PERANCANGAN PLASMID *SINGLE GUIDE RNA* (SGRNA) AKTIVASI-CRISPR GEN ANHIDRASE KARBONAT *Chlorella sorokiniana* Shihira dan Krauss

SKRIPSI

diajukan untuk memenuhi sebagian syarat untuk memperoleh gelar Sarjana Sains
Program Studi Biologi

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PROGRAM STUDI BIOLOGI
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UNIVERSITAS PENDIDIKAN INDONESIA
BANDUNG
2024

PERANCANGAN PLASMID *SINGLE GUIDE RNA* (SGRNA) AKTIVASI-CRISPR GEN ANHIDRASE KARBONAT *Chlorella sorokiniana* Shihira dan Krauss

Oleh
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Sebuah skripsi yang diajukan untuk memenuhi salah satu syarat memperoleh gelar
Sarjana Sains pada Program Studi Biologi,
Fakultas Pendidikan Matematika dan Ilmu Pengetahuan Alam

Agustus 2024

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PERANCANGAN PLASMID *SINGLE GUIDE RNA* (SGRNA) AKTIVASI-CRISPR GEN ANHIDRASE KARBONAT *Chlorella sorokiniana* Shihira dan Krauss

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LEMBAR PERNYATAAN

Dengan ini, saya menyatakan bahwa skripsi yang berjudul "Perancangan Plasmid *Single Guide RNA* (sgRNA) Aktivasi-CRISPR Gen Anhidrase Karbonat *Chlorella sorokiniana* Shihira dan Krauss" beserta seluruh isinya adalah karya asli saya sendiri. Saya tidak melakukan penjiplakan atau pengutipan dengan cara yang tidak sesuai menurut etika ilmiah yang berlaku dalam masyarakat keilmuan. Saya siap menanggung sanksi jika di kemudian hari ditemukan adanya pelanggaran etika keilmuan atau klaim dari pihak lain mengenai keaslian karya saya ini.

Bandung, Agustus 2024
Yang membuat pernyataan,

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ABSTRAK

Peningkatan emisi karbon dioksida (CO_2) akibat pembakaran bahan bakar fosil telah menyebabkan perubahan iklim global dan memerlukan solusi untuk mengurangi CO_2 di atmosfer. Salah satu solusi berkelanjutan adalah penangkapan CO_2 menggunakan mikroalga, seperti *Chlorella* sp., yang dikenal memiliki kemampuan fotosintesis yang tinggi. Penelitian ini bertujuan untuk mendapatkan sekuens gen anhidrase karbonat yang tepat sesuai dengan spesies *Chlorella* yang digunakan agar dapat merancang *single guide RNA* (sgRNA) dan plasmid aktivasi-CRISPR untuk gen anhidrase karbonat yang berperan penting dalam mekanisme konsentrasi CO_2. Penelitian dilakukan melalui kultur *Chlorella* sp., isolasi DNA menggunakan metode CTAB, amplifikasi gen 18S rRNA dengan PCR, dan sekuensing untuk identifikasi spesies. Hasil penelitian menunjukkan bahwa *Chlorella* sp. memiliki pola pertumbuhan dengan empat fase, yaitu lag, eksponensial, stasioner, dan kematian. Identifikasi morfologi spesimen mengkonfirmasi bahwa mikroalga yang dikultur adalah bagian dari genus *Chlorella*. DNA mikroalga berhasil diisolasi dengan kualitas yang baik dan gen 18S rRNA berhasil diamplifikasi dengan produk amplifikasi yang utuh. Analisis filogenetik mengidentifikasi spesimen sebagai *Chlorella sorokiniana* dengan dukungan *bootstrap* 21%. Sekuens gen anhidrase karbonat dari *Chlorella sorokiniana* digunakan untuk merancang empat sgRNA dengan pertimbangan kandungan GC dan panjang optimal. Rancangan plasmid CRISPR, pHSE401, dimodifikasi secara *in silico* dengan menambahkan dCas9-VP64 (aktivator transkripsi) dan sgRNA untuk aktivasi gen anhidrase karbonat. Temuan ini memberikan pendekatan baru yang berpotensi meningkatkan efisiensi fiksasi CO_2 oleh mikroalga, meskipun uji coba baru dilakukan secara *in silico* dan belum diuji di laboratorium, tetapi memiliki potensi aplikasi dalam bidang bioteknologi untuk mendukung upaya mitigasi perubahan iklim di masa mendatang.

Kata Kunci: 18S rRNA, Anhidrase Karbonat, *Chlorella* sp., CRISPR, Fiksasi CO_2

ABSTRACT

Increased carbon dioxide (CO_2) emissions due to fossil fuel combustion have led to global climate change and require solutions to reduce CO_2 in the atmosphere. One sustainable solution is CO_2 capture using microalgae, such as Chlorella sp., which is known to have high photosynthetic ability. This study aims to obtain the right carbonic anhydrase gene sequence according to the Chlorella species used in order to design single guide RNA (sgRNA) and activation plasmid-CRISPR for carbonic anhydrase gene which plays an important role in CO_2 concentration mechanism. The research was conducted through Chlorella sp. culture, DNA isolation using the CTAB method, 18S rRNA gene amplification by PCR, and sequencing for species identification. The results showed that Chlorella sp. has a growth pattern with four phases, namely lag, exponential, stationary, and death. Morphological identification of the specimens confirmed that the cultured microalgae were part of the genus Chlorella. Microalgal DNA was successfully isolated with good quality and the 18S rRNA gene was successfully amplified with intact amplification products. Phylogenetic analysis identified the specimen as Chlorella sorokiniana with 21% bootstrap support. The carbonic anhydrase gene sequence of Chlorella sorokiniana was used to design four sgRNAs with consideration of GC content and optimal length. The CRISPR plasmid design, pHSE401, was modified in silico by adding dCas9-VP64 (transcriptional activator) and sgRNAs for activation of the carbonic anhydrase gene. These findings provide a new approach that has the potential to increase the efficiency of CO_2 fixation by microalgae, although the trials have only been conducted in silico and have not been tested in the laboratory, but have potential applications in the field of biotechnology to support climate change mitigation efforts in the future.

Keywords: *18S rRNA, Carbonic Anhydrase, Chlorella sp., CRISPR, CO_2 Fixation*

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