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Prospective of Emerging DNA and RNA Editing Tools for Environmental Challenges

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Abstract

In a span of a decade, CRISPR-based system has been exponentially exploited in a wide range of applications. The majority of its utilization comes from the therapeutic field, serves as powerful gene therapy tools for many untreated diseases, and accelerates the understanding of complex diseases. In account of the robustness and the discovery of Cas enzymes with their distinctive properties, now it is possible to widen CRISPR application to another study field, including in tackling environmental issues. In this review paper, the most recent development of CRISPR utilization in environmental-linked studies is discussed in four different aspects, which are (1) role of CRISPR in biofuel production; (2) role of CRISPR in agricultural science; (3) role of CRISPR in the ecological study; and (4) role of CRISPR in water contaminant detection. The studies show that CRISPR can serve as a powerful genomic editing and detector tool in environmental studies, and the rooms for development are still wide open to improving its versatility in this field.

Keywords: *CRISPR, microalga-based biofuel, food security, CRISPR-based detector.*

1. INTRODUCTION

Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) DNA sequences was reported for the first time by Dr. Nakata group at 1987 [1]. This DNA segment that comprises of DNA repeat separated by non-repeating sequences spacer was found in 40% of sequenced bacteria [2] and determined as evolutionary product of the protective immune system of bacteria against bacteriophage viruses [3]–[6]. When a virus attacks bacteria, a pair of nucleases called Cas1 and Cas2 cleave part of virus' genome and integrate it in bacterial chromosome as a spacer in the CRISPR array, allowing the bacteria to "remember" the virus and utilize it as a "reference" for upcoming attacks. When the similar virus attacks the bacteria, the CRISPR array then transcribed as short CRISPR RNAs (crRNA), then tandem with a nuclease called Cas9, bind complementarily to the virus' genome and cleave it, hinder virus' replication in bacteria's cell. Upon the elucidation of its mechanism, there were questions raised if the CRISPR/Cas9 system can serve as genomic editing tools by substituting crRNA to any desirable DNA target segment.

Encouraged by the finding of protospacer-adjacent motifs (PAMs) as a sequence in DNA target that is critical for CRISPR system [7], demonstrations of reprogrammable CRISPR as a genetic editing tool were

successfully proceeded in 2012 [8], [9]. in 2013, scientists have adapted the system in eukaryotic genome editing *in vivo* [10], [11], marking the beginning of exponential utilization of CRISPR/Cas as genome editing tool. The versatility of CRISPR/Cas9 then triggered scientist to discover and examine Cas enzyme family available in the nature. Cas12a (formerly Cpf1) was characterized in 2015 [12], provides alternative PAM sequence and sticky ends product of nuclease activity. In 2016, Cas13 (formerly C2c2) was discovered. Unlike Cas12a and Cas9, Cas13 target RNA instead of DNA as its substrate, allowing scientists to utilize CRISPR/Cas system in transcriptomic level [13], [14]. Cas12a and Cas13 are characterized by "collateral cleavage" in which after cleave its oligonucleotide target, Cas12a and Cas13 continue to be activated and cleave other ssDNA or RNA, respectively. These properties, which at the first place are thought to be a drawback in comparison with Cas9, are immediately exploited in diagnostic tools development.

CRISPR/Cas system has been used in wide range of application in therapeutics field. Potential of CRISPR/Cas system for numerous disease therapy has been examined, including but not limited to Duchenne Muscular Dystrophy (DMD) [15], β-thalassemia [16], and hemophilia [17]. CRISPR/Cas system has also credited for accelerating cancer research. CRISPR/Cas13 based diagnostic tool, dubbed SHERLOCK

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(Specific High-sensitivity Enzymatic Reported Unlocking)[18] has been developed as genome-specific diagnostic tools for various viruses, including Zika virus [19], Ebola virus [20], and Sars-Cov-2 [21]. Besides its wide application in therapeutic field, CRISPR system has also been adopted to answer challenge in environmental science. In this paper, various developments of CRISPR system in environmental science are discussed, with the intention of encouragement to expedite CRISPR system application in wider range of area study.

2. METHODS

This paper is a review of the recent development of CRISPR system application to tackle challenges in environmental science. The topics covered in this review comprises of (1) Development of CRISPR-based System to Enhance Biofuel Production; (2) Development of Adaptive Organism; (3) Development of RNA-Based CRISPR System in Ecological Study; and (4) Collateral Cleavage CRISPR System as a Portable Detector of Water Contaminant. In each section, a summary of the recent development and author's perspective are presented.

3. DISCUSSION

Development of CRISPR-based System to Enhance Biofuel Production.

Microalga-based biofuel, has emerged as a potential substitution for petroleum-based fuels. In order to maintain its economic feasibility, the microalga must be improved genetically in order to have an ability to produce biomass with a high-yield lipid content. The effort using CRISPR/Cas9 to specifically knockout 18 transcription factor known as negative regulator for lipid production in *Nannochloropsis gaditana* has been doubled lipid production [22].

The biggest drawback of Microalga-based biofuel is its low-yield biomass production. Many strategies have been applied to overcome this problem, including adjustment in the cultivation processes, that comprises modulating light intensity, CO_2 content, and nutrition [23], [24]. On the other hand, generating enhanced microalga through genetic modification by various method such as Transcription Activator-Like Effector Nucleases (TALEN), Zinc-Finger Nucleases (ZFN), and CRISPR/Cas9 is another approach to address this issue. CRISPR/Cas9 system is proven to be a robust method, judging by its ability to edit multiple genes expression simultaneously. However, there are challenges still remain in genetic modification pathway. For instance, there is a limitation in knowledge about which genes is correlated to lipid production and the underlying effect after being edited is still widely unknown. Thus, to the date, genetic modification is not a favorable approach in this issue. Nevertheless, once the elucidation of lipid production in microalga begin to unravel, genome editing may have an impact as significant as cultivation adjustment. CRISPR system can be employed for the study of this aspect too.

Biofuels are believed to play a vital role as substitution for fossil-based fuel in an effort to tackle global warming issue. The mass production of biofuels in Indonesia now is still dominated by plant-based biofuel, exploit plants like palm oil as biomass source [25]. This production scheme actually affects supply on human consumption food, threaten food security, and caused deforestation. Therefore, a new source of biomass is desirable in order to facilitate biofuel production. In this context, microalga-based biofuel has advantages, that its production would not disturb food security since it is not considered as edible food, and does not need an arable land for its cultivation. Moreover, the microalga can be rapidly grown [23], therefore the biomass production rate is faster compared with others plant-based biofuel. Studies of improvement in microorganism-based biofuel production to be more feasible in the future may one of the critical steps to support Indonesia's fuel consumption and its commitment to low-carbon economic growth.

Development of Adaptive Organisms.

The challenge that coming along with climate change is the sustainability of food chain. Climate change can cause extreme weather including flood and drought, thus reduce an arable land. Other issues that cause this problem become more complex is the growing human population that can cause imbalance of demand and supply in food production. Moreover, decreasing in crop species cultivated can result in vulnerability of crops towards diseases or pest outbreak [26]. Thus, there is a growing concern among the scientist to address this problem by engineering plants to be more resilient.

Application of CRISPR/Cas9 system to target SAPK2 gene in rice and ARGOS8 gene in maize has improved drought tolerance in the respective crops [27], [28]. CRISPR/Cas9 system also improved virus resistance in cucumber, fungal

resilience in tomato, and herbicide resistance in watermelon [29-31]. To overcome availability of arable land, CRISPR/Cas9 has also improved harvest yield by increasing grain weigh and amylose content in rice [32].

Indonesia has been grappling with food insecurity despite its solid economic growth in recent years. Moreover, Indonesia's geolocation that is vulnerable to climate change, added with urban sprawl in arable lands, the challenge to provide food for its citizen can be even greater in the future. CRISPR application in agricultural improvement, especially for grain plants might provide an approach to answer the challenge. However, there is a growing concern about the risk of consuming genetically modified organism. Despite its wide utilization in agricultural, the nature of CRISPR/Cas9 to induce double-stranded break (DSB), triggered two different repair pathways: homology-directed repair (HDR) and nonhomologous end-joining (NHEJ) [33]. NHEJ are often caused errors in CRISPR-based genome editing, thus scientists are struggle to generate method in lowering NHEJ and enhance HDR. Any off-targets in the genomic editing can cause catastrophic effect when it comes to the plants engineering. Therefore, CRISPR/Cas9 or any other genetic editing tools must be carefully managed in order to minimize the risk. Nevertheless, CRISPR/Cas9 has been proven as one of the multipurposed genomic editing tools to improve the quality of cultivated crops.

Development of RNA-Based CRISPR Systems in Ecological Studies.

The discovery of Cas13 enzyme has unlocked distinctive properties of CRISPR system. Cas13 targets RNA instead of DNA as their substrate and proceed collateral cleavage upon activated. This property then utilized as detector tools. Baerwald et. al has developed a detector of species identification in genomic-level, makes it an accurate detector compare with the prior arts. Cas13-based analysis conducted in San Francisco Estuary has been successfully distinguish three fish species that have similar morphology, which are *Hypomesus transpacificius*, *Sprirrinchus thaleichthys*, and *Hypomesus nipponensis*. The analysis can be conducted on one tube in twenty minutes, makes it exceptional tools since ecological studies are often performed outside laboratories [34].

Sensitive and rapid species detection is valuable for ecological studies, especially when the climate change can cause huge shift in ecological web. Proof-of-concept research from Baerwald *et. al* (2020) has been shown the application of CRISPR system beyond its conventional use as genetic editing tools, on account of the discovery of non-canonical property of Cas13 [34]. The utility of CRISPR/Cas13 in ecological study and environmental DNA samples is still underperformed. To the knowledge of author, recent development of CRISPR utilization in this aspect is still limited to the proof-of-principle research [35]. This might occur due to the complexity of the ecological study itself that often hundreds of species are involved in relatively narrow piece of spaces. Nevertheless, the study is successfully shows that CRISPR system can potentially be utilized to generate meaningful data in ecological studies.

Collateral Cleavage CRISPR system as a Detector of Water Contaminant.

The versatility of CRISPR/Cas12 and CRISPR/Cas-13 with its collateral cleavage property encourages its further development. By coupling CRISPR/Cas12 with sequences correspond to Allosteric Transcription Factor (aTF), Jung *et al*. have developed a kit to detect contaminants in municipal water. Allosteric Transcription Factors (aTF) are proteins that expressed in bacteria that involved in the transcription process [36]. Upon binding with its specific substrate, aTF regulates genes expression, by turning a specific gene expression "on" or "off" due to its conformational changes. Several aTFs respond to the metal in its environment as the substrate. For instance, Zn^{2+} ion is a substrate for SmtB, aTF from ArsR/Smtb family. Ion Zn^{2+} would bound to the SmtB and reduces SmtB binding affinity towards smtA gene, thus enhances the transcription of the gene. This resulting in Zn^{2+}-dependent gene expression, which harnessed by Lucks et.al in their biosensor kit. By coupling CRISPR/Cas12 with SmtA-specific gRNA, the activation of CRISPR/Cas12 nuclease would depend on the SmtA expression rate, hence the fluorescence intensity itself represents concentration of Zn^{2+} in the sample. Numbers of biosensors were developed using similar principle to respond to the other pollutants, including organic chemicals and other metals such as Zn^{2+}, Cu^{2+}, Pb^{2+}, and Cd^{2+}. The application is even taken further by detection of antibiotics, a potential pollutant emerges by the unregulated medical reagents dumping. The biosensors system, dubbed as

ROSALIND (RNA Output Sensors Activated by Ligand Induction) was then advanced to the design of cell-free kit, making it is possible to analyze water sample in suburban area where there is no adequate laboratory equipment [36]. CRISPR/Cas13 has also developed in similar manner to produce riboswitch-based biosensor. This system, dubbed as SPRINT (SHERLOCK-based Profiling *in vitro* transcription), has successfully proven to be a robust portable kit for fluoride ion detection in water samples [37].

The development of ROSALIND and SPRINT biosensors is a solid prove of the robustness of RNA-Based CRISPR system, CRISPR/Cas12 and CRISPR/Cas13. ROSALIND and SPRINT are developed by harnessing unique gene regulations, with ROSALIND utilizes transcription factor linked to protein, and SPRINT exploit riboswitch, a mRNA that can interact with small molecule. Considering that RNAs have many roles in gene regulation, the development of Collateral cleavage utilization-based CRISPR system is expected to be significantly increase in coming years. ROSALIND and SPRINT are also proven that RNA-based CRISPR system is capable to be performed in cell-free manner, which coherent with demand in environmental science where the analysis sometimes must be taken place immediately after sampling to prevent any damage in sample or measurement inaccuracy. By using a portable cell-free kit such as ROSALIND or SPRINT, a real-time, fast, and robust water quality inspection now is possible to be proceeded, emphasizing role of CRISPR in answering challenges in environmental sciences.

4. CONCLUSION

This paper has shown that although it might still have to be improved in many aspects, CRISPR system has a rather solid role in the environmental science. CRISPR system has developed rapidly in a relatively short time and the quest of other Cas enzymes opens almost unlimited probability in the future.

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