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Review Article

Bacterial Detection Using the CRISPR-Cas System

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Abstract

The rapid and accurate detection of pathogenic bacteria is critical for public health, yet traditional methods like culture-based techniques, polymerase chain reaction (PCR), and Enzyme-linked immunosorbent assay (ELISA) often face challenges as long turnaround time, high costs, and the necessity of specialized infrastructure. The latest advances in Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-related proteins (Cas) CRISPR-Cas based diagnostics have transformed the detection of bacteria by providing extremely sensitive, selective, and economical solutions. Its ability to recognize and cleave nucleic acids makes it possible to identify infections precisely. Several Cas proteins are used in diagnostic systems, including Cas9, Cas12, Cas13, and Cas14. This review explores the mechanisms and applications of CRISPR-Cas technologies in bacterial detection based on these Cas proteins as CRISPR-Cas9-Based Detection, CRISPR-Cas12-Based Detection, CRISPR-Cas13-Based Detection and CRISPR-Cas 14-Based Detection techniques. Ongoing innovations and integration with emerging technologies will further enhance the sensitivity, scalability, and accessibility of CRISPR-based diagnostics.

Keywords: *CRISPR-Cas system, Bacterial detection, Cas9, Cas12, Cas13, Cas14*

Introduction

Pathogenic microorganisms and parasites are widely distributed in the natural environment which can cause a diversity of diseases to organisms increasing the morbidity and mortality which is considered to be a global threat. This could be prevented by early detection and timely diagnosis of these infectious microorganisms through treatment (Gomes *et al.*, 2018). The conventional methods are imaging techniques, culture tests, biochemical identification, serological examination, PCR and ELISA. But these prevailing diagnostic methods have disadvantages such as low accuracy, high cost and slow detection speed. Therefore scientists are working on the development of existing methods while exploring new diagnostic methods to detect emerging infections (Lazcka *et al.,* 2007).

Bacterial infections are very common among all the other infections, and need a proper diagnostic method since the infected person is treated with empiric antimicrobial medicine due to non- specific and less sensitive diagnosis which leads to antimicrobial resistance (Selvam *et al.,* 2022) Due to this, a diagnostic technique based on Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-related proteins (Cas) has been adapted recently. The CRISPR- Cas method exhibits specificity, sensitivity and rapidness which could result in timely treatment and intervention for the infection (Lou *et al.,* 2022)

Classification of CRISPR- Cas System

The CRISPR- Cas system is divided into 2 types according to the effector protein in the interference phase which are further divided into 6 types and 33 subtypes. The effectors of the first class of CRISPR system are composed of Cas proteins that will bind and process the target protein while the second class is composed of only one protein with multiple domains. The most prevalent type is class 1 system of about 90% which includes types I, III and IV. The types class I and III systems aid in targeting and DNA cleavage which are common while type IV systems are rare and lack components such as adaptive molecules and nucleic effector enzymes. The class 2 system which is more widely studied and applied than class 1 system, consists of type II, IV and VI. The 3 effector proteins are Cas9, C2c1 and Cpf1 (Masi *et al.,* 2023).

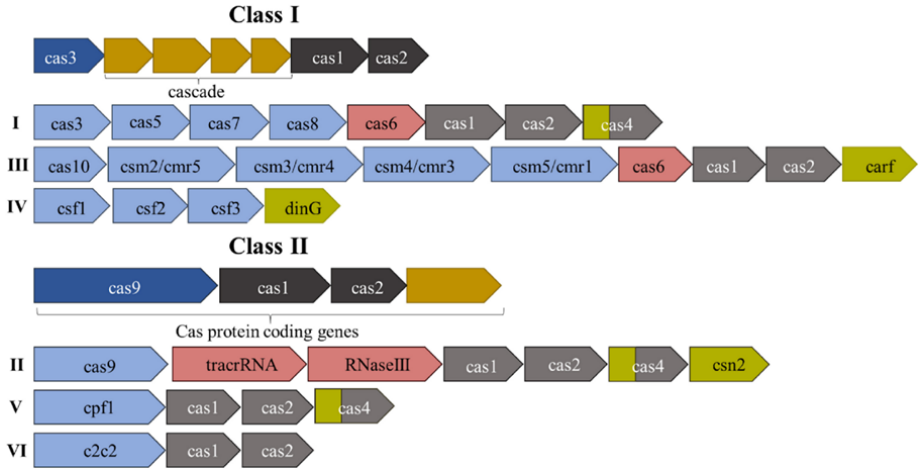

Figure 1. *Functional organization of CRISPR systems.*

Biology and Principal of CRISPR- Cas System

CRISPR- Cas system is an adaptive immune defense mechanism found in bacteria and archaea to invade pathogens which is first discovered in *Escherichia coli.* The CRISPR sequence is made up of spacer sequences and repeat sequences. That is the repeating palindromic sequences are separated by spacer sequences one alternatively (Miaowen *et al.,* 2017). This can be transcribed into CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) and a set of CRISPR associated (Cas) genes which encode Cas protein. This system can recognize specific exogenous nucleic acid sequences and cut them specifically and the DNA fragments will be attached into the CRISPR sequence as a new spacer. The helicase and nuclease activities of Cas protein aid to cut the DNA strands. When the same foreign organism invades again crRNA recognizes and pairs with its DNA causing the Cas protein to cleave target sequence of pathogen's DNA (Huan *et al.,* 2021).

The macromolecule spacer sequences are stored in CRISPR genomic locus, therefore it is considered to be an efficient storage system. These sequences are obtained from invading genetic elements and sequenced. To remove targeted foreign pathogens the Cas proteins are directed upon the subsequent calling of these sequences. To ensure the smooth functioning at the molecular level some mechanisms such as maturation of crRNA, adoption processes and interference by biological diversity across systems are employed by the CRISPR- Cas systems (Ekwebelem *et al.,* 2021)

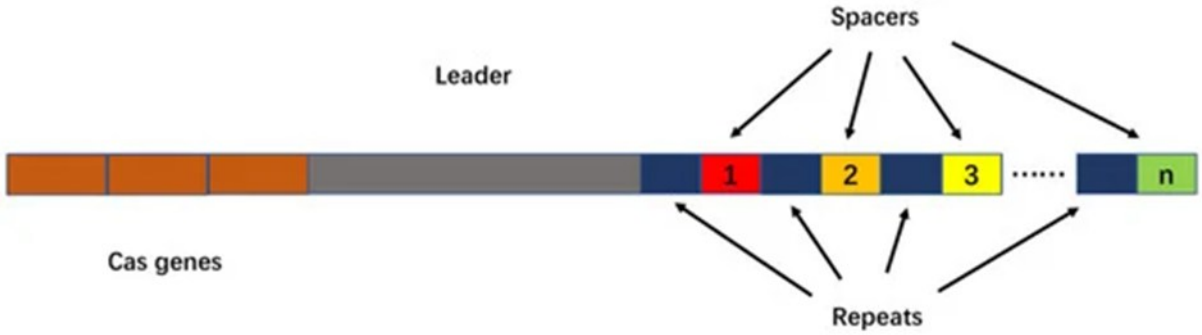

Figure 2. *CRISPR-Cas locus structure diagram. CRISPR-Cas includes transactivating crRNA, genes encoding Cas-related proteins (Cas genes), repeat sequences and spacer sequences.*

CRISPR-Cas Based Bacterial Detection

In Bacterial detection, Cas proteins are particularly used to target bacterial DNA. The Cas protein will attach or cleave the target DNA if it is present in the sample, producing a detectable signal which includes color changes and varied DNA fragments. The presence of the target bacterium is specifically identified by analyzing these signals (Huang *et al.,* 2023).

CRISPR-Cas9 Based Detection

Cas9 is the most commonly used CRISPR-associated protein and functions through a Single guide RNA (SgRNA) composed of CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA). It directs Cas9 to a specific sequence of DNA which is adjacent to a protospacer adjacent motif (PAM) resulting in a blunt-ended double-strand break (DSB) (Nishimasu *et al.,* 2014).

This aspect has been used in detection of nucleic acid by combining Cas9 with signal amplification techniques. The specificity and ability to differentiate single nucleotide polymorphisms (SNPs) helps to identify nucleic acids by combining signal amplification techniques with Cas9. Cas9 mediated triggers produce detectable signals through electrochemical or fluorescence methods which are effective for detecting genetic variations and pathogen-specific sequences.

The Cas9 nickase-based amplification reaction (Cas9nAR) and dCas9 Luminescence detection are the most prominent CRISPR-Cas9 Based Detection techniques. According to the previous studies, Huang *et al.,* (2018) detected DNA methylation and total RNA of Listeria monocytogenes by combining the CRISPR-Cas9 with an isothermal exponential amplification reaction. This technique showed a high specificity in finding the single mismatch with a detection limit of 0.82 αmol.

Subsequently, a Cas9 nickase-based amplification reaction (Cas9nAR) was developed by Wang *et al.,* (2019). A cyclic process of initiation, extension, nicking and replacement reactions was applied where Cas9nAR uses a sgRNA-Cas9 complex with single-stranded nicking properties and a strand displacing DNA polymerase and two primers with Cas9n cleavage sequences. The amplification of target sequence in the sample genomic DNA was done at a constant temperature of 37 °C. A detection limit equivalent to that of qPCR was attained in the detection of Salmonella typhimurium.

Zang *et al.,* (2022) produced a pair of sgRNAs that target the upstream and downstream components of the targeted DNA by splitting the luciferase into two halves and combining it with the dCas9 protein. The activation of luciferase activity initiates the emission of highly enhanced fluorescence signal, When the dCas9 on both sides identifies the targeted DNA and is adjacent resulting in the detection of Mycobacterium tuberculosis. Several DNA detection techniques have been developed based on this property.

CRISPR-Cas12 Based Detection

The versatility of CRISPR based detection is expanded by Cas12 proteins such as Cas12a. This protein shows trans cleavage activity when bound to its target DNA where single stranded DNA (ssDNA) is cleaved nonspecifically (Yamano *et al.,* 2016). The diagnostic assays as DETECTR uses this cleavage activity producing a fluorescent signal upon the activation of Cas12 to cleave DNA reports in the presence of target DNA (Li *et al.,* 2018).

A quick and precise technique for identifying AMR bacteria is required, as evidenced by the emergence and spread of NDM-producing genes in food producing animals. Shin *et al.,* (2024) proposed a fluorescent test based on CRISPR/ Cas12a and PCR that may identify bacteria that produce NDM (blaNDM). This CRISPR/Cas12a system's engineered gRNA allowed it to cleave PCR amplicons and ssDNA-FQ reporters at the same time, producing fluorescence signals. When tested against other foodborne pathogens that do not include blaNDM, this method was found to be extremely specific. It also showed great capacity to detect single-nucleotide polymorphism. With a detection limit of 2.7 × 100 CFU/ mL, the assay outperformed traditional PCR with gel electrophoresis by a factor of 100 in the case of blaNDM-1 harbouring E. coli. Furthermore, the new technique outperformed previously published real-time PCR methods in detecting AMR bacteria in food samples.

CRISPR-Cas13 Based Detection

Cas13 is a RNA directed ribonuclease that produces multiple cleavage sites in a single stranded region of a RNA target that has a certain base preference. Notably, after cleavage the Cas13 remains active (Son, 2024). In target RNA-dependent situations Cas13 performs trans-cleavage of bystander RNA exhibiting "Collateral cleavage" activity. Cas13 has a protospacer flanking site and it does not need a PAM sequence as Cas12. The complementation between crRNA and the target single-stranded RNA (ssRNA) is negatively affected by a guanine base that directly follows the protospacer (Wu *et al.,* 2023).

Similar to DETECTR, SHERLOCK (Specific High Sensitivity Enzymatic Reporter Unlocking) is a tool for diagnosis based on type VI CRISPR-Cas system acts by depending on the principles as DETECTR (Gootenberg *et al.,* 2017). Cas13 identifies and cleaves only to RNA. The in vitro transcription of the isolated aids in the detection of Target DNA. Using isothermal amplification, RPA can enrich target molecules and improve sensitivity. The fluorescent RNA and the Cas13 protein crRNA combine with the RNA fragments, which are amplified. Cas13 through crRNA detects the target molecules in the sample and cleaves the fluorescent RNA probes through collateral activity and destroys the association of the fluorophore and the quencher (Fapohunda *et al.,* 2022).

CRISPR-Cas14 -Based Detection

The extreme small size and ability to target ssDNA without a PAM sequence in Cas14 proteins make them significant. Due to the high specificity and sensitivity of Cas14 protein small and fragmented DNA could be easily detected using them. In the presence of target ssDNA the Cas14 shows trans cleavage activity producing detectable signals.Additionally, Cas14's small size makes it easier to integrate into different diagnostic platforms, which could improve the scalability and portability of CRISPR-based detection methods (Son, 2024).

For pathogenic detection the Cas14a1 advanced LAMP assay (CALA) and the Rapid Extraction of Bacterial Genomic DNA (REBGD) are combined.For artificial samples, the fluorimetric and visual methods (fCALA and vCALA) were both tested for bacterial identification with a limit of detection as low as 10 aM target. Three unique characteristics were present in the quick CALA strategies: (a) the operation was made simpler and faster by the quick extraction method; (b) the aggregated AuNPs sediments gave a clear and unambiguous visual readout (Wang *et al.,* 2024).

Limitations and Conclusion

CRISPR-Cas based detection method has some limitations such as sequence restriction, Sample pre-treatment, Contamination during the process and the lack of uniform standard. Despite all these limitations, this method has Strong specificity, high sensitivity, ease of use, and the ability to detect nucleic acids without the need for instruments under some conditions are the benefits of nucleic acid detection techniques based on CRISPR/Cas biosensors. These methods can detect even trace amounts of genetic material and distinguish between different subtypes or mutations. To meet the demands of many situations, they can also be integrated with other technologies.

Conflict of Interest

The author declare no conflict of interest.

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