## ABSTRACT

Laura Amelia Putri. 24020120140069. **Cloning, Expression, and Purification of Laccase Recombinant Protein from the Bacteria *Arthrobacter psychrolactophilus***. Thesis. Supervisor: Nurhayati and Fina Amreta Laksmi.

Laccase (benzenediol: *oxygen oxidoreductases*; EC 1.10.3.2) is an enzyme that has an application role in the textile field as bioremediation by decolorizing textile dyes that are difficult to degrade and toxic for the environment. Laccase can be produced from the psychrophilic extremophile bacterium *Arthrobacter psychrolactophilus* which is able to adapt to low temperatures. The production of laccase produced by bacteria is low, so recombinant technology is needed to produce laccase in larger quantities and facilitate the purification of laccase obtained after the cloning and expression of recombinant proteins. Cloning of the laccase enzyme coding gene with a size of 1.536 bp was carried out through *Escherichia coli* DH5-α host cells in the pET-28a(+) vector using the In-Fusion® HD Cloning Kit. Expression of recombinant laccase protein was carried out for folding to form a functional protein in *Escherichia coli* BL21 Star (DE3) with Isopropyl-β-D-thiogalactopyranoside (IPTG) induction at 14℃. The expression results were visualized by SDS-PAGE method which showed a recombinant laccase protein band measuring 54,547 kDa. Enzyme purification was carried out on 1 liter production through Immobilized Metal Affinity Chromatography (IMAC) with a 1 mL his-tag column that produced target protein elution peaks on the graph from fractions 8-16. Pure enzyme was indicated by the appearance of a single band after SDS-PAGE visualization. Total laccase protein was measured using Bicinchoninic Acid (BCA) Protein Assay with a value of 2.722 mg from 1.940 mg of purified sample A (fractions 12-15) and 0.782 mg of purified sample B (fractions 11 and 16).



**Keywords**: Laccase, *Arthrobacter psychrolactophilus*, Cloning, Expression, Purification