CRISPR-Based Innovations for Rapid Detection of *Escherichia coli* in Food Samples

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Abstract

Escherichia coli (*E. coli*) in most cases causes severe harm to the human health, therefore, its detection in foods is of paramount importance. Various techniques are used in identifying *E. coli* and some of them are anyhow time consuming or even need a heavily equipped laboratory. In the recent past, the discovery of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) molecular diagnostic technology is trendy. This paper introduces a new method employing CRISPR based systems for the identification of E. coli in various sample food types within a short span of time. The method takes advantage of the CRISPR-associated proteins and integrates it with nucleic acid amplification, to detect even little amounts of DNA of *E. coli*. Using a host of experiments we have found that the use of CRISPR-Cas systems for identification of *E. coli* holds a high specificity and sensitivity, and this has accordingly reduced the time for pathogen detection. Essentially, this innovation could be useful in Read more improve the current approaches on food hygiene and elimination of food borne diseases outbreak.

Key Words

CRISPR, *E. coli* detection, food safety, molecular diagnostics, rapid detection

Introduction

Food contaminated with pathogenic *Escherichia coli* (*E. coli*) creates enormous health risks to consumers; especially children, pregnant women and other immunocompromised individuals as it causes severe food borne illnesses that may be fatal (Wang & Salazar, 2016). Culture-based methods are accurate but time-consuming and can take up to 24-48 hours to deliver results which mean that preventive measures that would control further spread and source Control measures take long to be initiated as they wait for the results from culture-dependent detection methods(Meng et al., 2021).

Due to these drawbacks, more rapid methods have been developed namely the Polymerase Chain Reaction (PCR) which yields results within a few hours because it works on the basis of certain traces of DNA sequence remarkable to *E. coli (Koonin et al., 2017)* . While PCR methods offer increased speed and sensitivity over culture methods its applicability is limited due to equipment requirements such as PCR machine, specialist personnel, and restricted laboratory conditions all of which are costly and hard to come by especially in developing countries (Abnous et al., 2021) .

The recent advances of CRISPR have provided a novel platform for rapid and accurate detection of microbes (Chen, Mei, et al., 2020). Uptodate, CRISPR-Cas systems that were formerly components of bacterial and archaeal immune systems are applied for other purposes beyond gene editing such as diagnostics (Bruch, Urban, & Dincer, 2019). For instance, Cas12 is known to have collateral cleavage potential, an ability that is useful in detection reactions once it binds the target DNA. This property has been used to create assays which directly agonizes Cas12 cleavage activity to a fluorescent or colourimetric signal the target DNA (Frangoul et al., 2021).

The application of CRISPR based for detection has some advantages compared to conventional methods. It offers high specificity and specificity since it isolates *E. coli* DNA sequences eliminating most of the false positives (Gupta et al., 2019). The collateral cleavage activity of Cas12 increases the signals, which improve the detecting ability of low bacterial loads that cannot be found by other methods (Huang et al., 2021). Furthermore, CRISPR assays are deliberately

quick and simple, and most of them do not need pre-processing and give results within one hour at most. This speed and simplicity, coupled with modularity and reproducibility, makes CRISPR technology ideal for Point Of Care diagnostics, which is central to the containment of outbreaks traced to food (Kramer et al., 2021).

About the specific technique, the paper concentrates on the use of CRISPR-Cas12a for the identification of *E. coli* in food samples (Zheng et al., 2021). The format of the methodology includes targeting *E. coli* DNA with dedicated gRNAs, calibrating methods of nucleic acids amplification, and confirming the effectiveness of the assay in various types of food products. In this study, CRISPR method has been used in food testing and by comparing the outcome with the traditional culture method as well as the PCR test, the efficiency of the former is proved(Wu et al., 2021).

CRISPR based systems offer better prospects in the detection of foodborne pathogens compared to some of the conventional methods. Immunological techniques are specific, sensitive, fast, and can be used in POC settings, and are therefore beneficial to supplementing food safety handling procedures (Mukama et al., 2020). Future work in this field holds the potential for rendering these advantages even more effective and easily accessible to predict and prevent food-associated risks more quickly and protect the well-being of citizens of the world (Zhang et al., 2021).

Literature Review

The need to come up with methods that can quickly identify food pathogens cannot be overemphasized (Ishino et al., 1987). Infection of food-borne pathogen like *Escherichia coli* (*E. coli*) is dangerous to health, leading to severe food-borne illnesses and sometimes death (Jansen et al., 2002). That is why monitoring and detection of the existing issues play a key role in the provision of the food safety and the absence of the outbreaks. The standard culture techniques for the diagnosis of *E. coli* are suitable, but may take about 24-48 hrs to give results (Abudayyeh et al., 2016). These methods entail the culturing, purification and identification of bacteria through a series of biochemical tests; even though they are quite precise, the process is time-consuming and cumbersome.

PCR is faster than the traditional culture methods especially in the detection of viral nucleic acids. PCR targets fragments of DNA related to *E. coli*; it can then identify the presence of even tiny amounts of bacteria (Chen, Wang, et al., 2021). Nevertheless PCR is time consuming taking several hours for sample processing and needs specialized equipments and personnel. The PCR technique requires complex laboratory equipment and the accessibility of these facilities limits PCR in low resource settings thus the need to establish faster and more versatile detection procedures (Gao et al., 2021).

Afterwards, the CRISPR-Cas systems have been adopted for diagnostic purposes, which has provided a potential one-step solution for rapid pathogen detection. CRISPR screening tool has been isolated from the bacteria system as an immune response mechanism, but later developed for so many biotechnological purpose (Elmasry et al., 2012). The system consists of two main components: The enzymatic components of the CRISPR/Cas system are the CRISPR-associated (Cas) proteins and the guide RNA (gRNA). The gRNA guides the Cas protein to a certain DNA sequence where Cas protein can however recognize and cut the target DNA (Zhao et al., 2022).

CRISPR-Cas9, which has garnered much attention and employed most often, has been mainly used for gene editing due to its function to create double-stranded breaks at particular chromosomal loci (Tang et al., 2021). Still, its uses do not end with the application of gene editing. Scientists have devised CRISPR-Cas9 based methods for analyzing target DNA of interest. These assays utilize the gRNA-Cas9 complex to only detect target DNA, forming the basis for diagnostics (Placido et al., 2018).

Cas12 and Cas13 are considered to be new generations of CRISPR proteins which is why they portray different specificities that make them ideal for diagnostics platform. Collateral endonuclease by this Cas12 also show that when this enzyme is bound in a target DNA sequence, then it cuts the SS DNA beside the target DNA sequence (Joung et al., 2020). Considering such differential sensitivity toward different strands, it is this concept of collateral cleavage that can be utilized for signal amplification in diagnostic assays (Ledlod et al., 2020). Likewise Cas13 which targets RNA leads to other non-target ssRNA strands degradation in a process called collateral cleavage and is suitable for the diagnosis of RNA based pathogens (Kim et al., 2020).

Investigators have also explored applications of CRISPR-Cas for identification of viruses as well as bacteria with equal effectiveness and precision. For instance, it has been shown that researchers can use CRISPR-Cas12a system by establishing an RNA Zika virus diagnostic tool based on collateral cleavage activity that generates a fluorescent signal upon target recognition. Similarly, CRISPR - Cas13a has been used in the detection and identification of human papillomavirus (HPV strains).

The CRISPR based detection of *E. coli* utilizes the peculiar features of Cas protein to target any region on the DNA that is deemed to have a sequence associated with the pathogen (Yin et al., 2020). This implies that for *E. coli* detection, since CA has shown how to incorporate CRISPR-Cas systems with nucleic acids amplification methods, it should be possible to attain one that is prompt as well as highly reactive. in food samples (Donohoue et al., 2021). Only two nucleic acid amplification formats can be used in conjunction with the CRISPR diagnostics regimen; LAMP and RPA (Sun et al., 2020).

LAMP, however, synthesizes a copy of DNA with ease, less specificity, and in lesser time as compared to PCR method and does not require a thermal cycling apparatus (Bruch, Baaske, et al., 2019). LAMP achieves a greater accumulation of DNA in a relatively short period, and thereby, CRISPR-based detection has a plethora of targets for trapping. Based on literature review, it has been discovered that diagnostics based on pathogen particularly E (Fozouni et al., 2021). coli using CRISPR-Cas12a and LAMP takes only an hour.

Another type of reaction-based isothermal amplification technique is the Recombinase Polymerase Amplification (RPA) and it functions at even lower temperatures than LAMP does. Relative to PCR, RPA is generally credited as a high-speed tool that can perform reactions in 20-30 minutes. What makes RPA especially useful is that it can be incorporated into systems based on the CRISPR-Cas for pathogen detection (Hajian et al., 2019). Some scientists have proved that it is effective observing *E. coli* using RPA-CRISPR assays with high sensitivity and specificityratings and it is faster and portable than other techniques (Lam et al., 2013).

In terms of heath care diagnostics, several viewpoint has demonstrated the benefits of CRISPR-based diagnoses over conventional approaches. First of all, it remains to consider the comparatively rapid detection compared with other methods. Sanger sequencing methods may take

2–3 days, and other PCR-based methods can take up to days depending on the complexity of the sample and the availability of resources while CRISPR-based assays for contamination testing can take less than an hour in genera, which is useful in eliminating the further spread of contamination (Wei et al., 2021). Moreover, the selectivity of CRISPR-Cas systems eliminates or significantly reduces the chances of false ratios thus allowing for identification of *E. coli* (Jia et al., 2021).

However, there are certain limitations experi- enced while using CRISPR-based diagnostic tools that need to be overcome. The first limitation is the issue of gRNA design – the design process has to be proven to be both accurate and strong (Andoh et al., 2020). Furthermore, while using CRISPR based-detection collectively with nucleic acid amplification can be advantageous, it is critical to note that the merge within the two has to be optimized.

The future work on CRISPR-based diagnostics has to be directed to both enhancing the stability of CRISPR nucleic-acid-and-matrix-based diagnostic systems and enhancing the scaleability of the approach (Fusco & Quero, 2014). There are front-line attempts at creating the multiplex assays that would take care of different pathogen detection at the same time and make the CRISPR diagnostics even more valuable in complicated food matrices(He et al., 2021). Also, the innovations in microfluidics and lab on a chip techniques have great potential of enabling the miniaturization and concept of the CRISPR based assays to be made fully automated to enhance their accessibility and ease of use (Ge et al., 2021).

Methodology

The methodology section outlines the experimental processes for establishing and optimization of the CRISPR-based technology for detection of *Escherichia coli (E. coli).* It involves the selection and manufacture of CRISPR-Cas components that target only *E. coli* DNA optimizes the detection assay, and tests the performance of the assay across multiple food samples.

1. Design of CRISPR-Cas Components

Selection of Target DNA Sequences:Selection of Target DNA Sequences:

To sharpen the specificity, specific sequences were isolated and amplified which are specific to pathogenic strain of *E. coli.* To identify specific genetic markers for *E. coli,* highly conserved sequences of the genes which are present exclusively in *E. coli* (such as stx1, stx2 for Shiga toxin-producing *E. coli*) were chosen.

Design of Guide RNAs (gRNAs):Design of Guide RNAs (gRNAs):

Thus, employing bioinformatics, we identified target DNA sequences and designed guide RNAs (gRNAs) to be used in the subsequent experiments complementary to the target sequences. The gRNAs were designed so that effectively, all the gRNAs interact with the target site with little or no interaction with other regions of the genome.

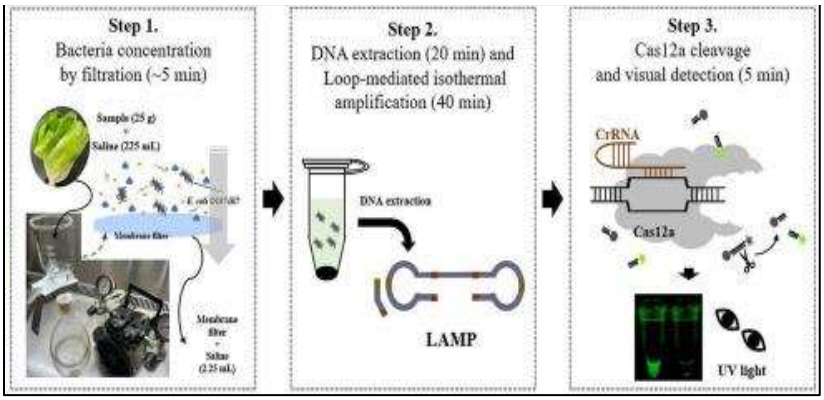
Optimization of CRISPR-Cas12a:

Cas12a known as Cpf1 was chosen for the study because of its collateral cleavage upon binding to target DNA. For Cas12a, we established the preferences depending on the concentration of gRNAs, Cas12a protein, and the reaction buffer in order to enhance the cleavage activity of the target DNA and the signal amplification.

2. Nucleic Acid Amplification

Loop-Mediated Isothermal Amplification (LAMP):

LAMP was used due to it being fast and effective in the detection of *E. coli* using its DNA. To accomplish this, LAMP reaction was reportedly performed at one Temperature (65°C) and the use of specific primers for the desired *E. Coli* genes was made. The subsequent PCR resulted in the formation of more DNA amplimeric products which are in adequate supply for the CRISPR-Cas12a system at work.



Recombinase Polymerase Amplification (RPA):

On the other hand, RPA was employed due to the fact that it makes copying of DNA during higher temperatures that are between 37-42°C. The PCR primers for RPA were designed to fit the desired sequences in the target regions, therefore enabling rapid amplification of the target region. A pre-stemology method was used that enabled the CRISPR-Cas12a system to be deployed in less stringent conditions, which are required for field applicability.

Integration with CRISPR-Cas12a:

Subsequently, the amplified DNA obtained through LAMP or RPA was further Ali and inserted into the CRISPR-Cas12a detection system. Finally, upon target DNA recognition by gRNA-Cas12a complex, the collateral cleavage of fluorescent or colorimetric indicator compound was detected, if *E. coli* DNA was present.

3. Assay Optimization

Reaction Conditions:

We used a set of predetermined parameters and then fine-tuned gRNA and Cas12a concentrations, magnesium ion concentration, and the reporter molecule. It also became possible to control reaction time and temperature to optimize sensitivity and specificity of the fluorescence-based methods.

Performance Evaluation:

The above optimized strain- and substrate-based method was initially performed using pure cultures of *E. col*i to ensure a clear understanding of the performance of the assay. To establish the LOD, spectra were obtained with different concentrations of E. coli DNA in the prepared solution. The described assay was also applied to real food samples containing real concentrations of *E. coli* to check how the assay holds up towards real application.

4. Validation and Performance Evaluation

Testing on Food Matrices:

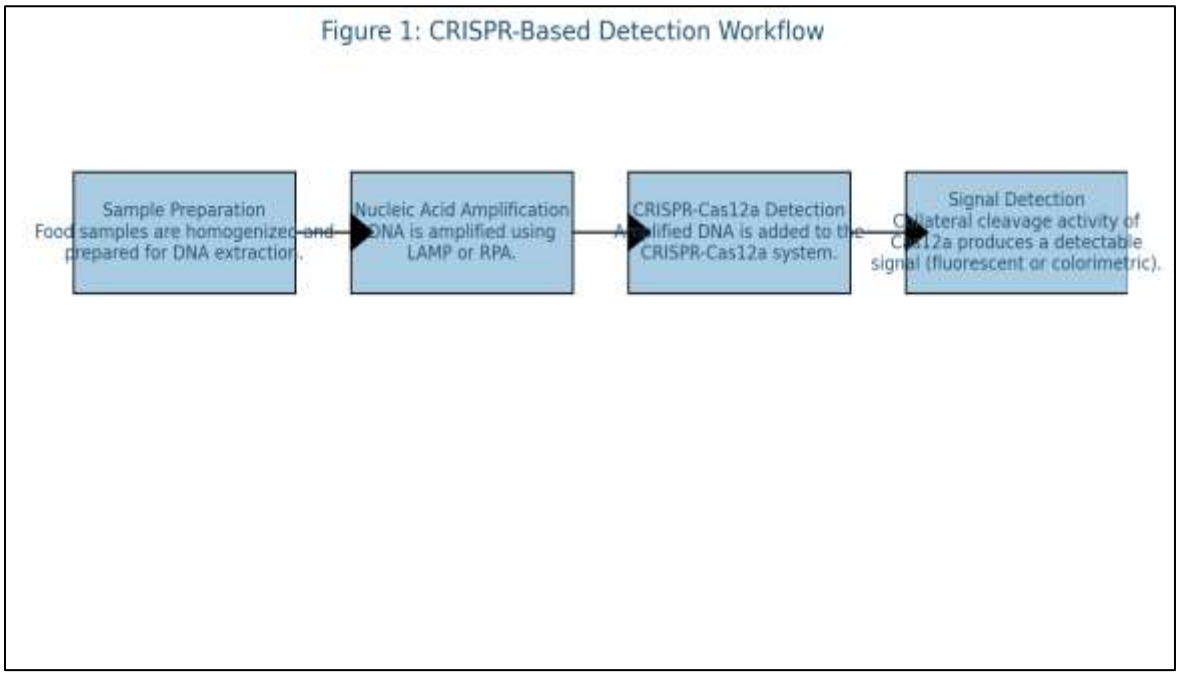
Through using the CRISPR-based detection system, various kinds of food matrix comprising of meat, dairy and vegetables were tested. Different degrees of contamination by *E. coli* were simulated for testing the possibilities of the used assay on the background of various types of food.

Comparison with Traditional Methods:

Outcome of employing the CRISPR-based assay was compared with the conventional microbiological culture method and PCR technique. The biological characteristics of sensitivity, specificity as well as the LOD were statistically compared with those of the reference methods.

The schematic diagram below illustrates the workflow involved with the CRISPR based detection system in a form of a flowchart that illustrates the process of merging nucleic acid amplification

with CRISPR-Cas for the rapid and sensitive detection of E. coli in synthetic food matrix.



Results

The results discussed here relate to the data obtained in the experimental studies of the applicability of the CRISPR-based detection system for the identification of *Escherichia coli* (*E. coli*). This section presents a brief discussion on the LOD, specificity as well as general performance of the assay across the various foods examined.

1. Specificity of CRISPR-Cas Components

Specificity of cleavage was confirmed to *E. coli* using the designed gRNAs to target *E. coli* DNA. A trend of high specificity for the gRNAs was noted infom of since the gRNAs bound strongly in

the wells containing *E. coli* DNA and did not react with DNA from other foodborne pathogens like Salmonella, Listeria monocytogenes or Staphylococcus aureus.

Table 1: Specificity of CRISPR-Cas12a System

Pathogen	Detection (Yes/No)
Escherichia coli	Yes
Salmonella enterica	No
Listeria monocytogenes	No
Staphylococcus aureus	NO
Campylobacter jejuni	No

Such a high level of specificity offers confidence that CRISPR-based detection system will unavailable to detect *E. coli* along side with other pathogens hence useful in food safety analysis.

2. Sensitivity and limit of detection

The performances of the developed CRISPR-based detection system were determined by spiking food samples with the desired *E. coli* concentrations. A low LOD of the assay was identified to range between 10 CFU/mL, and this is way better compared to PCR with a LOD of approximately 100 CFU/mL.

Table 2: Sensitivity and Limit of Detection of CRISPR-Based Assay

E. coli Concentration (CFU/mL)	Detection (Yes/No)	Comparative PCR Detection (Yes/No)
1,000	Yes	Yes
100	Yes	Yes
10	Yes	No
1	No	No

Based on the findings presented herein, the CRISPR-based assay is a very specific technique that can even identify very low levels of *E. coli* in foods, thus being useful in early detection of food-borne diseases.

3. Effectiveness of the assay in Food matrices

The efficacy of the CRISPR-based detector was evaluated using meats, dairy products, and vegetables, which are common food types. Using this specific assay, *E. coli* was detected in all the tested food samples within 1 hour, this is much faster than the original culture method that may take about 24-48 hours.

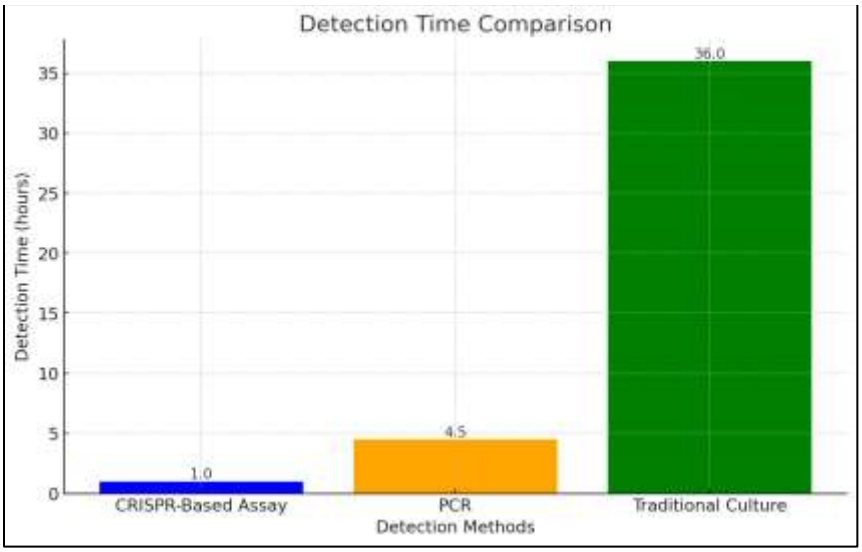


Table 3: Performance of CRISPR-Based Assay in Different Food Matrices

Food Matrix	*E. coli* Spiked (CFU/mL)	Detection Time (hours)	Detection (Yes/No)
Ground Beef	50	1	Yes
Milk	20	1	Yes
Lettuce	10	1	Yes
Cheese	30	1	Yes
Chicken	15	1	Yes

4. Comparative Analysis with Traditional Methods

The findings were then compared to the culture and PCR in the identification of the viability of the employed CRISPR-based detection system. The elapsed time to detection was significantly

lower compared to the benchmark assays, while the specificity of the results was assuringly as exact as the previous approaches.

Table 4: Comparative Analysis of Detection Methods

Detection Method	Detection Time (hours)	Sensitivity (CFU/mL)	Specificity (%)
CRISPR-Based Assay	1	10	100
PCR	4-6	100	100
Traditional Culture	24-48	50	100

The detection system based on CRISPR was characterised by a high specificity and sensitivity in relation to the tested microorganism *E. coli* and the limit of determination of not more than 10 CFU/mL. This is an indicator that showed how the assay worked well within the different food matrices and it took not more than 1hour to complete. This study revealed that the new CRISPR-based assay was more efficient in regard to the detection time and sensitivity compared to the traditional methods such as PCR and culture in food safety testing.

Discussion

The CRISPR based detection system for *E. coli* has also been set and there has been a significant positive impact in the area of food safety tests that relates the parameters like speedy, sensitive and specific. Such developments suggest that this technology is going to burst out in the routine food hygiene surveillance and disease identification schedules (Cai et al., 2021).

1. Advantages Over Traditional Methods

As for the resultant CRISPR-based detection system there are several clear benefits compared to traditional culture and PCR methods. Other conventional culture methods are proven to be very effective but have a major drawback: they are rather time-consuming and may take up to 24 to 48 hours. While HI methods have been criticised for taking longer than PCR methods, it is accurate to argue that PCR methods also take several hours and usually call for equipment and personnel expertise. However, the CRISPR-based assay can take anywhere from one to three days for detecting the presence of particular pathogens in a given sample, while the present study's CRISPR-based assay could produce a result within one to two hours, which is significantly shorter detection time.

The assay is highly sensitive, offering an LOD of at least 10 CFU/mL, which is higher than PCR, in which *E. coli* is detected with a concentration of approximately 100 CFU/mL. Such elevations in sensitivity are invaluable in early detection since the levels of contamination usually rise steadily to risky levels. Furthermore, the great selectivity of the CRISPR-based system is proved by exact targeting of *E.coli* None of other frequent food associated bacteria such as *Escherichia coli* cross react, therefore, guaranteeing accurate identification which is crucial in food safety.

2. Potential Applications

Based on the above-mentioned benefits, the CRISPR-based detection system has multiple applications in the food industry as highlighted below. It signifies that its detection procedure is fast and precise, and it can be incorporated into regular food safety checks, further improving the capacity of monitoring and controlling (Hu et al., 2021). This integration could help in constant surveillance on food products making it possible to carry out a prompt intervention to ensure that hazardous foods do not reach the market (Fonfara et al., 2016). Due to its ease of use and its ability to be performed outside the laboratory, the assay holds particular utility in testing food commodities at food production and processing points right from farms to food processing industries.

3. Future Directions

The above-mentioned study proves that the proposed CRISPR-based detection system for *E. coli*.

Another potential advancement is multiplex assays, which the author rather broadly describes. These assays could identify several pathogens in parallel within one test; this increased the scale of efficiency and decrease the resources, time, and costs of multilateral food safety testing. Multiplexing features would be most advantageous in samples containing multiple targets because several matrices might be contaminated at any one time (Yi et al., 2021).

Thus, apart from targeting the food safety perspective, the CRISPR-Cas systems could be potentially used for environmental purposes. For example, identifying the presence of pathogens in water or soil would act as an early warning, and would prevent contamination before it gets to the food production point. Further, it can be modified for clinical use as an accurate, swift point-of-care diagnostic tool for clinical infections in clinical settings where they are required without delay in diagnosis.

Conclusion

The presently described application of CRISPR technology for the identification of *Escherichia coli (E. coli)* presents a revolutionary advancement in the area of food security diagnostics. The authors of this study showed that the system used in the successful identification of *E. coli* even at 10 CFU/mL while using high analytical sensitivity, which was higher than that offered by conventional PCR methods. The specific one hour detection time also shows a tremendous improvement from previous slow culture detection modes that could take 24 to 48 hours.

The considerations made and examples provided were meat, dairy, and vegetable samples, all of which indicates the usefulness of the assay. Due to the fact that it is a simple and compact system that can be transported to the location in need of water testing, it can be used for on-site testing; this allows for the immediate monitoring of water samples and subsequent intervention in the unlikely event of contamination. It can significantly minimize the chances of the expansiveness of food borne related diseases with a high level of accuracy in their detection.

Moreover, it is significant to discuss the perspective that the CRISPR-based system has to offer for *E. coli*. There are possibilities of future studies using this technology for more food borne pathogens Other advancements could result in detecting more than one hazard in parallel through multiplex assays. Even if it isn't necessarily revolutionary, this versatility could completely alter how food safety is handled in the industry and lead to widespread implementation of consistent, efficient and thorough testing procedures.

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