KANYANUT ADULJIT: Isolation and Functional Characterization of Gene Encoding Xylanolytic Enzyme from Metagenomic Library of Termite Gut: THESIS ADVISOR: Dr. TATSAPORN TODHANAKASEM, THESIS CO_ADVISOR: Dr. THIDARAT NIMCHUA

Metagenomes of uncultured microorganisms represent an unlimited biodiversity of genetic materials for discovery of novel biocatalysts. In this study, isolation and characterization of gene encoding xylan-degrading enzyme from the already established metagenomic fosmid library of termite gut was completely explored. Briefly, determination of xylanase activity of the selected positive fosmid clone, namely Xyn14.3, on LB agar plate was carried out using functional-based approach at pH ranging from 7.0 to 12.0. The enzyme showed optimal activity at pH 9.0 as it showed the most intense blue-color zone around the colony. In order to identify the gene encoding this enzyme, subcloning and sequencing of Xyn14.3 was performed. Further analysis of the obtained sequence showed an ORF encoding protein of 273 amino acids which contained a conserved domain of glycoside hydrolase family 11 (GH11) with 56% identity to known xylanase in database. The retrieved gene was overexpressed in *E. coli* and the corresponding 30.14 kDa protein was subsequently purified using His-trap chromatography. Biochemical characterization of the recombinant xylanase displayed a wide range of pH activity with a maximum at pH 7.0 and the optimum temperature is 45 °C. The stability of enzyme was highly maintained in pH 7.0 at 40 °C for 2 hours. Enzyme pretreatment of agricultural wastes (e.g. bagasse, rice straw, and rice husk) was investigated using DNS method. The reducing sugar released from these lignocellulosic wastes was increased in a proportion of the amount of enzyme applied. From the overall results, this study provides evidences that metagenome analysis is considered to be powerful tool n order to explanation of novel genes with potential utilization in several industries.

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