# ABSTRACT

Leoni Handoyo, 24020220140077, **Gene Expression and Production Optimization of Xylanase GH Family 11 from *Kitasatospora* sp. in *Escherichia coli* BL21 (DE3)**. Under the guidance of Anto Budiharjo dan Nanik Rahmani

Xylanase is an enzyme capable of hydrolyzing xylan into xylooligosaccharides (XOS) and xylose. This enzyme has many applications in the pharmaceutical, food and feed, bakery, paper and pulp, textile, and bio-refinery industries. The use of enzymes in industry is required in high quantities and is able to survive in industrial processes so that potential microbes capable of producing xylanase are needed. Previous research on xylanase (GH-10 and GH-11) from *Kitasatospora* sp. ID06-480 strain expressed in *Streptomyces lividans* 1326 efficiently hydrolyzed xylan and produced XOS. *Escherichia coli* BL21 (DE3) is a good host cell in recombinant protein expression due to its ability to overexpress proteins. Research on the expression of heterologous xylanase GH11 derived from *Kitasatospora* sp. ID06-480 in *Escherichia coli* BL21 (DE3) bacteria needs to be done to determine its expression and activity. The purpose of this study was to determine the expression of the GH family 11 xylanase gene from *Kitasatospora* sp. in *Escherichia coli* BL21 (DE3). In addition, the optimum production media to increase the activity of recombinant GH family 11 xylanase enzyme was analyzed. The optimum temperature, IPTG concentration, and post induction time simultaneously during production were analyzed using Response Surface Methodology-Box-Behnken Design (RSM-BBD) to increase the activity of recombinant GH family 11 xylanase enzyme. Plasmid transformation with GH11 xylanase gene insertion into *Escherichia coli* BL21 (DE3) was carried out, followed by selection of the best colonies based on visualization of protein expression through SDS PAGE. Selected colonies were used for production media optimization (LB, TB, 2x YT, and SC 100%) and production optimization with RSM-BBD (temperature, IPTG concentration, and post induction time simultaneously) in increasing enzyme activity. The results showed that xylanase GH family 11 from *Kitasatospora* sp. was successfully expressed in *Escherichia coli* BL21 (DE3). TB media became the optimum media with the largest activity value of 27 Units/mL at the 12th hour of culture. Temperature, IPTG concentration, and post induction time were simultaneously optimum in increasing activity sequentially, namely 26,4˚C, 0,5 mM, 12 hours with an activity value of 84,4237 Units/mL.

*Keywords : Escherichia coli BL21 (DE3), Xylanase GH11, Heterologous expression, Production optimization, RSM-BBD*