Umam, M. Khotibul, 2019, Construction of Recombinant Plasmid Carrying *β-1,3-glucanase* gene (pPICZαA-*MKAFGlu1*) and Its Expression in *Pichia pastoris* KM71H. The script was under guidance of Prof. Dr. Afaf Baktir, M.S., Apt. and Ali Rohman, M.Si., Ph.D., Department of Chemistry, Faculty of Science and Technology, Airlangga University.

ABSTRACT

β-1,3-glucanase is an enzyme that plays an important role in the health field especially as an anti-biofilm. The MKAFGlu1 gene is a gene encoding β-1,3-glucanase, obtained from metagenomic exploration of digestive gland of *Achatina fulica*. This study aims to carry out the construction of the plasmid pPICZαA-*MKAFGlu1* and express the MKAFGlu1 enzyme in *Pichia pastoris* KM71H. The *MKAFGlu1* gene was isolated and amplified from the plasmid pTriplEx2-*MKAFGlu1* by using the PCR method. The recombinant plasmid pPICZαA-*MKAFGlu1* was propagated in *E. coli* TOP10 then transformed into *P. pastoris* KM71H. The transforman was expressed extracellularly by methanol induction every 24 hours until the 144th hour incubation. The enzyme was harvested out every 24 hours to test its expression level. The activity assay for β-1,3-glucanase was carried out by colorimetric method, reducing sugar test with DNS. Amplification of the *MKAFGlu1* gene with PCR produced a band with a size of 737 bp which was the size of the *MKAFGlu1* gene. The transformation of isolated plasmids from recombinant *E. coli* TOP10 pPICZαA-*MKAFGlu1* into *P. pastoris* KM71H resulted in three positive colonies carrying pPICZαA-*MKAFGlu1*. The β-1,3-glucanase activity continued to increase with the highest activity of 2.2 U/mL at 120 hours. Therefore, from this study the best harvest time for methanol-induced β-1,3-glucanase enzyme expression was at the 120th hour of incubation time, with an activity of 2.2 U / ml.

Keywords: Expression, recombinant β-1,3-glucanase, Pichia pastoris, methanol induction, MKAFGlu1 gene