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Review Article

The CRISPR-Cas System as a Technology to Redefine Industrial Biotechnology

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Abstract

CRISPR technology has revolutionized the field of Industrial Biotechnology, producing numerous miracles. Several CRISPR/Cas9-based methodologies help simple, quick strain development in yeast and investigate their potential for the synchronous presentation of different hereditary alterations, thus promoting and increasing the efficacy of several industrial technologies. CRISPR/Cas9 framework aimed at fast genome altering of *Clostridium ljungdahlii* is a known wide target intended for business creation and increasing ethanol's productivity from union gas by several folds. CRISPR-Cas frameworks have prospective for numerous microbes designing solicitations and bacterial strain composing inoculation of societies, self-immunity or auto-focused on cells execution, plus the designing or regulator of metabolic paths enhanced biochemical combination. CRISPR-related protein 9 (Cas9), by the RNA-guided endonuclease specifically, stood out for its guarantee in fundamental examination and quality altering built therapeutics. This review focuses on various industrial and pharmaceutical Biotechnology advancements, including the top qualities for CRISPR-Cas frameworks featuring how these highlights can be utilized in the modern scenario.

Keywords: CRISPR; Industrial Biotechnology; RNA-guided; Yeast; Bacteriophages

Introduction

Microorganisms have played a vital role in various fields, including pharmaceutical, agricultural, industrial, and even in several mechanical processes. They produce a massive number of valuable products possessing great commercial importance and even for the production of chemically synthesized compounds which are not possible to be artificially made even in the laboratories [1]. Additionally, it is also observed that the artificial synthesis of these compounds is associated with very high costs. The utilization of microorganisms has made this easy, affordable, and very simple, and thus Industrial Biotechnology and Microbiology have always targeted microorganisms for improving and hike in yield and productivity. Earlier traditional production methods yielded unsatisfactory results, and therefore, this led researchers to focus on various novel and alternative strategies for improving quality and quantity. The development of novel and enhanced techniques like process engineering, novel downstream processes and fermentation technologies have given new hopes. DNA sequencing has made it possible to acquire knowledge about the various DNA sequences [2].

Additionally, with the integration of several Bioinformatics tools, it has also helped us get a crystal-clear understanding of the

various regulatory elements and manipulate the microorganisms' genome. On the other hand, Recombinant DNA Technology has utilized these data to efficiently engineer the genome and, therefore, remove unwanted genes or integrate different genes to enhance various desirable products [3]. The different genetic engineering techniques have helped achieve increased production of various beverages, fuel, nutraceuticals, food additives, and even multiple products essential for animal and human health. The production of alcohols and acetone has reached new heights with improved procedures and techniques [4].

Industrial biology has progressed altogether as of late because of enhancements in genomic designing procedures; explicitly, and improvements have risen out of changing genomic successions through the addition, erasure, and change of nucleotide bases after controlling the transcriptomic and epigenomic features. The mechanical biotechnological field is dependent on several strategies satisfying the developing needs for rising various synthetic substances, biomolecules, and multiple metabolites that the maturation can deliver of the microbes [5].

Demonstration and revelation of CRISPR and associated proteins qualities have powered the late advancement of an adaptable democratized genome-designing tool kit dependent on CRISPR-Cas frameworks' computational focusing. Specialists have utilized these frameworks in unicellular microorganisms keeping up genomic respectability through relieving the impacts to unfamiliar or portable genomic components to alter and control genomic DNA by exhibiting double breakdowns offering ascent to grouping changes when fixed by endogenous fix pathways and to control quality clarification using the development of counterfeit record factors [6].

Besides, these frameworks empower the concurrent focusing of numerous target points and display potential as adaptable stages for extensive genome-wide adjustments of microorganisms [1]. These utilization methods portray importance and efficacy in the hereditary control of modern microorganisms where the frameworks can aid either to supplement prior procedures or give another arrangement of abilities for living beings where instruments for genetic control are deficient. Additionally, CRISPR technology can be an efficient tool for curing a number of cancers like prostate cancer, breast cancer, and lung cancer to name a few [7,8].

CRISPR technology for efficient biofuel production

When combined with computational technology, CRISPR has paved the way for the efficient production of several bioactive compounds. The technology has also encouraged several novel biomanufacturing processes. The efficient usage of computer algorithms has aided in detecting specific genes that can be switched on-off to enhance the target compound's productivity. Experiments conducted with *Pseudomonas putida* by engineering its genome have helped obtain higher quantities of indigoidine. CRISPR interference technology has helped to successfully block or nullify the expression of as many as 14 genes [9].

Isopentenol can be an efficient and up-and-coming source of biofuel, and also, several replacements for petroleum-derived products have been successfully developed by this mechanism [9]. Studies have so indicated that several fungal strains can be engineered with the aid of CRISPR strategy to produce a complex mixture of enzymes which can enhance the breakdown of the carbohydrates commonly found in biomasses and can successfully convert it into a form of sugar which can be easily fermented to produce various types of biofuel and even produce second-generation ethanol [10].

Modification of the fungal strain *Trichoderma reesei* by introducing several improvements into the RUT-C30 locus has shown positive results for enzymes that can degrade the plant cell walls. This process mainly involved the modification of several transcription factors, deletion of proteases, and even the addition of several enzymes, which the fungus lacks typically. This guided the path for the cheap production of cellulosic ethanol and several other bio-renewable products [11]. Additionally, *Issatchenkia orientalis* has been engineered to harvest several organic acids by deleting specific genes and utilizing autonomously replicating sequences obtained from *Saccharomyces cerevisiae* to construct an episomal plasmid. CAS9 guide RNAs have been effectively used on Yarrowia lipolytic to identify several essential genes, enhancing the production of lipids and enhancing biofuel production [12].

CRISPR3/Cas system cloning in *E. coli*

Until this point in time, productive genomic designing in microorganisms solely depended upon utilization of genetic material benefactors portrayed utilizing endogenous DNA fix hardware, exogenous hybrid frameworks, selectable pointers, site-explicit

recombinase, bunch II intron retrotransposition, using the counterfeit chromosomes. CRISPR enhances the immune potential of *E. coli*. It is known to render adaptive immunity based on RNA and is particularly effective against the virus, mainly the bacteriophages [13].

CRISPR-associated protein commonly referred to as (Cas) is an endonuclease known to cleave foreign DNA. This cleavage's main criterion to occur is a motif called protospacer adjacent motif (PAM), ensuring highly efficient targeting [13]. Researchers use this property to modify the host genome genetically. CRISPR/Cas9 is highly simple and versatile. Upstream of PAM sequences, Cas9 nucleases are generally guided to the target DNA. It results in double-stranded breaks of around three to four bases. Two processes, namely Non Homologous End Joining (NHEJ) and the Homology-Directed Repair (HDR) process, respectively, join these breaks. NHEJ mechanism is more prone to mistakes and is applicable when donor DNA is not present. On the other hand, HDR is a precise, helpful method when the donor DNA is present [14].

CRISPR3/Cas system to prevent plasmid transformation

When the CRISPR 3 framework's functional movement is tested in the heterologous *E. coli*, it is observed that they utilized a plasmid DNA change measure. Using pUC18, which is viable with pACYC184 in the *E. coli*, the researchers designed plasmid sp1 and plasmid sp2, which had proto-spacer arrangements indistinguishable from the spacers as mentioned earlier of CRISPR 3 exhibit, along relating PAM 50 - TGGTG-30 ahead to the proto-spacer understanding. They tried the plasmid change proficiency in beneficiary *E. coli* [15].

CRISPR to design novel antibiotics

CRISPR technology has proved to be the new torchbearer for the pharmaceutical industry. It has helped develop better antibiotics and increase the antibiotics' production simultaneously [16]. Newly developed CRISPRi has widely helped to guide gene expression and has also been designed to control the levels of a protein that can be produced, which have helped the scientists detect the various antibiotic targets, thus aiding in the development of better antibiotics. CRISPR has improved the status of the existing antibiotic drugs and has also helped develop new ones. It has successfully helped us identify the bacteria's weaknesses and target these points for an efficient drug development process [17].

The problem of antimicrobial resistance is a severely growing problem nowadays. This technology has helped reduce the output of protein from various genes, therefore assisting scientists in figuring out the mechanism behind the inhibition of microbial growth by applying antibiotics. CRISPRi does not generally cut the DNA [18], but leads to the deflection of several proteins leading to the inhibition of several genes' activation. It results in lower expression of the gene, helping the researchers understand the mechanism behind bacteria's behavior to be highly stimulated by less or light levels of the drug used [19]. It widely proves the complex but clear-cut connection between the gene and the drug, thus leading to the screening of thousands of genes as crucial antibiotic targets. This method has been applied to *E. Coli* and several disease-causing species like *Salmonella, Pseudomonas, Staphylococcus* by the commonly known conjugative transfer of Mobile-CRISPRi [18].

Genetic alterations in *Saccharomyces cerevisiae*

Various strategies in *S. cerevisiae* have been created solely for strain designing. Though, at this point, after numerous hereditary controls are required, strain development is still a complex process. Where past investigations essentially centered around the utilization of CRISPR/Cas9 for not being active qualitatively, the adaptability of this technology-based process to design yeast by accomplishing a concurrent mix of a vast number of genes develop joined with quality erasure and the simultaneous presentation of two mononucleotide changes at various positions as shown [20]. Groups of normalized plasmids, just as the online Yeastriction target-arrangement identifier and primary plan instrument, are made accessible to the yeast investigation network to encourage quick, normalized, and effective utilization CRISPR/Cas9 framework [6].

Articulation and improvement of heterologous item paths in several strains of *S. cerevisiae* [21] needs a presentation of different (progressive) hereditary adjustments, including the incorporation of item pathway qualities at numerous genetic loci also, overhauling focal digestion by adjusting properties of direct metabolic responses (for example through quality erasure, changing administrative properties or substitution of local qualities by heterologous partners). Presentation of necessary hereditary alterations has continued to be tedious and work the entire cycle, as every individual adjustment wants a pattern of change, choice, and affirmation. Microscopic organisms have built up a few frameworks to corrupt unfamiliar DNA. Quickly after their revelation, limitation compounds turned into the 'workhorses of atomic science [22].

Genetic engineering of filamentous fungi

Today with the population explosion and our over-dependence on perishable resources for the production of energy leads humanity to a future where we soon will face a massive energy crisis as the non-renewable sources are limited and will quickly get exhausted. Therefore, the idea of utilizing various non-perishable resources to produce energy is now gaining tremendous momentum [23]. Among these, one of the most challenging areas is eco-friendly fuel production. Genome engineering is a dream of humanity by utilizing the CRISPR mechanism. This technology has been successfully employed to engineer various microorganisms and even different plants for generating biofuels and specific chemicals. These chemicals have been widely used in plastic product production and even in lubricants and the textile industry [24].

CRISPR has revolutionized the fermentation industry by bringing about ample genetic manipulations and has successfully bioengineered yeast and bacterial strains and a significantly faster rate. This has enhanced the screening process of several novel strains and has made it possible to increase biofuel yield and several other hydrocarbon derivatives [23].

The quantity of wholly sequenced parasitic genomes is quickly expanding. Since hereditary instruments are inadequately created for most filamentous organisms, it is hard to utilize hereditary designing for understanding the science of these growths and to misuse them mechanically completely [25]. Therefore, there is an interest in creating adaptable strategies that can be used to control non-model filamentous changes hereditarily. Scientists have built up a CRISPR-Cas9 oriented framework adjusted for usage in filamentous growths. The framework now contains four common CRISPR-Cas9 vectors that are furnished by commonly utilized parasitic markers considering determining an expansive scope of organisms [26]. Besides, scientists have built up content that permits distinguishing proto-spacers targeting quality homologs in numerous species encouraging presentation of essential changes in distinctive filamentous parasites [27].

Additionally, for a commonly known wild-type *A. aculeatus* strain, the CRISPR Cas9 framework is utilized to create a strain encompassing an AACU pyrG marker displaying the idea that subsequent strains may be used quality focusing [10]. Henceforth, it permits coordinated mutagenesis by changing an objective host by only one plasmid, which covers various qualities and is known to encode the framework's two segments. RNA-guided DNA double-stranded breaks might be fixed wrongly by the application NHEJ mechanism [28].

Commercial wine production is one of the biggest challenges that the world is facing nowadays. With the increase in demand for various alcoholic beverages, researchers aim to find novel strategies for increasing production and maintaining the quality at the same time. *Trichoderma* and *Aspergillus* are the most common and widely used sources for wine production. These can remain active over a wide pH range and SO_2 conditions, which are primarily encountered in the wine. Genetic engineering has helped increase quantity and quality simultaneously [29].

Production of secondary metabolites using CRISPR tool

The overall yield and utilization of the various types of different secondary metabolites in the industry are not uncommon. Initially, the scientists faced several challenges to improve the productivity and yield of such metabolites. CRISPR technology has solved this problem to a great extent. It has opened several paths for the various discoveries of several novel compounds and, at the same time, has aided in the successful engineering of the genomes of several organisms [30].

CRISPR technology has helped discover a novel gene that can help produce polyketide and non-ribosomal proteins in the genus *Talaromyces. T. Atroroseus* has been engineered using the CRISPR Technology to make secondary metabolism in fungus, which has helped discover a novel gene known to produce a polyketide non-ribosomal peptide turn stimulates ZG-1494-alpha which owes a medically relevant property. It can be considered a rare example of genetic engineering's reverse process [31].

Genome editing in *Clostridium ljungdahlii*

Acetogenic microorganisms can change over solitary gases like carbon monoxide and carbon dioxide to mass synthetics and powers. Acknowledging its maximum capacity is being blocked through the non-appearance of successful hereditary apparatuses for significant throughput genome adjustment. This paper reports the improvement of an exceptionally proficient CRISPR/Cas9 framework for the only alteration of *C. ljungdahlii*, which presents a picture to increase and enhance the productivity of C2H5OH from union gas [32].

The framework defeats the insufficiencies of right now accessible devices (faster, no other anti-toxin obstruction quality, scar-less and insignificant polar impacts), then it will discover value in different acetogenins, including pathogen *C. difficile*. The utilization of petroleum products is not, at this point, valid [33]. A limited asset, their utilization is harming the climate through contamination and an unnatural weather change. Elective, earth well-disposed wellsprings of synthetic substances and powers are obligatory. Be that as it may, its hard-headedness to deconstruction is making the advancement of financial cycles incredibly testing. One option is to catch carbon previously fuse into lingo cellulosic biomass straightforwardly [34]. The acetogenins' true potential mainly dwells in their ability to deliver synthetic compounds and energizes other than ethanol [35].

The main provisional strides towards such objectives have been made as of late through the turn of events and execution of conventional apparatuses for the change of acetogenins, for example, *C ljungdahlii* and *C autoethanogenum* [36]. The instruments used to date, be that as it may, has been restricted to either the presentation of independent plasmids encoding chose enzymes engaged with item arrangement, the age of insertional mutants utilizing ClosTron mutagenesis or the inclusion of anti-toxin opposition qualities by the process known as homologous recombination and single hybrid reconciliation of self-destruction plasmids [35].

In further studies, the creation of butyrate from *C. acetobutylicum* was performed and was coordinated into the *C. ljungdahlii* genome through homologous recombination. From that point adjusted by extraction of the catP anti-toxin opposition marker utilized for determination of the incorporated DNA [36]. Notwithstanding this advancement, the created strategies and instruments are primarily lumbering and tedious, dependent on various work escalated screening stages to recognize the ideal cell lines. Also, their sending brings about mutants with possessions that are not exactly ideal. Then again, the occurrence of embedded DNA in those mutants prepared to utilize the ClosTron [35] or through twofold hybrid coordination of freak alleles conveying anti-infection opposition qualities work as a safe framework, presenting protection from exogenous hereditary components, for example, plasmids and phages [37].

CRISPR immunization against bacteriophages

CRISPR and Cas qualities structure the CRISPR–Cas safe framework that gives grouping explicit versatile insusceptibility against unfamiliar hereditary components in microorganisms. Reconciliation of short expanses of obtrusive DNA as novel 'spacers' into CRISPR loci demonstrates its resistance [38,39]. True to form, dynamic CRISPR loci advanced through spellbound expansion of a few novel spacers succeeding presentation to bacteriophages. Although examining the draft genome arrangement uncovered an assortment of single nucleotide polymorphisms and inclusions/cancellations, Sanger sequencing rejected a large portion of the various in silico contrasts [40]. Likewise, 2 SNPs and two little INDELs were recognized and followed in the transitional variations. In general, building CRISPR-encoded insusceptibility does not altogether influence the genome, which permits significant practical possessions in isogenic CRISPR freaks [41].

It is fundamental for the turn of events and detailing maintainable and vigorous cutting edge starter societies with expanded modern life expectancies [38]. Of essential significance, bacteriophages' universal presence (phages) in the climate has adversely and overinfluenced maturation measures in mechanical settings. Phages continue producing offices given their protection from sanitization, airborne dispersal. The down-to-earth provokes natural disinfection procedures in food-grade making settings [40].

Plenty of phage guard frameworks happen in lactic corrosive microorganisms, including anticipation of adsorption, hindering infusion, fruitless disease, R-M (limitation change), poison neutralizing agent frameworks, and the as of late described CRISPR loci. Furthermore, these guard frameworks might be joined at times designed to get control over phage populaces in modern settings [42].

Though conventional guard systems depended initially on frameworks, for example, the avoidance of adhesion and obstructing of nucleic acid infusion, then consequently on R-M frameworks and failed contamination, the significance of the recently portrayed CRISPR–Cas (CRISPR-related) frameworks for giving phage opposition in *S. thermophiles* had an emotional effect on the administration of phage-related problems [43]. By and large, three different kinds of CRISPR-Cas frameworks have been set up based on the succession of general cas1 and cas2 qualities, just as the event of mark qualities, specifically cas3, cas9, and cas10 for Types I, II, and III separately [44].

Conclusion

Exploring and utilizing CRISPR-based procedures for improving bacteriophage opposition in different *S. thermophilus* strains

and utilizing various isogenic variations in turn plans will guarantee manageable, more, perpetual utilization of the most productive and attractive strains with broadened life expectancies [45]. Saddling CRISPR invulnerability may be upgraded further by mix with another productive and viable phage-opposition instrument. These outcomes further our comprehension of infection have elements, particularly concerning CRISPR insusceptibility, and give a developmental system for investigating the communications among microbes and their phages and their environmental impact [46].

In a long time since the trial portrayal of CRISPR-Cas as versatile, safe frameworks, the comprehensive utilization of CRISPR-Cas frameworks is without a doubt because of their simplicity of execution, quick iterative plan, and different strategies for use [47]. The ongoing revelation of new CRISPR-Cas frameworks, each with one-of-a-kind capacities and possible uses in mechanical organisms, recommends the presence of different sub-atomic machines covered up in the genomes of crude microscopic organisms and Archaea that can additionally grow the sub-atomic tool kit. The advantages of CRISPR-Cas frameworks will affect mechanical biotechnology [44].

Similarly, CRISPR-Cas frameworks' arrangement depends on an entire assortment of setting up advertisers, selectable markers, and vectors to encourage its hearty use. Aimed at specific alteration types, an earlier comprehension of the endogenous DNA fix apparatus helps designing endeavors. Dubious is the capacity to play out the synchronous designing of consortia, or explicit altering of person strains in a blended animal group biome [48].

Despite these impediments, utilizing these frameworks to control organisms applicable to modern biotechnology will probably proceed. More extensive appropriation of CRISPR-Cas frameworks will speed up the goal of such problems. Additionally, these frameworks provide another strategy to exploit this data to improve alluring organic operators' mechanical and business creation. It is pronounced in the instance of filamentous growths, where the enthusiasm for bioactive mixes and proteins has kept on filling as of late, and generally, scarcely any apparatuses have been created for hereditary solid designing [49]. These frameworks' capacity playing a role in a quality presentation, endogenous quality cancellation, and transcriptional control help advance the creation of a different exhibit of mixes in an assortment of microbial life forms.

Highlights

- CRISPR/Cas9-based methodologies help simple, quick strain development in yeast and investigate their potential for the synchronous presentation of different hereditary alterations.
- CRISPR-Cas frameworks have a prospective for numerous microbes designing solicitations and bacterial strain composing societies' inoculation.
- CRISPR-related protein 9 (Cas9), by the RNA-guided endonuclease specifically, stood out for its guarantee in fundamental

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