Transformasi, ekspresi, dan purifikasi protein betaglukosidase rekombinan dari Thermotoga neapoliatana dalam Pichia pastoris = Transformation, expression and purification recombinant protein of betaglukosidase from Thermotoga neapoliatana in Pichia pastoris.

Dian Fairuza, author

Abstrak

Lignoselulosa dapat dijadikan sebagai biomassa untuk menghasilkan produk bahan bakar. Hidrolisis biomassa lignoselulosa menggunakan enzim selulase. Selulase mengandung dari 3 komplek enzim yaitu, eksoglukanase, endoglukanase dan betaglukosidase Namun, betaglukosidase memiliki jumlah lebih sedikit daripada eksoglukanase dan endoglukanase. Semakin sedikit betaglukosidase dapat memicu proses hidrolisis selulosa terhambat, oleh karena itu pengembangan betaglukosida perlu dilakukan dengan diekpresikan ke dalam Pichia pastoris. Transformasi plasmid pLIPI-TnBgl1A dilakukan dengan metode elektroporasi, sedangkan ekspresi gen dan hasil purifikasi protein rekombinan dianalisis menggunakan SDS-PAGE dan Western blot. Gen betaglukosidase dari Thermotoga neapolitana berhasil ditransformasikan kedalam Pichia pastoris. Transforman yang telah diseleksi menghasilkan 2 koloni positif. Berat molekuler protein diperkirakan sekitar 53 kDa dan jumlah protein estimasi 1 mg/mL dan 1,4 mg/mL. Hasil analisis kemurnian protein rekombinan melalui SDS PAGE dan western blot memperlihatkan pita tepat di 53 kDa. Jumlah yield protein yang terpurifikasi didapatkan sekitar 21,4 % dan 24,1%. Hasil menunjukkan bahwa gen TnBgl1A telah berhasil ditransformasi dan terekspresikan dengan baik di Pichia pastoris dan protein rekombinan berhasil dipurifikasi dengan kemurnian yang cukup baik.

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Lignocellulose can be used as biomass to produce fuel products. Hydrolysis of lignocellulosic biomass using the cellulase enzyme. Cellulase contains 3 enzyme complexes, there are exoglucanase, endoglucanase and betaglucosidase. However, betaglukosidase has less amount than exoglucanase and endoglucanase. The less betaglucosidase can trigger the cellulose hydrolysis process is inhibited, therefore the development of betaglucoside needs to be done by expressing it into Pichia pastoris. Transformation of the pLIPI-TnBgl1A plasmid was performed by electroporation method, while gene expression and recombinant protein purification results were analyzed using SDS-PAGE and Western blot. The betaglucosidase gene from Thermotoga neapolitana was successfully transformed into Pichia pastoris. Transformants that have been selected produce 2 positive colonies. The molecular weight of protein is estimated to be around 53 kDa and the estimated protein amount is 1 mg/mL and 1.4 mg/mL. The results of the analysis of recombinant protein purity through SDS PAGE and western blot show the right band at 53 kDa. The amount of purified protein yield was around 21.4% and 24.1%. The results showed that the TnBgl1A gene was successfully transformed and well expressed in Pichia pastoris and the recombinant protein was purified with good purity.