ABSTRACT

Wardah Kamilia Zulfa. 24020120130123. Cloning, Expression, and Purification of L-Asparaginase Recombinant from *Arthrobacter psychrolactophilus*. Under the guidance of Wijanarka and Fina Amreta Laksmi.

L-asparaginase, classified under EC 3.5.1.1, is an enzyme that catalyzes the hydrolysis of L-asparagine into L-aspartate and ammonia. This enzyme plays a role in the food industry by reducing the formation of acrylamide, a potential carcinogen. It is also widely used in the pharmaceutical industry for its antitumor properties, particularly in the treatment of acute lymphoblastic leukemia (ALL). Preliminary studies conducted using genome mining methods have found that the Asparaginase gene can also be found in the bacterial strain *Arthrobacter psychrolactophilus*. In this study, cloning was performed using the In-Fusion® HD Cloning Kit, followed by expression stages using E. coli BL21 Star (DE3) as the host and pET28a(+) as the expression vector with Isopropil-β-D-tiogalaktopiranosid (IPTG) induction in 18 ℃ incubation. Immobilized Metal Affinity Chromatography (IMAC) method is employed for purification. Based on PCR analysis, the target gene length aligns with in-silico analysis at 972 bp. The cloning procedure was successfully executed, as indicated by the presence of the pET28a(+).ApL-Asn fragment, which measures 1257 bp. Expression and purification results were confirmed using SDS-Page, revealing the enzyme's monomer molecular weight of 35.72 kDa. Furthermore, the purified protein total obtained in a single production (500 mL) was 336,26 mg for crude enzyme samples and 12,24 mg for IMAC-purified samples.

Keywords: Isopropil-β-D-tiogalaktopiranosid (IPTG), *Immobilized Metal Affinity Chromatography* (IMAC), *Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis* (SDS-Page), *Escherichia coli* BL21 Star (DE3), pET28a(+)