is:

$$NSE\,(E6) = (1/e_p)(1/Q_{max})\,\Sigma_N\,[Q(N) - Q_{max}\,EXP\{-\alpha(N-1)\}]^2,$$

where $e_p = 10^{-4}$ is quantization step; Q(N) is the quantity of amino acids depending on the number of ordering.

For viruses of both high oncogenic risk (20 viruses) and low oncogenic risk (153 viruses), NSE function has the same dimension as Q(Am). Therefore, NSE is a generalized function (of some concentration). The function NSE may be considered as "the width" of α-level. It is significant that NSE values are unique for each E6 oncoprotein.

Generalized function NSE of E6 oncoproteins

Separately, for viruses of high and low oncogenic risk there was built NSE dependence of E6 oncoproteins on the number of reversed ranking. NSE function is a generalized function of papillomavirus DNA, which contains E6 gene.

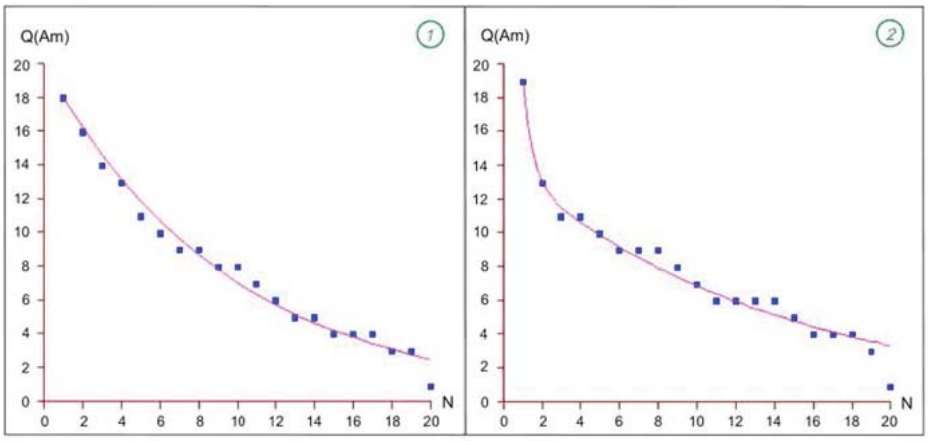

Fig. 1. 1 — **Amino acid content in E6 oncoprotein of HPV16 virus and its approximation with exponent:**
$Q(Am) = 18\ EXP\{-0.1043248(N-1)\}$
2 **—Amino acid content in E6 oncoprotein of HPV82 virus and its approximation with 2-exponent:**
$Q(Am) = 13.2\ EXP\{-0.073(N-1)\} + 5.8\ EXP\{-2.049177(N-1)\}$

NSE function may be considered as decreasing with time concentration of papillomavirus DNA at spontaneous elimination of virus infection. As increasing function, NSE represents the growth of virus loading at approaching to the epithelium border.

Decreasing NSE function is always a simple exponent, so as elimination of virus DNA is an irreversible process, irrespective of whether cancer cell transformation takes place or no, and therefore it has a maximally simple functional form. It should be pointed out that disappearance of virus DNA and its incorporation into the cell genome is described by an exponentially damped curve as expected at spontaneous elimination of virus DNA.

As epithelial cells become mature and move towards the epithelial surface, production of virus particles intensifies. That is why quantitative growth of virus DNA, and, accordingly, E6 genes, from the basal to surface layer follows exponential law (space form of the exponent). Spontaneous disappearance of the virus infection follows time exponential law.

Figs. 2 and 3 shows NSE plots, separately for viruses of high and low oncogenic risk. NSE function for viruses of high oncogenic risk differs little from simple exponent:

$$NSE_{HRSE} = 35.265\ EXP\{-0.1047024(N-1)\}.$$

As a generalized function, NSE is proportional to the probability of E6 oncoprotein decomposition.

Exact arrangement of E6 virus proteins at 2-exponential dependence of NSE requires experimental data. Generalized function determines only the image of NSE.

If 2-exponente is chosen as a main ensemble function, NSE function for viruses of high oncogenic risk will be reduced by half, and for viruses of low oncogenic risk — three fold decreased. Arrangement of E6 oncoproteins with NSE function will also change.

Integrated plot of NSE function for all analyzed papillomaviruses is shown on the Fig. 4. One may see from the plot that structural characteristics of E6 oncoproteins for both high and low oncogenous risk are mutually confused.

Hartley cumulative entropy

At productive infection, the quantity of virus DNA continuously increases when getting nearer to the epithelial surface. This process leads to the entropy growth in cells, since total information in them grows. Inverse process is spontaneous elimination of viruses. At cancer, cell entropy decreases, as there is no virus production. Intermediate forms of the infection may take place, but the main event leading to cancer is the incorporation of virus DNA into cell genome.

For productive virus infection, NSE function is defined as exact 2-exponent increasing from a basal cell layer to the epithelial surface. Decreasing cancer NSE function is the rough exponent, as events in a cell cannot be reversed. Logarithm of numerical values of NSE function is proportional to the cell entropy associated with the presence of virus DNA.

An integer value of NSE may be defined as the number of parameters (symbols) that characterize properties of E6 protein. Then LOG(NSE) is Hartley entropy (information measure) [53].

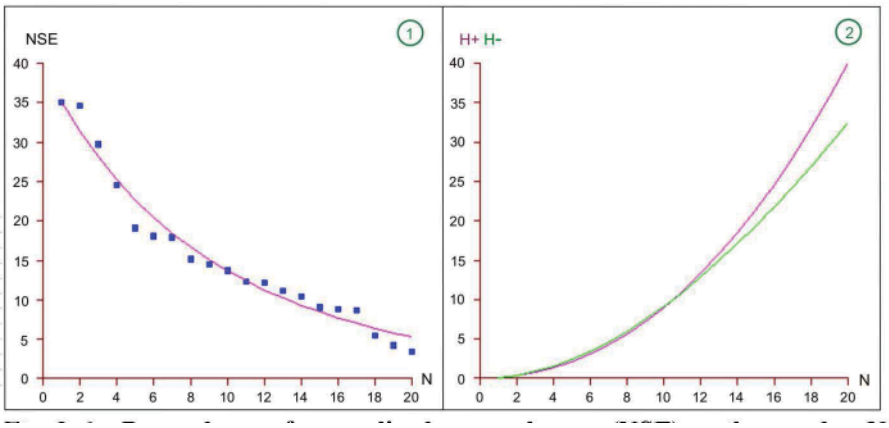

***Fig. 2. 1* — Dependence of normalized squared error (NSE) on the number N of reverse ranking of viruses with high oncogenic risk and exponential ap-proximation:**

$NSE_{HR} = 28.4\ EXP\{-\ 0.088999(N - 1)\} + 6.865\ EXP\{-\ 0.2197541(N - 1)\}$.

Arrangement of papillomaviruses with high oncogenic risk in decreasing order:

18, 45, 82, 59, 70, 53, 68, 56, 33, 73, 66, 85, 58, 51, 52, 31, 39, 26, 35, 16.

***2* — Plots of H^+ and H^- functions of cumulative entropy $d = 2$ for viruses of high oncogenic risk, N = 1...20:**

$H^+(N) = N^d LOG\ [28.4\ EXP\{-\ 0.088999(20 - N)\} + 6.865\ EXP\{-\ 0.2197541(20 - N)]$.

$H^-(N) = N^d LOG\ [35.265\ EXP\{-\ 0.1047024(N - 1)\}]$. Crosspoint of H^+ and H^- is at $N_0 = 10$.

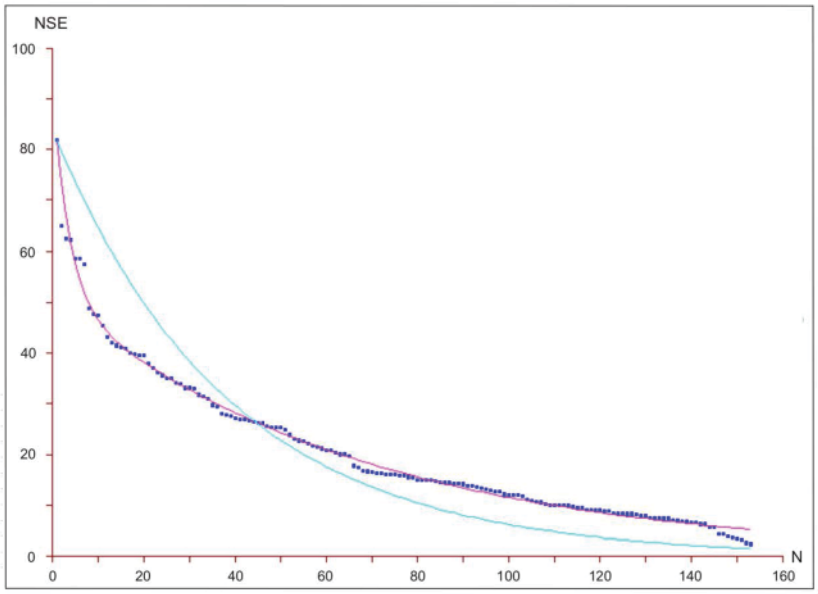

Fig. 3. **Dependence of normalized squared error (NSE) on the number N of reverse ranking of low oncogenous risk viruses:**

exponential approximation (lilac-coloured curve)

$NSE_{LR} = 50.5\ EXP\{-\ 0.0149(N - 1)\} + 31.352\ EXP\{-\ 0.2812865(N - 1)\}$

rough simple exponent (blue curve) is given by

$NSE_{LRSE} = 81.852\ EXP\{-\ 0.02603004(N - 1)\}$

arrangement of papillomaviruses of low oncogenic risk in decreasing order:

96, 204, 131, 129, 47, 197, 126, 171, 202, 140, 122, 121, 112, 105, 169, 1, 159, 115, 134, 141, 95, 151, 125, 22, 153, 113, 99, 9, 150, 63, 30, 111, 136, 201, 98, 104, 86, 199, 149, 96, 204, 131, 129, 47, 197, 126, 171, 202, 140, 122, 121, 112, 105, 169, 1, 159, 115, 134, 141, 95, 151, 125, 22, 153, 113, 99, 9, 150, 63, 30, 111, 136, 201, 98, 104, 86, 199, 149, 154, 23, 209, 109, 142, 48, 163, 117, 37, 180, 123, 3, 28, 145, 42, 17, 4, 87, 165, 20, 166, 173, 128, 179, 78, 168, 90, 60, 152, 69, 119, 100, 67, 175, 158, 12, 24, 184, 107, 65, 147, 50, 200, 32, 91, 11, 205, 170, 157, 161, 124, 138, 83, 21, 27, 167, 172, 81, 137, 133, 71, 49, 156, 120, 2, 74, 178, 92, 106, 44, 162, 94, 80, 38, 43, 36, 10, 34, 19, 84, 143, 77, 97, 61, 174, 76, 54, 110, 132, 7, 15, 144, 139, 130, 135, 164, 41, 5, 25, 88, 6, 75, 62, 55, 57, 72, 118, 89, 40, 114, 29, 93, 13, 102.

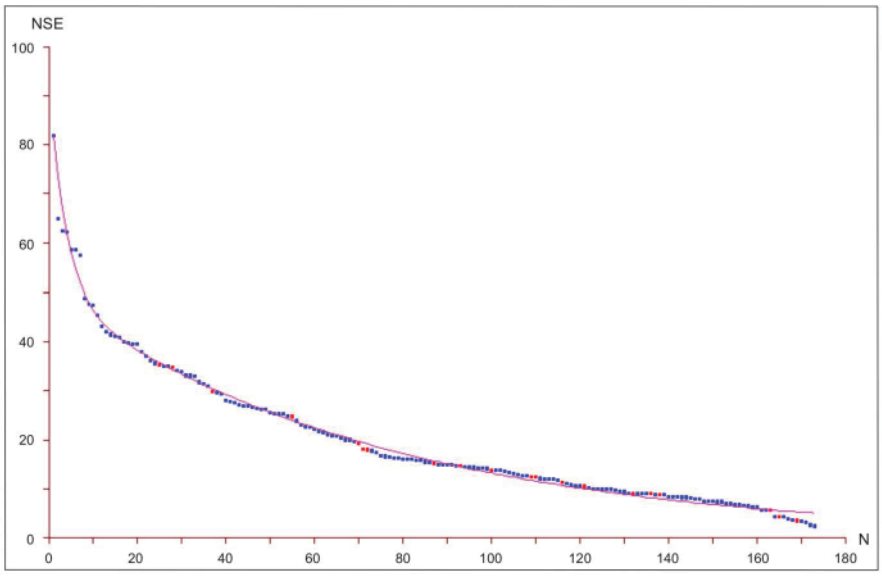

Fig. 4. **Dependence of the normalized squared error (NSE) on the number N of reverse ranking of papillomavirus oncoproteins E6 (173 viruses). Marked with red are E6 oncoproteins of viruses with high oncogenic risk. 2-exponential approximation is:**
$NSE_{HLR} = 49\ EXP\{-0.0132(N-1)\} + 32.852\ EXP\{-0.2639727(N-1)\}$

Assume that d designates the dimension (integer or fractional) of an informational cell that contains NSE parameters of E6 oncoproteins. Then Hartley cumulative entropy is proportional to $N^d LOG(NSE)$. If dimension d is fractional then the informational cell will be fractal; in the case when dimension d is integer, we may suppose that d is the number of linked cells.

Graphics of H^+ и H^- functions of cumulative entropy at $d = 2$ for viruses of high and low oncogenous risk are shown on Figs. 2.2 and 5.

Let us consider the connection of informational cell dimension with features of papillomavirus infection. If $d > 4$, any structural cell changes cannot be observed; that is, the virus infection is in the latent period. It is natural, as multidimentional informational cells just cannot be observed. We may also suppose that dimension d passes all fractional stages from $d = 4$ to $d = 1$.

In the case when $3 < d < 4$, one may observe the productive virus infection for papillomaviruses of high and low oncogenic risk; however for viruses of high oncogenic risk, neoplasia may develop. Virus DNAs are distributed in three-dimensional space of cells for viruses of low oncogenic risk and in four-dimensional space (taking into account the incorporation of virus DNA into the cell genome) for viruses of high oncogenic risk.

Region $2 < d < 3$ defines the fractal dimension of papillomas and condylomas as benign tumors for viruses of low oncogenic risk and epidermoid intraepithelial lesions (precancerous conditions) for papillomaviruses of high oncogenic risk.

Region $d < 2$ for viruses of low oncogenic risk does not exist. Value $d = 2$ may be attributed to pointed condylomas because of their rather soft consistency.

In the region $1 < d < 2$, preinvasive carcinoma is observed when the fractal dimension of cancerous tumour is less then observed two-dimensional one as cancerous tumours are weakly differentiated.

At $d = 1$, metastases appear. Detaching of cancer cells is possible at the cutting of last (single) bond with the cancer tumour.

Before crossing point N_0, we have H– (N) > H+ (N), that is, the entropy of virus DNA elimination or cancer transformation is more than the entropy of quantitative growth of episome DNA.

We will designate the area between curves H– (N) and H+ (N) at $N < N_0$ as S–, and the area between curves H+ (N) and H– (N) at $N > N_0$ — as S+. Then, the probability of cancer cell transformation may be defined as $P_c = S-/S+$.

Table shows probability values of cancer cell transformation for of high and low oncogenic risk.

Note that decreasing rough exponential function NSE (elimination of virus DNA or cancer) corresponds to the return for cell into the past, but not in the least for the cell evolution.

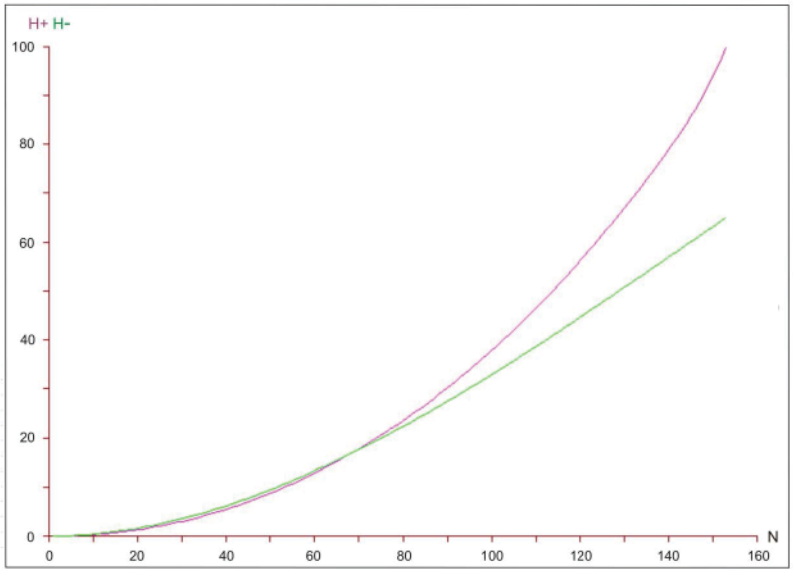

Fig. 5. **Graphics of H^+ и H^- functions of cumulative entropy at $d = 2$ for viruses of low oncogenous risk, N = 1...153:**
$H+ (N) = N^d LOG [50.5 EXP\{- 0.0149(153 - N)\} + 31.352 EXP\{- 0.2812865(153 - N)\}]$.
$H^- (N) = N^d LOG [81.852 EXP\{- 0.02603004(N - 1)\}]$.
Crosspoint of H^+ and H^- is at $N_0 = 67.5$

Values of probability of cancer cell transformation for papillomaviruses of high and low oncogenic risk

d	High risk	Low risk
1	$1.94230300 \cdot 10^{-1}$	—
2	$5.72016772 \cdot 10^{-2}$	$2.92785191 \cdot 10^{-2}$
3	$1.98637659 \cdot 10^{-2}$	$9.06198389 \cdot 10^{-3}$
4	$7.55089981 \cdot 10^{-3}$	$3.06071257 \cdot 10^{-3}$
5	$3.03657242 \cdot 10^{-3}$	$1.09113459 \cdot 10^{-3}$
6	$1.26838686 \cdot 10^{-3}$	$4.03370226 \cdot 10^{-4}$
7	$5.44360273 \cdot 10^{-4}$	$1.53033009 \cdot 10^{-4}$
8	$2.38393159 \cdot 10^{-4}$	$5.91933751 \cdot 10^{-5}$

Evolution for a cell is its transformation at productive virus infection, since at that entropy growth in the cell is the largest.

It is also significant that exponential function NSE for viruses of high oncogenic risk is not too rough. Therefore, external factors heavily influence on cancer cell transformation, as opposed to rough exponent NSE for viruses of low oncogenic risk, when external factors do not influence on cancer cell transformation.

Conclusions

Using a statistical ensemble of E6 proteins separately for viruses of high and low oncogenic risk makes it possible to assess the probability of cancerous transformation of cells, which is proportional to the ratio of the area of entropy of cancer transformation to the area of the productive entropy region papillomavirus infection.

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REFERENCES

1. *Walboomers J.M.M., Jacobs M.V., Manos M.M., Bosch F. X., Kummer J. A., Shah K. V., Snijders P.J., Peto J., Meijer C.J., Muñoz N.* Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol.* 1999, V. 189, P. 12–19. https://doi.org/10.1002/(SICI)1096-9896(199909)189:1<12::AID-PATH431>3.0.CO;2-F
2. *Harald zur Hausen.* Papillomaviruses in the causation of human cancers – a brief historical account. *Virology.* 2009, V. 384, P. 260–265. https://doi.org/10.1016/j.virol.2008.11.046
3. *Crosbie E. J., Einstein M. H., Franceschi S., Kitchener H. C.* Human papillomavirus and cervical cancer. *Lancet.* 2013, V. 382, P. 889–999. https://doi.org/10.1016/S0140-6736(13)60022-7
4. *Senapati R., Senapati N. N., Dwibedi B.* Molecular mechanisms of HPV mediated neoplastic progression. *Infectious Agents and Cancer.* 2016, V. 11, P. 59. https://doi.org/10.1186/s13027-016-0107-4

5. *Castellsagué X, Munoz N.* Chapter 3: Cofactors in human papillomavirus carcinogenesis — role of parity, oral contraceptives and tobacco smoking. *J. Natl. Cancer Inst. Monogr.* 2003, V. 31, P. 20–28.
6. *Castle P. E.* How Does Tobacco Smoke Contribute to Cervical Carcinogenesis? *J. Virol.* 2008, 82 (12), 6084–6086. https://doi.org/10.1128/JVI.00103-08
7. *Long Fu Xi, Hughes J. P., Castle P. E., Edelstein Z. R., Wang C., Galloway D. A.,. Koutsky L. A., Kiviat N. B., Schiffman M.* Viral Load in the Natural History of Human Papillomavirus Type 16 Infection: A Nested Case–control Study. *J. Infect. Dis.* 2011, 203 (10), 1425–1433. https://doi.org/10.1093/infdis/jir049
8. *Yetimalar H., Kasap B., Cukurova K., Yildiz A., Keklik A., Soylu F.* Cofactors in human papillomavirus infection and cervical carcinogenesis. *Arch. Gynecol. Obstet.* 2012, 285 (3), 805–810. https://doi.org/10.1007/s00404-011-2034-3
9. *Jalil E. M., Bastos F. I., dos Santos Melli P. P., Duarte G., Simoes R. T., Yamamoto A. Y., de Morais R. A. A., Quintana S. M.* HPV clearance in postpartum period of HIV positive and negative women: a prospective follow-up study. *BMC Infect. Dis.* 2013, V. 13, P. 564. https://doi.org/10.1186/1471-2334-13-564
10. *Sundstrom K., Ploner A., Dahlstrom L. A., Palmgren J., Dillner J., Adami H.-O., Ylitalo N., Sparén P.* Prospective Study of HPV16 Viral Load and Risk of In Situ and Invasive Squamous Cervical Cancer. *Cancer Epidemiol. Biomarkers Prev.* 2013, 22 (1). 150–158. https://doi.org/10.1158/1055-9965.EPI-12-0953-T
11. *Del Río-Ospina L., Soto-De León S. C., Camargo M., Moreno-Pérez D. A., Sánchez R., Pérez-Prados A., Patarroyo M. E., Patarroyo M. A.* The DNA load of six high-risk human papillomavirus types and its association with cervical lesions. *BMC Cancer.* 2015, V. 15, P. 100. https://doi.org/10.1186/s12885-015-1126-z
12. *Tulay P., Serakinci N.* The role of human papillomaviruses in cancer progression. *J. Cancer Metastasis Treat.* 2016, V. 2, P. 201–213. https://doi.org/10.20517/2394-4722.2015.67
13. *Doorbar J., Quintb W., Banksc L., Bravo I. G., Stoler M., Broker T. R., Stanley M. A.* The Biology and Life-Cycle of Human Papillomaviruses. *Vaccine.* 2012, 30 (5), F55–70. https://doi.org/10.1016/j.vaccine.2012.06.083
14. *Doorbar J., Egawa N., Griffin H., Kranjec C., Murakami I.* Human papillomavirus molecular biology and disease association. *Rev. Med. Virol.* 2015, 25 (1), 2–23. https://doi.org/10.1002/rmv.1822
15. *Egawa N., Doorbar J.* The low-risk papillomaviruses. *Virus Res.* 2017, V. 231, P. 119–127. https://doi.org/10.1016/j.virusres.2016.12.017
16. *Kajitani N., Satsuka A., Kawate A., Sakai H.* Productive Lifecycle of Human Papillomaviruses that Depends Upon Squamous Epithelial Differentiation. *Front Microbiol.* 2012, V. 3, P. 152. https://doi.org/10.3389/fmicb.2012.00152
17. *Akagi K., Li J., Broutian T. R., Xiao W., Jiang B., Rocco J. W., Teknos T. N., Kumar B., Wangsa D., He D., Ried T., Symer D. E., Gillison M. L.* Genome-wide analysis of HPV integration in human cancers reveals recurrent, focal genomic instability. *Genome Res.* 2014, 24 (2), 185–199. https://doi.org/10.1101/gr.164806.113
18. *McBride A. A., Warburton A.* The role of integration in oncogenic progression of HPV- associated cancers. *PLoS Pathog.* 2017, 13 (4), e1006211. https://doi.org/10.1371/journal.ppat.1006211
19. *Pol S. B. V., Klingelhutz A. J.* Papillomavirus E6 oncoproteins. *Virology.* 2013, V. 445, P. 115–137. https://doi.org/10.1016/j.virol.2013.04.026
20. *Roman A., Munger K.* The papillomavirus E7 proteins. *Virology.* 2013, 445 (1–2), 138–168. https://doi.org/10.1016/j.virol.2013.04.013
21. *Maglennon G. A., Doorbar J.* The Biology of Papillomavirus Latency. *Open Virol. J.* 2012, 6 (Suppl 2: M4), 190–197. https://doi.org/10.2174/1874357901206010190
22. *Gravitt P. E.* Evidence and Impact of Human Papillomavirus Latency. *Open Virol. J.* 2012, 6 (Suppl 2: M5), 198–203. https://doi.org/10.2174/1874357901206010198
23. *Brown C. R., Leon M. L., Muñoz K., Fagioni A., Amador L. G., Frain B., Tu W., Qadadri B., Brown D. R.* Human papillomavirus infection and its association with cervical dysplasia in Ecuadorian women attending a private cancer screening clinic. *Braz. J. Med. Biol. Res.* 2009, 42 (7), 629–636. https://doi.org/10.1590/s0100-879x2009000700007
24. *Kaspersen M. D., Larsen P. B., Ingerslev H. J., Fedder J., Petersen G. B., Bonde J., Höllsberg P.* Identification of Multiple HPV Types on Spermatozoa from Human Sperm Donors. *PLoS One.* 2011, 6 (3), e18095. https://doi.org/10.1371/journal.pone.0018095
25. *McBride A. A.* Oncogenic human papillomaviruses. *Phil. Trans. R. Soc. B.* 2017, V. 372, P. 20160273. https://doi.org/10.1098/rstb.2016.0273
26. *Gravitt P. E., Winer R. L.* Natural History of HPV Infection across the Lifespan: Role of Viral Latency. *Viruses.* 2017, 9 (10), 267. https://doi.org/10.3390/v9100267
27. *Plummer M., Schiffman M., Castle P. E.* A 2-Year Prospective Study of Human Papillomavirus Persistence among Women with a Cytological Diagnosis of Atypical Squamous Cells of Undetermined Significance or Low-Grade Squamous Intraepithelial Lesion. *J. Infect. Dis.* 2007, V. 195, P. 1582–1589. https://doi.org/10.1086/516784
28. *Burd E. M.* Human Papillomavirus and Cervical Cancer. *Clin. Microbiol. Rev.* 2003, 16 (1), 1–17. https://doi.org/10.1128/CMR.16.1.1-17.2003

29. *Marks M. A., Castle P. E., Schiffman M., Gravitt P. E.* Evaluation of Any or Type-Specific Persistence of High-Risk Human Papillomavirus for Detecting Cervical Precancer. *J. Clin. Microbiol*. 2012, 50 (2), 300–306. https://doi.org/10.1128/JCM.05979-11
30. *Sudenga S. L., Shrestha S.* Key considerations and current perspectives of epidemiological studies on human papillomavirus persistence, the intermediate phenotype to cervical cancer. *Int. J. Infect. Dis*. 2013, 17 (4), e216–e220. https://doi.org/10.1016/j.ijid.2012.12.027
31. *Schiffman M., Wentzensen N.* Human Papillomavirus Infection and the Multistage Carcinogenesis of Cervical Cancer. *Cancer Epidemiol. Biomarkers Prev*. 2013, 22 (4), 553–560. https://doi.org/10.1158/1055-9965.EPI-12-1406
32. *Santiago D. N., Heidbuechel J. P. W., Kandell W. M.* Fighting Cancer with Mathematics and Viruses. *Viruses*. 2017, 9 (9), 239. https://doi.org/10.3390/v9090239
33. *Pongsumpun P.* Mathematical Model of Cervical Cancer due to Human Papillomavirus Infection. *Mathematical Methods in Science and Engineering. Proceedings of the 1st Int. Conference on Mathematical Methods & Computational Techniques in Science & Engineering (MMCTSE 2014) Athens, Greece – November 28–30, 2014*. P. 157–161.
34. *Ribassin-Majed L., Lounes R.* A SIS model for Human Papillomavirus transmission. MAP5. 2011-03. 15 pages. 2010. https://hal.archives-ouvertes.fr/hal-00555733v1
35. *Obeng-Denteh W., Afrifa R. T., Barnes B. at al.* Modeling the Epidemiology of Human Papilloma Virus Infection and Vaccination and Its Impact on Cervical Cancer in Ghana. *J. Sci. Res. Rep*. 2014, 3 (19), 2501–2518. https://doi.org/10.9734/JSRR/2014/11019
36. *Baussano I., Franceschi S., Plummer M.* Infection transmission and chronic disease models in the study of infection-associated cancers. *Br. J. Cancer*. 2014, V. 110, P. 7–11. https://doi.org/10.1038/bjc.2013.740
37. *Angstmann C. N., Henry B. I., McGann A. V.* A Fractional Order Recovery SIR Model from a Stochastic Process. *Bull. Math. Biol*. 2016, V. 78, P. 468–499. https://doi.org/10.1007/s11538-016-0151-7
38. *Lee S. L., Tameru A. M.* A Mathematical Model of Human Papillomavirus (HPV) in the United States and its Impact on Cervical Cancer. *J. Cancer*. 2012, V. 3, P. 262–268. https://doi.org/10.7150/jca.4161
39. *Dasbach E. J., Elbasha E. H., Insinga R. P.* Mathematical Models for Predicting the Epidemiologic and Economic Impact of Vaccination against Human Papillomavirus Infection and Disease. *Epidemiol. Rev*. 2006, V. 28, P. 88–100. https://doi.org/10.1093/epirev/mxj006
40. *Goldhaber-Fiebert J. D., Stout N. K., Ortendahl J., Kuntz K. M., Goldie S. J., Salomon J. A.* Modeling human papillomavirus and cervical cancer in the United States for analyses of screening and vaccination. *Popul. Health Metr*. 2007, V. 5, P. 11. https://doi.org/10.1186/1478-7954-5-11
41. *Smith R. J., Li J., Mao J., Sahai B.* Using within-host mathematical modeling predict the long-term outcome of human papillomavirus vaccines. *Canadian Applied Mathematics Quarterly*. 2013, 21 (2), 281–299.
42. *Haeussler K., Marcellusi A., Mennini F. S.* Cost-Effectiveness Analysis of Universal Human Papillomavirus Vaccination Using a Dynamic Bayesian Methodology: The BEST II Study. *Value Health*. 2015, 18 (8), 956–968. https://doi.org/10.1016/j.jval.2015.08.010
43. *Myers E. R., McCrory D. C., Nanda K., Bastian L., Matchar D. B.* Mathematical Model for the Natural History of Human Papillomavirus Infection and Cervical Carcinogenesis. *Am. J. Epidemiol*. 2000, 151 (12), 1158–1171. https://doi.org/10.1093/oxfordjournals.aje.a010166
44. *Bureau A., Shiborski S., Hughes J. P.* Applications of continuous time hidden Markov models to the study of misclassified disease outcomes. *Stat. Med*. 2003. 22 (3), 441–462. https://doi.org/10.1002/sim.1270
45. *Kang M., Lagakos S. W.* Statistical methods for panel data from a semi-Markov process, with application to HPV. *Biostatistics*. 2007, 8 (2), 252–264. https://doi.org/10.1093/biostatistics/kxl006
46. *Mitchell C. E., Hudgens M. G., King C. C. at al.* Discrete-time semi-Markov modeling of human papillomavirus Persistence. *Stat. Med*. 2011, 30 (17), 2160–2170.
47. *Spitoni Cristian, Verduijn Marion and Putter Hein*. Estimation and Asymptotic Theory for Transition Probabilities in Markov Renewal Multi–State Models. *Int. J. Biostat*. 2012, 8 (1), 23. https://doi.org/10.1515/1557-4679.1375
48. *Longet S., Schiller J. T., Bobst M., Jichlinski P., Nardelli-Haefliger D.* A Murine Genital-Challenge Model Is a Sensitive Measure of Protective Antibodies against Human Papillomavirus Infection. *J. Virol*. 2011, 85 (24), 13253–13259. https://doi.org/10.1128/JVI.06093-11
49. *De Azambuja K., Barman P., Toyama J.* Validation of an HPV16-mediated Carcinogenesis Mouse Model. *In vivo*. 2014, V. 28, P. 761–768. PMID: 25189887. PMCID: PMC5214601
50. *Hu J., Budgeon L. R., Cladel N. M., Balogh K., Myers R., Cooper T. K., Christensen N. D.* Tracking vaginal, anal and oral infection in a mouse papillomavirus infection model. *J. Gen. Virol*. 2015, 96 (12), 3554–3565. https://doi.org/10.1099/jgv.0.000295
51. *Jiafen Hu, Nancy M. Cladel, Lynn R. Budgeon, Balogh K. K., Christensen N. D.* The Mouse Papillomavirus Infection Model.

Viruses. 2017, V. 9, P. 246. https://doi.org/10.3390/v9090246
52. *Mandelbrot B.* An information theory of the statistical structure of language. In *Communication Theory. ed. W. Jackson, Betterworths*. 1953, P.486–502.
53. *Hartley R. V. L.* Transmission of information. *Bell Syst. Techn. J.* 1928, 7 (3), 535–563. https://doi.org/10.1002/j.1538-7305.1928.tb01236.x

ВИЗНАЧЕННЯ ЙМОВІРНОСТІ РАКОВОЇ ТРАНСФОРМАЦІЇ КЛІТИН ЗА ПАПІЛОМАВІРУСНОЇ ІНФЕКЦІЇ ЛЮДИНИ

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Мета. Оцінити ймовірності ракової трансформації клітин вірусами високого та низького онкогенних ризиків.

Методи. Використовували статистичні ансамблі визначення ймовірності трансформації ракових клітин.

Для аналізу було відобрано онкопротеїни E6, які безпосередньо впливають на процес трансформації ракових клітин.

Результати. За допомогою нормальної квадратичної помилки (NSE) для вірусів високого (20 штамів) і низького (153 штами) онкогенних ризиків було побудовано рангову статистику 2-експоненціального типу. Для продуктивної папіломавірусної інфекції функцію NSE визначали як зростаючу точну 2-експоненту клітинного шару базальної поверхні епітелію. Логарифм числових значень NSE пропорційний ентропії клітин, яка пов'язана з наявністю вірусної ДНК. Для підрахунку ентропії використовували узагальнену формулу Хартлі з інформаційною коміркою розмірності d: H = NdLOG (NSE), де N — узагальнена координата комірки.

Висновки. Використання статистичного ансамблю протеїнів E6 окремо для вірусів високого та низького онкогенних ризиків дає змогу оцінити ймовірність ракової трансформації клітин, яка пропорційна відношенню площі ентропії ракової трансформації до площі продуктивної ентропії папіломавірусної інфекції.

Ключові слова: папіломавірусна інфекція людини, канцерогенне переродження клітин, кумулятивна ентропія Хартлі.

ОПРЕДЕЛЕНИЕ ВЕРОЯТНОСТИ РАКОВОЙ ТРАНСФОРМАЦИИ КЛЕТОК ПРИ ПАПИЛЛОМАВИРУСНОЙ ИНФЕКЦИИ ЧЕЛОВЕКА

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Цель. Оценка вероятности раковой трансформации клеток вирусами высокого и низкого онкогенных рисков.

Методы. Использовались статистические ансамбли для определения вероятности трансформации раковых клеток.

Для анализа были отобраны онкопротеины E6, непосредственно влияющие на процесс трансформации раковых клеток.

Результаты. С помощью нормализованной квадратичной ошибки (NSE) для вирусов высокого (20 штаммов) и низкого (153 штаммов) онкогенного риска была построена ранговая статистика 2-экспоненциального типа. Для продуктивной папилломавирусной инфекции функцию NSE определяли как возрастающую точную 2-экспоненту клеточного слоя базальной поверхности эпителия. Логарифм числовых значений NSE пропорционален энтропии клетки, связанной с наличием вирусной ДНК. Для вычисления энтропии использовалась обобщенная формула Хартли с информационной ячейкой размерности d: H = NdLOG (NSE), где N — обобщенная координата ячейки.

Выводы. Использование статистического ансамбля протеинов E6 отдельно для вирусов высокого и низкого онкогенных рисков позволяет оценить вероятность раковой трансформации клеток, которая пропорциональна отношению площади энтропии раковой трансформации к площади продуктивной энтропии папилломавирусной инфекции.

Ключевые слова: папилломавирусная инфекция человека, канцерогенное перерождение клеток, кумулятивная энтропия Хартли.