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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

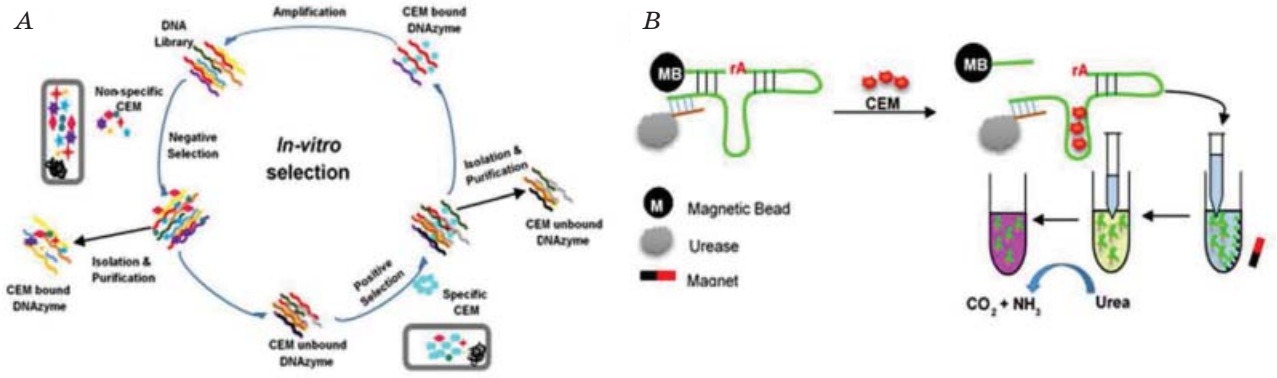

Fig. 1. ***A* — Schematic illustration of the *in vitro* selection of bacteria-specific DNAzyme using CEM**

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.

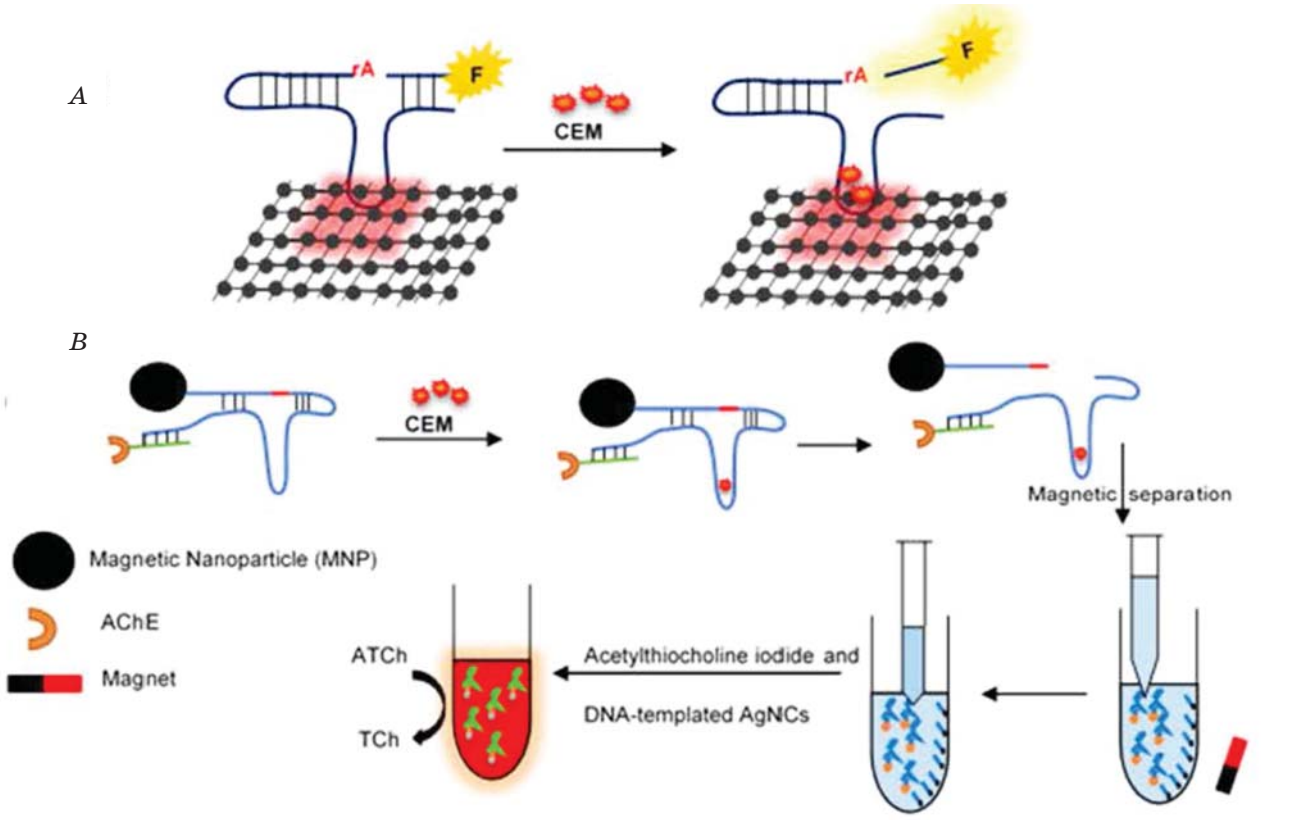

Fig. 2. A — **Schematic illustration of the graphene-based DNAzyme biosensor to detect *E. coli* bacteria [27]**
Attachment of the DNAzyme onto the graphene quenched the fluorescence emission of the fluorophore. Upon the cleavage reaction,the release of fluorophore tagged DNA fragment enhanced the fluorescence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

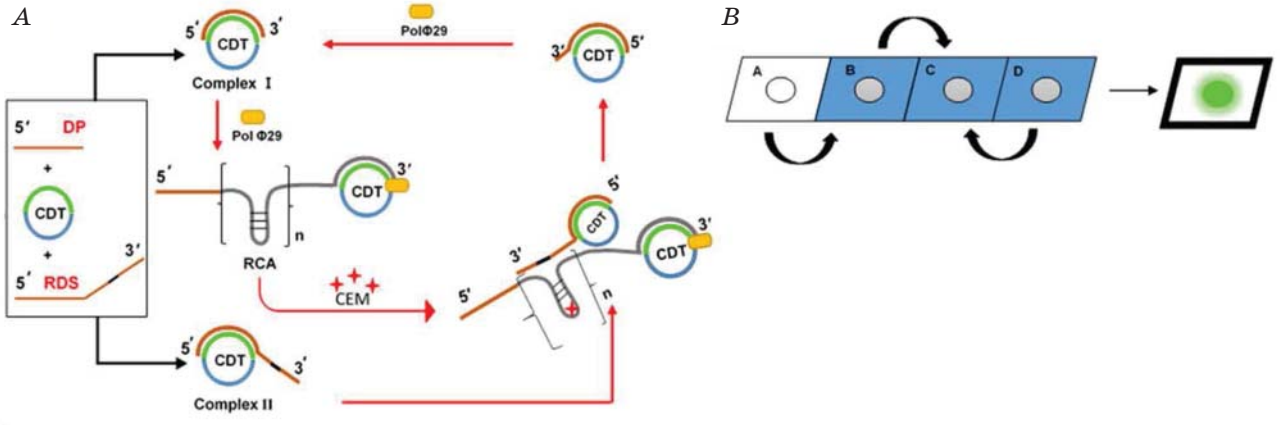

Fig. 3. ***A* — Schematic representation of RCA-based DNAzyme detection of *E.* coli [30]**

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.

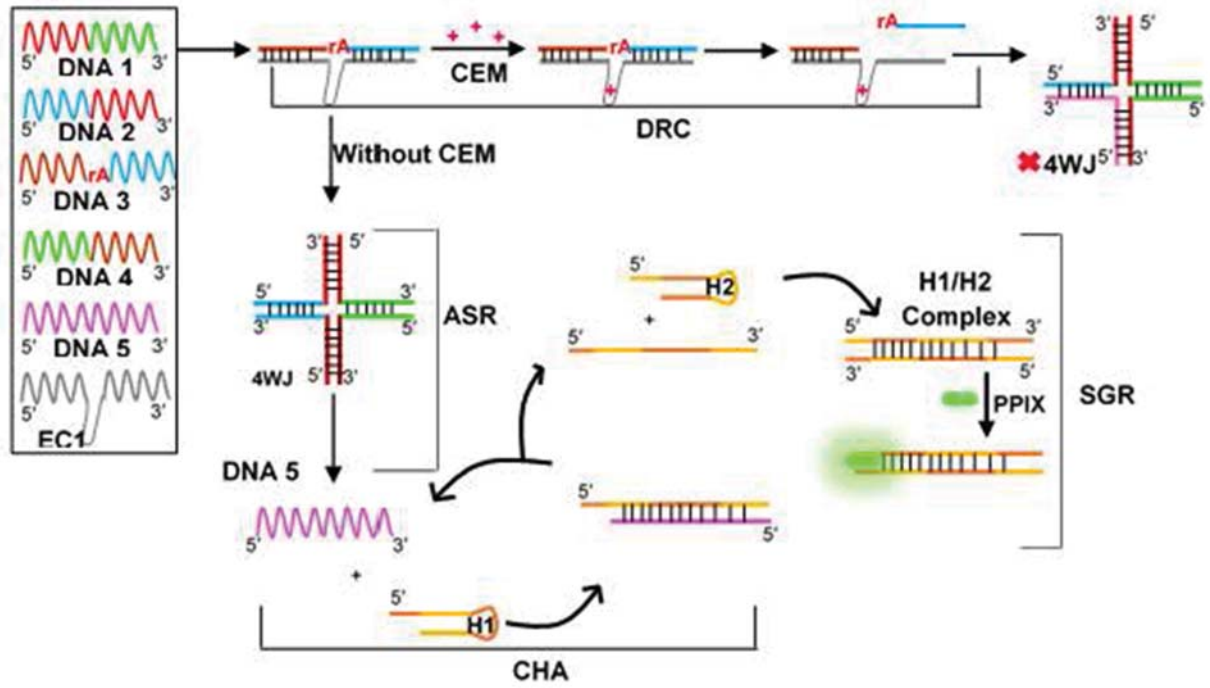

Fig. 4. **Multi-component all-DNA biosensing system based on DNAzyme for *E. coli* detection [33]**

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.

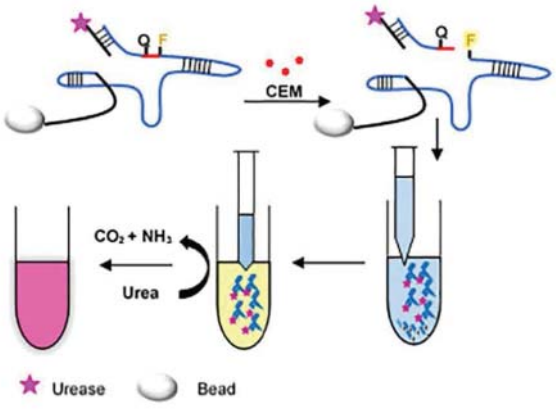

Fig. 5. **Schematic illustration of the *H. pylori* pathogen detection by the DNAzyme [19]**

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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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.