Penyisipan Gen Selulase pada Vektor Ekspresi *Yeast* pYY1 dengan ADH1 Promoter sebagai Pembentuk Kandidat Strain Penghasil Selulo-etanol

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INTISARI

Produksi etanol dari bahan berbasis selulosa pada dunia industri sering menhadapi kendala karena membutuhkan *pretreatment* yang cukup ekstensif dan juga dibutuhkan enzim selulase dengan jumlah cukup besar, sehingga diperlukan suatu metode lain yang lebih efektif dan efisien. Salah satunya dengan melakukan penyisipan gen selulase dari mikroorganisme selulolitik seperti *Aspergillus niger* yang memiliki enzim selulase yang dikode oleh gen *cbhA*. Gen pengkode enzim selulase ini disisipkan ke dalam host yang akan digunakan untuk menghasilkan kandidat strain penghasil selulo-etanol. Penelitian ini bertujuan untuk menyisipkan gen selulase dari *Aspergillus niger* ke dalam vektor ekspresi *yeast* dengan promoter ADH1 untuk membentuk kandidat strain penghasil selulo-etanol. RNA total diisolasi dari *Aspergillus niger*. RNA dirubah menjadi cDNA melalui teknik RT-PCR. Gen *cbhA* diamplifikasi dari cDNA yang telah ada. Gen *cbhA* dijadikan sebagai insert dan disisipkan pada vektor ekspresi *yeast* berupa pYY1. Fragmen DNA vektor dan insert dipotong menggunakan enzim restriksi *PstI* dan *SpeI*. Enzim T4 DNA ligase digunakan untuk meligasi vektor dan insert. Produk ligasi ditransformasi pada sel kompeten *Escherichia coli* DH10B dengan metode elektroporasi, hasil transformasi dikonfirmasi dengan *Colony PCR*. Plasmid rekombinan ditransformasi ke *Saccharomyces cerevisiae* INV*Sc*1. Dari penelitian diperoleh hasil yaitu amplifikasi gen *cbhA* telah berhasil dilakukan dengan menghasilkan pita DNA berukuran 1514 bp sesuai gen target. Gen *cbhA* berhasil disisipkan pada vektor pYY1 dan plasmid rekombinan berhasil ditransformasi secara *heat shock* ke *Saccharomyces cerevisiae* INV*Sc*1.

Kata kunci: RT-PCR, gen *cbhA*, pYY1, enzim restriksi, transformasi

Insertion of Cellulase Gene in Yeast Expression Vector pYY1 with ADH1 Promoter as Former Candidate for Cellulo-Ethanol Producing Strains

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ABSTRACT

The production of ethanol from cellulose-based materials in the industrial world often faces obstacles because it requires quite extensive pretreatment and large amounts of cellulase enzymes are also needed, so we need another method that is more effective and efficient. One of them is by inserting cellulase genes from cellulolytic microorganisms such as *Aspergillus niger* which possess cellulase enzymes encoded by the *cbhA* gene. This cellulase enzyme coding gene is inserted into the host which will be used to produce candidate strains producing cellulo-ethanol. This study aims to insert the cellulase gene from *Aspergillus niger* into a yeast expression vector with an ADH1 promoter to form candidate of cellulo-ethanol-producing strains. Total RNA was isolated from *Aspergillus niger*. RNA is converted to cDNA through the RT-PCR technique. The *cbhA* gene is amplified from existing cDNA, then used as an insert and inserted into yeast expression vector pYY1. Vector DNA fragments and inserts were cut using *PstI* and *SpeI* restriction enzymes. Ligation products were transformed into competent cells of *Escherichia coli* DH10B by electroporation method, the transformation results were confirmed by Colony PCR. Recombinant plasmids were transformed into *Saccharomyces cerevisiae* INV*Sc*1. The results obtained from the study are that the *cbhA* gene amplification has been successfully carried out by producing DNA bands with the size of 1514 bp according to the target gene. The *cbhA* gene was successfully inserted into pYY1 vector and the recombinant plasmid was successfully transformed by heat shock into *Saccharomyces cerevisiae* INV*Sc*1.

Keywords: RT-PCR, *cbhA* gene, pYY1, restriction enzyme, transformation