Comprehensive Analysis of CRISPR-Cas Systems in Microbial and Their Multifaceted Applications

Suriana Sabri [1], Md Kawsar Mustofa [2], M. T. Fouad [3], Saikat Mukherjee [4], Md Fakruddin [5*], Md Asaduzzaman Shishir [6]

Abstract

Scientific interest in CRISPR-Cas systems is immense due to their emergence as groundbreaking tools for genome editing and modification in various microbes. Initially recognized as bacterial defenses against viral invaders, CRISPR-Cas systems have been found in a wide range of microbial species, including bacteria and archaea. They are classified into two main classes: Class 1, consisting of multi-subunit complexes, and Class 2, characterized by single-protein Cas9 systems. These classes display remarkable diversity, with numerous subtypes and variants enabling adaptation to various ecological niches. The versatility of CRISPR-Cas systems is one of their most appealing attributes. They employ diverse genome editing strategies, reflecting their adaptability and evolution as adaptive immune systems in microorganisms, co-evolving in response to viral threats. Beyond viral defense, these systems contribute to genome stability and integrity in bacteria and archaea. CRISPR-Cas systems have become indispensable tools in laboratories for functional genomics, precise genome editing, and gene reprogramming. They play pivotal roles in synthetic biology and biotechnology, facilitating the engineering of microorganisms for environmental remediation and biofuel production. Furthermore, CRISPR-based diagnostics enable rapid and precise identification of infections and genetic alterations, promising a transformative impact on disease diagnosis. Additionally, the potential of CRISPR-based antimicrobials to combat drug-resistant microorganisms holds significant promise in medicine. In conclusion, the diverse applications of CRISPR-Cas systems underscore the remarkable adaptability of life and the potential for scientific and medical advancements. Continued exploration and optimization of these systems will unlock new avenues for research and transformative applications across various industries. Harnessing the defensive mechanisms of microbial CRISPR-Cas systems exemplifies both the power of nature and human ingenuity for societal benefit and scientific progress.

Keywords: Gut-Brain Axis, Microbiota, Neurological Health, Signaling Pathways, Microbiome-based Therapies

Significance | This review describes CRISPR-Cas systems as an advanced tool in microbial biotechnology with a great potential for genetic manipulation and disease intervention.

*Correspondence: Md Fakruddin, Department of Biochemistry & Microbiology, North South University, Bashundhara, Dhaka-1229, Bangladesh.
E-mail: fakruddinmurad@gmail.com

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Introduction

In 1987, Japanese researchers (Ishino et al., 1987) first observed the CRISPR-Cas system, though its function and significance remained unknown at the time. It wasn't until 2005 that the discovery of many inserted sequences in bacterial genomes being of viral or plasmidic origin (Mojica et al., 2005; Doudna and Charpentier, 201) sparked the realization of its importance in

Author Affiliation:
[1] Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia
[2] UT Southwestern Medical Center, USA,
[3] Department of Dairy Science, Division of Food Industries and Nutrition, National Research Centre,
12311 Dokki, Giza, Egypt
[4] Department of Biochemistry, Manipur University, Imphal,Manipur, India.
[5] Department of Biochemistry & Microbiology, North South University, Bashundhara, Dhaka-1229, Bangladesh.
[6] Department of Microbiology, Dhaka International University, Badda, Dhaka, Bangladesh

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immunity. Jinek et al. (2012) demonstrated the system's potency as a gene-editing tool in 2012, leading to the development of various technologies utilizing CRISPR-Cas. Since then, it has been utilized in treating neurological illnesses (Kolli et al., 201)], editing plant genomes (Samanta et al., 201)], and notably in cancer therapy (Zhen et al., 2014). Recently, it has been proposed as a tool for combating human viruses (Soppe and Lebbink, 2017) and harmful bacteria (Bikard and Barrangou, 2017).

Recent research predominantly emphasizes the utilization of CRISPR in combating retroviral infections, notably coronavirus disease 2019 (COVID-19) caused by the novel coronavirus SARS-CoV-2 (Li et al., 2020; Nguyen et al., 2020). While CRISPR-Cas boasts diverse applications, this article narrows its focus to its role in microbial systems. Microbes, encompassing bacteria and archaea, have long been subjects of scientific inquiry due to their significance in biology, ecology, and medicine. Microbes, vital to numerous ecosystems, play pivotal roles in bioremediation, nutrient cycling, and the production of industrial enzymes and antibiotics.

Moreover, various infections, such as antibiotic-resistant bacteria, pose significant threats to human health. Understanding the functionality of these microbes' CRISPR-Cas systems is not only intriguing from a scientific perspective but also potentially beneficial. These microbial CRISPR-Cas systems play vital roles in their interactions with foreign genetic material and their adaptability to the environment. Delving into microbial genetics and harnessing CRISPR-Cas's potential for diverse applications necessitate comprehension of how microorganisms utilize this technology (Barrangou et al., 2007).

The advent of CRISPR-Cas systems heralds a new era in molecular biology and genetics. Initially identified in microorganisms, these mechanisms have revolutionized our ability to precisely manipulate DNA and gene expression. This study explores the intricate interplay between microbes and CRISPR-Cas systems, shedding light on the significant implications of this groundbreaking technology.

CRISPR-Cas is useful for revolutionary medical treatments and therapies by targeting genetic issues at their core. Researchers are exploring its application in gene therapy due to its potential to precisely address genetic problems. Microbes play a role in this endeavor by facilitating the targeting of specific cells or tissues through acting as delivery mechanisms for CRISPR-Cas.

Furthermore, it is crucial to acknowledge the environmental impact of CRISPR-Cas in the realm of microbiology. Tackling global challenges such as pollution and climate change requires an understanding of how microorganisms interact with their surroundings, contribute to ecosystem function, and adapt to changing conditions.

CRISPR-Cas Structure

Viruses were thought to be the primary agents of life's evolution because they promoted genetic exchange and served as a selection pressure on bacteria from various habitats. Bacteriophage infection kills between 20 and 40 percent of bacteria every day (Hampton et al., 2020). It was later demonstrated that the selection of a variety of defense mechanisms in bacteria that enable the recognition of foreign DNA and differentiation from self DNA occurred after the acquisition of exogenous DNA via transduction, conjugation, and horizontal gene transfer (HGT) (Horvath and Barrangou, 2010). Since insertion in these regions is unlikely to be harmful, it is unclear whether clustering defense genes in islands offers a particular selective advantage upon “genomic junkyards,” in which the defense genes frequently acquired via HGT are accumulated(Doron et al., 2018). Bacteria have several different defense mechanisms.

As a result, many bacteria and most archaea evolved a variety of autoprotective mechanisms to fend off invading nucleic acids. Among them, the CRISPR-Cas immune system is made up of CRISPR arrays and related cas genes (Rath et al., 2015). The sequence-specific neutralization of invasive genetic elements, such as viruses and plasmids, is guided by information stored in CRISPR arrays (Jackson et al., 2017). Since its first description in 1987 (Ishino et al., 1987), the system has been found in 45% of bacteria and 85% of archaea (McGinn and Marraffini, 2019). According to Horvath and Barrangou (2010), CRISPR loci have a distinct location; they are present in both chromosomes and plasmids. The CRISPR-Cas locus is usually composed of an operon of cas genes and a CRISPR array consisting of a sequence of conserved, sequence-specific repeats (25–35 bp long), flanking unique inserts of similar length known as spacers (26–72 bp), according to Wright et al. (2016), Jackson et al. (2017) (Figure 1). Many CRISPR spacer sequences show similarities to DNA found outside of chromosomes (Mojica et al., 2005). These sequences are known as protospacers and are present in plasmids and viral genomes (Horvath et al., 2008). Having been acquired during previous infections, spacers are crucial elements of adaptive immunity (Pourcel et al., 2005). They ensure that invaders will be identified and eliminated in the case of subsequent attacks (Barrangou et al., 2007).

The majority of the repetitions had partially palindromic sequences, which provided stability and very conserved secondary structures. It is anticipated that palindromic repetitions would produce RNAs with stable hairpin structures (Kunin and Makarova, 2019; Kunin et al., 2007). Repeat sequences that are conserved are often found in a single CRISPR locus; however, variations in repeat length and sequence exist between distinct CRISPR loci (Kunin et al., 2007). The leader sequence, which is a DNA sequence rich in adenine and thymine, comes before each CRISPR array’s first repetition (Grissa et al., 2007).Cas proteins work to incorporate new spacers into the CRISPR array that are

produced from invasive MGEs (Jackson et al., 2017). Thus, the history of previous CRISPR-Cas-mediated interactions may be deduced from the spacer sequences of a particular bacterial strain. Both the total number of CRISPR loci and their individual lengths are changeable. Methanocaldococcus sp. FS406-22 is one of the bacteria that have eighteen CRISPR loci. A number of these loci might consist of hundreds of repeat-spacer units. Some of the tiniest microbial genomes (such as Nanoarchaeum equitans) include several CRISPR loci; the number and length of these loci are not associated with genome size (Sorek et al., 2013).

Cas Proteins

The components of CRISPR systems include neighboring CRISPR-associated cas genes, which code for proteins involved in DNA repair (Makarova et al., 2002) and the immune response (Brouns et al., 2008). CRISPR arrays are made up of spacers and repetitive sequences. With more genomes being annotated, diverse cas genes have been found in more taxa, making categorization extremely challenging. Four cas genes were initially found in genomes that contained the CRISPR-Cas system (Jansen et al., 2002). However, as genome sequences have been obtained, 93 cas genes have been found, which are categorized into approximately 45 distinct gene families according to the sequence similarity of the encoded proteins (Haft et al., 2005; Makarova et al., 2015). Cas1 and Cas2 are regarded as a signature in genomes that contain CRISPR loci. Six of these cas genes (cas1–cas6) are widely distributed (Haft et al., 2005) According to Haft et al. (2005), the examination of cas1 sequences indicates the existence of several separate CRISPR-Cas systems, each distinguished by a specific composition and conserved arrangement of cas genes.

Certain sets of CRISPR loci are interacting with the Cas proteins (Makarova et al., 2006). The CRISPR system of Escherichia coli was given the name Cse1 (CRISPR system of E. Coli gene1) for Cas proteins; other subtypes included Aeropyrum (Csa), Desulfovibrio (Csd), Haloarcula (Csh), Mycobacterium (Csm), Neisseria (Csn), Thermotoga (Cst), and Yersinia (Csy) (Haft et al., 2005). The interactions between homologous Cas proteins, the variety of cas operons, and animals with numerous CRISPR loci are too much for these first categories to handle with ease. The organization of the CRISPR-Cas system into two modules is suggested by a new classification scheme based on the evolutionary relationships between conserved proteins and the cas operon. The adaptation module requires the proteins Cas1 and Cas2, which are involved in spacer acquisition, and the effector module is necessary for the processing of primary CRISPR transcripts (crRNA), interference, and degradation of foreign nucleic acids. As a result, the Cascade proteins required by the various CRISPR subtypes to process crRNA may vary; some subtypes require numerous proteins, while others just need one multifunctional protein. The majority of protein complexes were found in the class 1 CRISPR-Cas system, where ribonucleoproteins with phylogenetically similar effector complexes of types I and III were discovered (Brouns et al., 2008; Makarova et al., 2015). As was noted in types II, V, and VI, the class 2 system, in contrast, has a single interference protein (Shmakov et al., 2017). Pre-crRNA processing appears to involve a number of other proteins that have at least one RNA recognition motif with uncertain function (RAMP) (Makarova et al., 2011).

Classification of CRISPR-Cas Systems

Given that the majority of prokaryotes acquired several CRISPR loci by frequent horizontal gene transfer (HGT) and that the CRISPR-Cas systems exhibit a high variety of Cas proteins, it is nearly difficult to classify these immune systems in a straightforward manner. Nevertheless, CRISPR-Cas systems were found in two classes and six kinds, each of which had many subtypes. Types I, II, and IV belong to class 1, while types II, V, and VI belong to class 2, based on the unique architecture of the effector modules (Koonin and Makarova, 2019).Multisubunit crRNA-effector complexes are present in class 1 systems, but in class 2 systems, a single protein, such as Cas9, performs all effector complex tasks (Table 1). Types IV and V, which are in classes 1 and 2, respectively, have evidence (Koonin and Makarova 2019). The expression, interference, and adaptability modules of the CRISPR-Cas systems differ in a number of ways (Makarova et al., 2015). While the majority of prokaryotes only have one kind of CRISPR-Cas system, it has also been documented that other types of systems may coexist. Systems classified as evolutionary ancestral systems are CRISPR-Cas systems in class 1.

Through the introduction of transposable elements encoding different nucleases, class 2 systems emerged from class 1 systems and are currently employed as genome editing tools (Mohanraju et al., 2016). The existence of the protein Cas3 unites all type I systems (Sinkunas et al., 2011). Six subclasses of the type I system are defined by the varied number of cas genes (type I-A to type I-F). The Cas1, Cas2, and Cas3 proteins are encoded by all type I systems as a complex resembling a cascade. Target localization, spacer acquisition, and crRNA processing are all facilitated by the cascade complex. The type I-A systems exhibit a specific Cascade complex that includes Cas3 [Rath et al., 2015]. Class 2 systems diverged from class 1 systems by the introduction of transposable elements encoding distinct nucleases, and they are being used as tools for genome editing (Mohanraju et al., 2016).

All type I systems are connected by the presence of the protein Cas3 (Sinkunas et al., 2011). Type I system subclasses (type I-A to type I-F) are distinguished by the different number of cas genes. All type I systems encode the Cas1, Cas2, and Cas3 proteins in a complex that resembles a cascade. The cascade complex aids in

target localization, spacer acquisition, and crRNA processing. According to Rath et al. (2015), the type I-A systems display a particular Cascade complex that contains Cas3.Type II CRISPR-Cas systems encode the Cas1, Cas2, Cas9, and occasionally Csn2 or Cas4 proteins (Barrangou et al., 2007; Heler et al., 2015; Wei et al., 2015). Cas9 has a role in target DNA cleavage, crRNA processing, and adaptation (Heler et al., 2015; Wei et al., 2015). Subtypes II-A, II-B, and II-C were further classifications for Type II systems. Csn2 and Cas4 genes are absent from Type II-C (Chylinski et al., 2013). Although the role of the protein Cas10 in type III CRISPR-Cas systems is yet unknown, it was discovered. The majority of Cas proteins are included in complexes that resemble Cascade, such as Type III-A's Csm complex or Type III-B's Cmr complex (Rouillon et al., 2013).

The target DNA in type I and type II systems and DNA and/or RNA in type III systems is another distinction between CRISPR-Cas systems. According to Makarova et al. (2011), the type I and type III systems are present in both bacteria and archaea, but the type II system is exclusive to bacteria. Multiple copies of the small subunit Cas11 (Csm2 or Cmr5) and the protein Cas7 make up the various subunit complexes that make up Type III systems exhibiting dual DNA/RNA interference activity [Staals et al., 2014]. The only system that effectively protects against DNA and RNA invaders using three distinct nuclease activities—specific DNA/RNA cleavage, nonspecific ssDNA cleavage, and so on—is the type III CRISPR-Cas system.After releasing the cleaved target RNA from the complex, the Cas7 protein may operate as a switch to reduce the DNAse activity of Cas10 (Samai et al., 2015). According to Maniv et al. (2016), the Type III system is adaptable to mutations in the protospacer sequence and can neutralize escape mutants from that specific system (Silas et al., 2017). Zhu et al. (2018) have studied the type III system's specific functions and structure. Many biotechnological applications based on type III systems have been created as a result of their unique characteristics. These applications include gene silencing and genome editing, among other genetic alterations (Liu et al., 2018).

Mechanism of CRISPR-Cas

Three phases comprise the CRISPR-Cas system-mediated defense process: adaptation, expression, and interference (Figure 2). Only when the cell comes into contact with fresh foreign DNA does the first stage take place. Every time a cell becomes infected, the second and third processes take place. The system recognizes the protospacer sequences of invasive DNA because of a brief conserved region termed Protospacer-Adjacent Motif (PAM) that is located within a few bases (2–5 bp) of the spacers (Mojica et al., 2009). The process of recognizing a protospacer involves creating and incorporating a new spacer sequence into the CRISPR array that is identical to the protospacer. This is followed by cellular repair proteins repairing the CRISPR array and duplicating the proximal repeat (Liu et al., 2017). Reverse transcriptase mostly linked to the Cas1 protein allows for spacer acquisition from RNA (transcripts of a DNA genome of an MGE via reverse transcription) (Silas et al., 2016, 2017).With the exception of type III CRISPR, which has variable spacer lengths, many CRISPR-Cas systems have very constant system-specific spacer lengths. In order to prevent alignment at the incorrect end of the crRNA target binding site, the PAM sequence present in the pre-spacer substrate enables the proper integration of additional spacers into the CRISPR array. The Cas1-Cas2 complex is the mediator of this mechanism. The CRISPR array is cleaved at the leader-repeat junction and the incoming spacer is joined in-between the repeat strands by the Cas1 protein; the coordinated nicking and ligation process is similar to a traditional integrase reaction (Arslan et al., 2014). A process like this seems sense given the Cas1 protein's anticipated integrase activity (Makarova et al., 2006). The Cas1–Cas2 complex catalyzes two cleavage-ligation processes after loading the incoming spacer: the first occurs at the leader end of the first repeat, and the second occurs at the spacer end of the repeat (McGinn and Marraffini, 2019). Non-Cas proteins engaged in DNA repair through Cas1's interaction with essential repair system components including RecB, RecC, and RuvB are also necessary for the acquisition process (Babu et al., 2011). The confirmation of this mechanism was made by Díez-Villaseñor et al. (2013). Jackson et al.'s evaluation of the Cascade complex's functionality is available (2017).

The spacer is translated into pre-crRNA, a precursor of CRISPR RNA, which is then processed into mature crRNA in the second stage. It is believed that the expressed spacer sequence that the crRNA provides identifies and directs the complex to engage the particular protospacer target. A unique complex of Cas proteins, a specific processing nuclease (Cas6), a single large Cas protein, or an external Rnase process the pre-crRNA to produce mature crRNAs during the expression-processing stage (Brouns et al., 2008; Sorek et al., 2013; Rath et al., 2015; Mohanraju et al., 2016; Barrangou and Horvath, 2017).

The foreign nucleic acid is identified and eliminated by crRNA and Cas proteins during the third step of interference. According to Rath et al. (2015), the complex of crRNA-Cas proteins finds the matching protospacer and causes certain Cas nucleases to degrade the target. The spacer transcript does not neutralize the DNA sequence if there is no match between the foreign DNA and the CRISPR spacer. A phage, on the other hand, can proliferate inside the cell, causing lysis and eventual death of the bacterium (Han et al., 2013). Numerous papers from the past have covered various facets of CRISPR-Cas biology (Sorek et al., 2013; Charpentier et al., 2015).

CRISPR-Cas Regulation of Virulence Gene Expression in Bacteria

It is anticipated that pathogenic bacteria would rely on effective defense mechanisms like CRISPR-Cas during their infection cycle since they face a variety of stressors and the presence of foreign DNA elements. The production of virulence genes is regulated by the CRISPR-Cas system and is a unique stress response exhibited by pathogens (Louwen et al., 2014). The majority of bacteria responsible for nosocomial infections have the capacity to form biofilms, which is important for their virulence and resistance to drugs. CRISPR-Cas-positive strains of P. Aeruginosa (Zegans et al., 2009) and E. Faecalis (Bourgogne et al., 2008) showed an enhanced capacity to build biofilms.In Serratia marcescens (Patterson et al., 2016), P. Aeruginosa (Høyland-Kroghsbo et al., 2017), and Clostridium difficile (Maikova et al., 2018), quorum sensing triggers the expression of the CRISPR-Cas gene. Certain genes involved in the development of biofilms in Acinetobacter baumannii are nearly exclusively found in strains that have been enriched in CRISPR-Cas systems [Mangas et al., 2019]. In Streptococcus mutans, deletion of the cas3 gene impacts biofilm formation [Tang et al., 2019]. This results in lower survival under heat shock and DNA-damaging conditions, improved growth under low pH, and decreased growth under oxidative stress (Serbanescu et al., 2015).According to Li et al. (2016), P. Aeruginosa uses the cas3 gene to upregulate virulence proteins that help the bacterium avoid being recognized by the host immune system. The cas2 gene is necessary for intracellular infection in Legionella pneumophila (Gunderson and Cianciotto, 2013). Cui et al. (2020) also showed that deletion of cas3 reduced intracellular invasion and downregulated the capacity of Salmonella enterica to generate biofilms. The Cas9 gene has a significant role in adhesion, invasion, and intracellular survival of Campylobacter jejuni (Louwen et al., 2013), Francisella novicida, and Neisseria meningitidis (Sampson et al., 2013).The absence of the cas9 gene in Streptococcus pyogenes has been associated with a decrease in the quantity of virulence factors and the manifestation of many virulence-regulating proteins. In comparison to the wild-type parent, the mutant strain exhibits decreased adhesion to epithelial cells as well as other changed characteristics including growth in human whole blood ex vivo and pathogenicity in a mouse necrotizing skin infection model (Gao et al., 2019). While it has been observed that the elevation of several virulence factors is correlated with the presence of cas genes, the intricate function of the CRISP-Cas systems in important pathogens is yet unknown. Although there is a positive link between P. Aeruginosa virulence and CRISPR-Cas system presence, CRISPR-Cas activity is neither required nor sufficient for enhanced virulence. The findings of Vasquez-Rifo et al.(2019) suggest that bacterial adaptive immunity and virulence are indirectly associated with the effects of physiological, ecological, and evolutionary factors.

Anti-CRISPR Defence

Bacteriophages and bacteria always put pressure on one another, much as in any other prey-predator interaction. This continual pressure causes co-dependent evolution, wherein phages create new strategies to evade or overcome bacterial defense mechanisms, while bacteria find new means to live. Innate immunity, which is present in all bacteria (Seed, 2015; Trasanidou et al., 2019), and acquired immunity, which is exemplified by the CRISPR-Cas system, are the foundations of bacterial defense against bacteriophages. While the CRISPR-Cas system significantly reduces the likelihood of phage invasion, innate immunity is not very effective. As a result, phages have evolved defense mechanisms against this defense by encoding proteins known as “anti-CRISPR proteins,” which deactivate the system (Bondy-Denomy et al., 2013; Pawluk et al., 2014; Bondy-Denomy et al., 2015).Phage that infect P. Aeruginosa with the type I-F CRISPR system has the first genes for anti-CRISPR proteins (Acr) (Bondy-Denomy et al., 2013). Since then, a great deal of research has gone into identifying proteins that also deactivate other kinds. As of right now, the following CRISPR types can be rendered inactive by known proteins: I-C, I-D, I-E, I-F, II-A, II-C, V-A, and VI-B. See Trasanidou et al. (2019) for further information on the protein structure, mechanism of action, and bacterial species where they have been found. It has been found recently that a bacteriophage may develop a structure like a nuclease, which allows it to hide from CRISPR immunity that targets DNA, but leaves it vulnerable to CRISPR-Cas systems that target RNA (Malone et al., 2020). Among such RNA-targeting systems is type III CRISPR-Cas, for which bacteriophages have recently discovered a way to defeat it (Athukoralage et al., 2020). The goal of the most recent research is to identify proteins that render the CRISPR-Cas9 genome editing mechanism inactive. As of right now, CRISPR-Cas9 stands as the most potent and targeted gene editing method known; yet, over time, the system’s nonspecific activity might become a concern. Finding the system’s off-switch under these circumstances is as helpful as it gets (Pawluk et al., 2016b; Rauch et al., 2017; Dong et al., 2017).

Biotechnological Applications

The most recent study focuses on employing the system to battle pathogens, such as bacteria or viruses, and many other biotechnological applications, in addition to the long-running CRISPR-Cas-based studies on genetic editing and cancer treatment (Carroll and Zhou, 2017). It has already been discovered that CRISPR-Cas self-targeting kills bacteria. Stern et al. (2010) discovered that self-targeting happens in 18% of all CRISPR-bearing species after examining 330 different organisms’ CRISPRs. Thus, it was proposed to transfer the CRISPR loci that target chromosomal areas linked to virulence or antibiotic resistance in order to employ CRISPR-Cas systems to tackle bacterial infections

(Bikard et al., 2012).The Cas9 endonuclease-based RNA-programmable genome editing technique, which was introduced by Jinek et al. (2012), has gained widespread acceptance. It was suggested by Stern et al. (2010) that self-targeting is a form of autoimmunity rather than a regulatory mechanism that has an autoimmune fitness cost. This could account for the abundance of degraded CRISPR systems across prokaryotes. Stern et al. (2010) observed the abundance of degraded repeats near self-targeting spacers and no conservation across species. As a result, harmful microorganisms have been effectively targeted using CRISPR-Cas systems. The extraction of individual bacterial strains from mixed populations of Escherichia coli provided new opportunities for the creation of antibiotics that are "smart," meaning they can distinguish between useful and harmful microbes and avoid resistance (Gomaa et al., 2014).

The CRISPR-Cas3 and CRISPR-Cas9 constructions are two such powerful tools. They may be engineered to target certain virulence genes and thus attack infections, or to target antibiotic resistance genes and so combat drug resistance. Research never ends. This approach works well for chromosomal genes, but not for plasmid-encoded virulence and resistance. Recently, a number of antibacterial nucleocapsids based on CRISPR-Cas13a that can identify antimicrobial resistance genes in resistant microbes and destroy them have been created. Sequence-specific Rnase activity was revealed by CapsidCas13a constructs made by enclosing Leptotrichia shahii's programmable CRISPR-Cas13a into a bacteriophage capsid. According to Kiga et al. (2020), the systems were suggested as diagnostic tools for identifying bacterial resistance genes as well as therapeutic drugs against bacterial illnesses.The potential for modified CRISPR-Cas systems to distinguish between infections and commensal bacteria is intriguing. This technology might be used to modify microbial communities, which include the human microbiome linked to many disorders.

With time and under the selection pressure of employing CRISPR as antimicrobials, a potential resistance may also arise here because of the Acr genes that were discussed in the preceding section. The ability to target distinct virulence gene locations was made possible by the development of self-targeting gene editing technology. Precise glucosyltransferase alteration led to reduced exopolysaccharide production and impaired biofilm-forming capacity in S. Mutans (Gong et al., 2018). Antimicrobials with the specific range of action selected by design can be produced by the application of CRISPR-Cas technology. A Galleria mellonella infection model's survival was increased by the effective delivery of RNA-guided nucleases that targeted certain DNA regions (Citorik et al., 2014). A CRISPR-Cas9 system that targets the chromosomal kanamycin resistance gene in Staphylococcus aureus was also delivered using a phage-based method. Strong growth inhibition of resistant bacteria was therefore obtained as a result of chromosomal breakage and consequent cell death. Antibiotic-resistant bacteria were also significantly reduced by the therapy in an in vivo mouse skin infection model (Bikard et al., 2014).

With the development of a novel base editing system and CRISPR-Cas9-mediated genome editing technique, a target gene can be rendered inactive by the cytidine base editor, which creates an early stop codon. Pseudomonas species' very effective genetic modification is thought to hasten a range of studies into bacterial physiology, therapeutic target discovery, and metabolic engineering (Chen et al., 2018).Furthermore, prokaryotic and eukaryotic cells can inhibit gene expression in a sequence-specific manner by the use of CRISPR interference (CRISPRi), a genetic perturbation approach (Qi et al., 2013). By suppressing the luxS gene, which codes for a synthase involved in the first step of biofilm development, the CRISPRi technique was used to prevent the production of bacterial biofilm in E. Coli (Zuberi et al., 2017).New groundwork was made possible by the discovery of guidelines for the targeted delivery of transcriptional repressors (CRISPRi) and activators (CRISPRa) to native genes using endonuclease-deficient Cas9 (Gilbert et al., 2014). CRISPR-Cas technologies provide a compelling alternative for programmable and sequence-specific antimicrobials as they have been successfully repurposed to target virulence factors and antibiotic resistance genes in bacteria (Bikard and Barrangou, 2017). In eukaryotes, self-targeting with CRISPR-based technologies led mostly to genome editing. In order to affect gene expression in human cells for regenerative medicine, genetic reprogramming, and cell and gene therapy, targeted gene regulation is increasingly employed.

It has been suggested that the CRISPR-Cas system be used to treat viral infections in addition to bacterial ones. CRISPR-Cas9 is designed to target human viruses, including DNA viruses like hepatitis B, human papillomavirus, and Epstein-Barr virus. CRISPR-Cas9 is ineffective in treating retroviral infections because Cas9 is unable to interact directly with RNA (Li et al., 2020). In light of the COVID-19 pandemic, which is now being produced by the retrovirus SARS-CoV-2, CRISPR research is concentrating on developing a system that can target RNA in order to treat the disease. According to recent research, the Cas13 proteins Cas13d (Nguyen et al., 2020) and Cas13a (Li et al., 2020) can target RNA.This manual Because they have no effect on the human transcriptome, RNAs are also harmless. According to a paper that is currently pending review and is accessible online as a preprint, Abbott et al. (2020) claimed to have designed a method that can target more than 90% of coronaviruses and could be easily applied to additional strains that are on the rise and might potentially spark a pandemic. In light of the current epidemic, disease surveillance presents a possible new use for CRISPR-Cas systems.

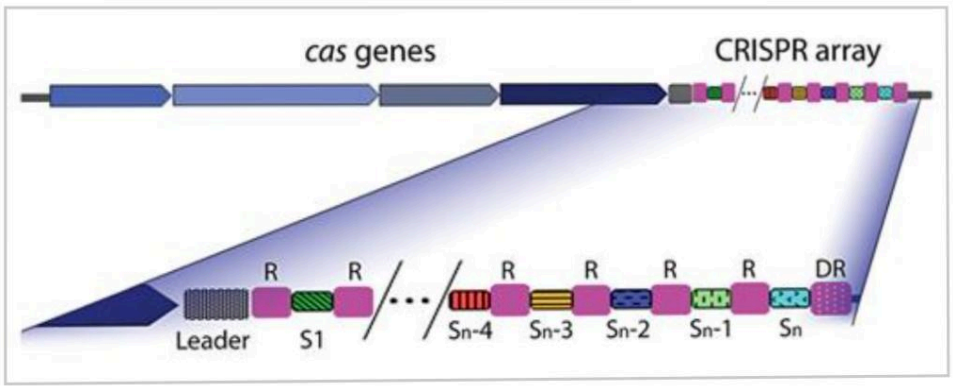

Figure 1. The structure of a CRISPR-Cas system. R, repeat; S, spacer; n, the total number of spacers in the array. A CRISPR array can have at least one spacer, each flanked by two repeats. The last repeat, which usually suffers mutations, is called degenerated repeat (DR).

Table 1. The six types of CRISPR-Cas systems

Class	Type	Spacer acquisition	Pre-crRNA processing	crRNP	Signature protein
1	I	Cas1, Cas2, Cas4	Cas6(Cas5d for I-C)	Cascade	
	III	Cas1, Cas2, RT	Cas6/Rnase E/ Rnase H	Csm/Cmr	
	IV	Unknown	Csf5	Csf	Csf1
2	II	Cas1, Cas2, Cas4/Csn2	Rnase III, Cas9	Cas9	Cas9
	V	Cas1, Cas2, Cas4	Cas12	Cas12	Cas12
	VI	Cas1, Cas2	Cas13	Cas13	Cas13

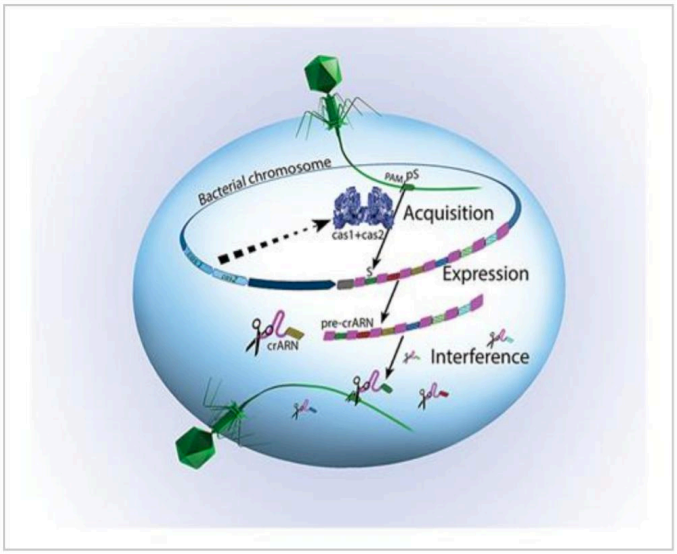

Figure 2. The action stages of the CRISPR-Cas system. In the first stage, adaptation, cas1 and cas2 genes are transcribed, and the resulting proteins form a complex that recognizes the protospacer adjacent motive (PAM) and copy the protospacer sequence.

Along with other RNA viruses, various diagnostic techniques for SARS-CoV-2 were proposed in the most recent research publications (Metsky et al., 2020; Ding et al., 2020; Curti et al., 2020). The SHERLOCK technique, which stands for "specific high-sensitivity enzymatic reporter unlocking," was created by Kellner et al(2019).

Biomedical Applications of CRISPR-Cas and Microbial Genome Editing in Human Health

The creative use of microbial genome editing methods and CRISPR-Cas systems has significantly enhanced biomedical research. These technologies provide a multitude of options for tackling diverse health-related issues. This review of the literature examines the important applications of CRISPR-Cas in genome editing, medication development, precision medicine, and current research trends with an eye toward future possibilities.The area of genome editing has undergone a paradigm change thanks to CRISPR-Cas systems. The CRISPR-Cas9 system was made possible by the groundbreaking work of Doudna and Charpentier (2014), and it now allows for precise DNA alterations. This innovative technology can be programmed and is controlled by RNA sequences that may be altered. It gives scientists a flexible tool to modify specific regions of the genome, potentially correcting genetic abnormalities that cause a wide range of illnesses. It is essential to comprehend the molecular mechanics of this system, as explained by Jinek et al. (2012), in order to fully use its potential. Their research demonstrated the potential for precise DNA editing using the system's RNA-guided endonuclease, which might lead to significant developments in human health.Innovative antibiotics and antivirals are desperately needed in the drug development field to fight antibiotic-resistant bacteria and new viral threats.

Wright (2017) highlighted this difficulty and said that CRISPR-Cas systems provide a glimmer of hope. Scholars are investigating the potential use of these systems in the development of new antibiotics that can successfully combat antibiotic resistance. De Clercq and Li (2016) offered an analogous historical overview of antiviral medications and deliberated on the ways in which novel technologies, such as CRISPR-Cas, are impacting the advancement of antiviral treatments. CRISPR-Cas has uses beyond drug development, potentially addressing some of the most urgent public health issues.A revolutionary new direction in healthcare is being pursued by precision medicine. The Precision treatment Initiative was unveiled by Collins and Varmus (2015) as a historic US endeavor that aims to improve healthcare through tailored treatment.The field of CRISPR-Cas technology is still developing in terms of present research and possible applications. In their examination of the most recent advancements in CRISPR-Cas, Knott and Doudna (2018) emphasized the technology's pivotal role in the direction of genetic engineering. This technology is always being improved to increase its specificity and efficiency, broadening its range of uses. It is not stationary. Furthermore, Hasin, Seldin, and Lusis (2017) investigated the role that multi-omics techniques have in comprehending complicated illnesses. In this case, CRISPR-Cas systems are essential for producing and evaluating enormous datasets that clarify the complex processes underlying a range of medical disorders. The integration of many technologies like as transcriptomics, proteomics, and genomes allows for a comprehensive comprehension of illnesses and expedites the creation of focused treatments.

Challanges and Future Recommendation

A new age in genetic engineering and microbial biotechnology has been brought about by CRISPR-Cas systems, which provide genome editing with unprecedented accuracy and flexibility. A number of important suggestions and difficulties will influence this field's course going forward.Regarding recommendations, the main need is to keep expanding the CRISPR toolkit. In order to target a wider range of microbial species and improve editing efficiency and specificity, this entails investigating and utilizing novel Cas proteins. Beyond that, ethical issues are also very much in the picture. The development of CRISPR technology has made it imperative to create and follow strict ethical standards and laws regulating its usage in microbiological applications. Public approval and ethical research methods are critical.

Another area of the CRISPR landscape that needs innovation is delivery techniques. To achieve precise genome editing in complicated settings like soil or the human gut, researchers need to devise new and effective methods for distributing CRISPR components. Moreover, it is critical to address the issue of microbial resistance to CRISPR-Cas systems. The development of anti-CRISPR proteins has made resistance methods imperative.It's crucial to take the surroundings into account as well. Thorough evaluation of CRISPR technology's environmental effect is essential, since it finds more and more applications in ecological and agricultural contexts. It is necessary to develop mitigation techniques to prevent unforeseen effects on ecosystems.

Notwithstanding these encouraging suggestions, there are still a lot of obstacles facing the study of CRISPR-Cas systems in microorganisms. Notably, off-target impacts continue to be a problem. It's a constant struggle to find ways to guarantee genetic modification accuracy and avoid unintentionally upsetting microbial ecosystems.Researchers have to overcome challenges posed by the constantly changing legal and regulatory environment, making sure that established protocols are followed and that rules are adjusted as needed. Furthermore, strong containment and biosafety protocols are essential to preventing unintentional discharges of genetically modified bacteria into the environment. Last but not least, having an open and knowledgeable public conversation is an essential bioethical

component. To ensure ethical and efficient use of CRISPR-Cas technology, it is imperative to cultivate public acceptance and comprehension of its consequences.

Conclusion

To sum up, the CRISPR-Cas systems found in microorganisms are an amazing and adaptable tool that have transformed the fields of microbial study and genetic engineering. Applications for these adaptive immune systems are numerous and include antimicrobial defense and gene editing. Our comprehension of the many processes and roles of CRISPR-Cas systems in various bacteria opens up new avenues for biotechnology, medicine, and our exploration of the microbiological world.It is impossible to overestimate the promise of CRISPR-Cas systems to solve important problems like infectious illnesses and antibiotic resistance. Furthermore, the continuous study in this area broadens our understanding all the time, opening doors for new discoveries and innovations.As we advance, it is crucial to take into account the moral and legal ramifications of using CRISPR-Cas technology, guaranteeing ethical and knowledgeable procedures. Given how quickly new information is being discovered and how CRISPR-Cas applications are developing, it is clear that this amazing technology will continue to influence research and medicine in the years to come. Staying up to speed with the constantly changing field of CRISPR-Cas systems in bacteria requires consulting references to the most recent research and publications.

Author Contributions

S.S., M.K.M., M.T.F., S.M., M.F., M.A.S. drafted the manuscript and made substantial contributions to the design of the study. L.E.A.H., S.M. reviewed and drafted the paper.

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