I. INTRODUCTION

1.1. Background

Biofilms have become a major concern in public health because microorganisms associated with biofilms are less susceptible to antimicrobial treatments (Arampatzi *et al.*, 2011). In addition, biofilm treatments with antibiotic method cause a problem as prolonged use of antibiotics may lead to antibiotic resistance (Mulya & Waturangi, 2021). Biofilms are known to cause nosocomial or hospital-acquired infections, as well as chronic and infectious diseases (Miquel *et al.*, 2016). In fact, it has been reported that around 50% of hospital-acquired infections in immunodeficient patients are attributed to biofilms (Asma *et al.,* 2022). Moreover, as much as 80% of chronic infections globally are associated with biofilms and are caused by bacteria that are resistant to antibiotics (Meroni *et al.,* 2021). Therefore, the effective control of microbial biofilm is necessary to overcome various health problems.

This study used four model organisms, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Chromobacterium violaceum* to investigate biofilm inhibition. Urinary tract infections (UTIs) associated with biofilms are often caused by *E. coli*, and Uropathogenic *Escherichia coli* (UPEC) is the primary etiological agent of UTIs (Alshammari *et al*., 2023; Zagaglia *et al*., 2024). UPEC has the ability to develop biofilms on the surface of catheters, bladder walls, and within bladder epithelial cells. UPEC biofilms are hard to eliminate because they show weak response to conventional antimicrobial treatments and are more resistant to the host immune response. Moreover, it has been reported that UPEC biofilms can survive even after treatment with various antibiotics (Castillo *et al*., 2023). *P. aeruginosa* is known

for its ability to form biofilms, which play a crucial role in establishing persistent lung infection. This process involves a transition from planktonic to sessile forms, attachment to organic tissues or abiotic surfaces, and virulence factors such as alginate, elastase, LPS and rhamnolipids (Davis & Brown, 2016). *S. aureus* biofilm infections are very common in orthopedic infections, and it has been demonstrated *in vitro* that *S. aureus* biofilms may cause bone loss during chronic osteomyelitis by diminishing osteoblast viability and increasing bone resorption (Sanchez *et al*., 2013; Yu *et al*., 2020). Moreover, *C. violaceum* can become a pathogen and possesses the ability to form biofilm, which may increases its resistance to antibiotics and promotes the initiation of infections in the host (Alisjahbana *et al*., 2021; Dimitrova *et al*., 2024). These four model organisms are recognized as the causative agents of many infections.

Bacterial biofilms, complex microbial communities embedded in extracellular polymeric substances (Zhao *et al*., 2023), are formed through quorum sensing (QS), a bacterial signaling system that controls virulence factors, including biofilm formation. Quorum sensing increases bacterial survival, virulence, resistance to host immune systems, and antibiotic resistance (Tritripmongkol *et al.*, 2022). Disruption of quorum sensing allows for alleviating undesirable bacterial traits controlled by signaling, including virulence and biofilm formation (Sikdar & Elias, 2020). Interestingly, quorum sensing disruption has been known to decrease virulence factor secretion without causing bacterial death or suppressing bacterial growth (Dimitrova *et al.*, 2023). A previous research has shown that a bacterium produces anti-quorum sensing substances that exhibit biofilm inhibition activity. For example, it was found that *Delftia tsuruhatensis* containing 1,2-benzenedicarboxylic acid, diisooctyl ester exhibit

anti-quorum sensing properties and hinder the biofilm formation by *P. aeruginosa* (Singh *et al.,* 2017). Hence, using bacteria could be an effective approach to control biofilm formation.

Cell-free supernatant (CFS), a microbiological culture medium containing metabolites that exclude bacterial cells, has great potential and broad application prospects against biofilms as are currently studied as antimicrobial alternatives (Liu *et al.,* 2023). Prior studies have found that cell-free supernatant from *Pseudomonas* and other bacteria exhibit biofilm inhibitory activity. Specifically, it has been reported that *Pseudomonas aeruginosa* CFS contains extracellular compounds such as Hexadecanoic acid, methyl ester and Octadecanoic acid, methyl ester, which have inhibitory effects on biofilm formation in *Agrobacterium tumefaciens* (Al-Barhawee & Al-Rubyee, 2024). Moreover, A study revealed that CFS of *Bacillus thuringiensis* can inhibit *Staphylococcus aureus* biofilm development because it contains squalene, a compound with antibiofilm properties and 3-methoxycinnamic acid, which serves as quorum sensing inhibitor (Ray *et al.,* 2023). Therefore, cell-free supernatant may become an effective solution for controlling biofilms.

A previous study found *Pseudomonas sp.* (MB273N), a novel bacterial species isolated from the Maliau Basin Conservation Area (data not published). The antibiofilm activity of *Pseudomonas sp.* (MB273N) cell-free supernatant against biofilms formed by *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. violaceum* has not been evaluated. Therefore, this study aimed to investigate whether there is a potency of *Pseudomonas sp.* (MB273N) cell-free supernatant as an antibiofilm agent against *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. violaceum*, and to observe the characteristics of

these four microbial biofilms both before and after treated with CFS.

1.2. Formulation of The Problems

1.2.1. How is the potency of *Pseudomonas sp.* (MB273N) cell-free supernatant as antibiofilm agent against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Chromobacterium violaceum*?

1.2.2. What are the characteristics of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Chromobacterium violaceum* biofilms with and without the treatment of *Pseudomonas sp.* (MB273N) cell-free supernatant?

1.3. Objectives

1.3.1. To investigate the potency of the cell-free supernatant of *Pseudomonas sp.* (MB273N) as an antibiofilm agent against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Chromobacterium violaceum.*

1.3.2. To observe the characteristics of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Chromobacterium violaceum* biofilms with and without the treatment of *Pseudomonas sp.* (MB273N) cell-free supernatant.

1.4. Benefits of The Study

1.4.1. This study will inform the ability of cell-free supernatant of *Pseudomonas sp.* (MB273N) to control biofilms of *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Chromobacterium violaceum.*

1.4.2. This study will inform the characteristics of bacterial biofilms both untreated and treated with *Pseudomonas sp.* (MB273N) cell-free supernatant.