ABSTRACT

Keysha Jamala Putri. 24020120140176. **Preliminary CRISPR/Cas12a Study for Rapid and Ultrasensitive Detection of Pseudomonas aeruginosa Bacteria with phzA2 Virulence Gene Causing Food-Borne Disease**. Supervised by Nurhayati and Achmad Dinoto

Food-borne diseases can pose significant global health problem. Food-borne disease is one of the diseases caused by the consumption of food or beverages that have been contaminated by pathogenic microorganisms or substances. *Pseudomonas aeruginosa* is a Gram-negative potential food-borne disease agent that can contaminate food. An accurate detection method of *P. aeruginosa* presence are needed to identify and control the spread of the pathogen. The CRISPR/Cas12a system is one candidate for specific nucleic acid-based detection methods. The virulence gene *phzA2* plays an important role in the fenazine biosynthesis pathway, which is responsible for the interaction and co-infection ability of bacteria toward host tissues. The *phzA2* gene is relatively conservative compared to other pathogenic microorganisms, therefore it can be used as potential marker of sensitive and specific CRISPR/Cas12a-based detection in identifying *P. aeruginosa* contamination. The purpose of this research is to design and analyze crRNA & forward primer and reverse primer of phzA2 gene in silico, optimize the annealing temperature of phzA2 gene primer, test the specificity and sensitivity of *phzA2* primer, test the detection limit (LOD) of *phzA2* gene on spiked sample, and analyze the sequencing of *phzA2* gene. The stages of this research are the search for *phzA2* gene sequences, design and analysis of crRNA and *phzA2* gene primers, cultivation and recultivation of bacterial isolates, bacterial DNA extraction, dilution of *phzA2* primers, *phzA2* gene PCR, specificity and sensitivity tests, serial dilutions and TPC, preparation of spike samples, electrophoresis of PCR products, *phzA2* gene sequencing analysis. The crRNA design was carried out using Benchling and Primer3 software, then analyzed based on the requirements. The annealing temperature optimization results at 59°C showed the brightest and clearest band. Specificity test results showed that *phzA2* gene was only expressed in *P. aeruginosa* and the sensitivity limit of detection of *P. aeruginosa* with the *phzA2* gene was 10^6. The detection limit of *P. aeruginosa* spiked sample is at 10^6.

Keyword: Food-borne Disease, CRISPR/Cas12a, *Pseudomonas aeruginosa*, *phzA2*, Biosensor