LITERATURE REVIEW: THE POTENTIAL OF BACTERIOPHAGES AS ANTIBIOFILM OF ESCHERICHIA COLI

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Abstract

Escherichia coli has the potential as a pathogenic bacterium and causes various diseases such as diarrhea, urinary tract infections, and meningitis. Other than that, *E. coli* also known to have the ability to form biofilm. Biofilm is an extracellular matrix secreted by bacteria that has function such as protecting bacteria from environmental stress, immune cells, and antibiotics. This causes problems for diseases related to *E. coli* infection because the structure of *E. coli* biofilm makes it difficult for antibiotics to penetrate so that bacteria become resistant to antibiotics. Therefore, an alternative to antibiotics is needed, and one of the prospective alternatives is bacteriophage. Bacteriophages are known to have characteristics as an antibiofilm since it is not affected by the ability of bacterial resistance, has the ability to produce enzymes that degrade biofilm in the bacteria and able to infect persister cells or bacterial cells that are dormant. Moreover, bacteriophages are also known as natural predators of bacteria. The purpose of this article is to explore and explain the potential of bacteriophages as antibiofilm of *Escherichia coli* bacteria.
Keywords: *Escherichia coli,* pathogen*,* antibiofilm.

1 INTRODUCTION

Biofilm is a group of microbial cells that live in a matrix of extracellular polymeric substance (EPS). EPS is a matrix secreted by microbes which consists of long chain of carbohydrates, DNA, and other biological macromolecules [1]. Biofilm help microbes attach to biotic or abiotic surfaces and have a role in increasing infection. More than 75% of diseases by microbial infections cause of biofilm formation, for example, bone infections, otitis media (inflammation of the middle ear), periodontitis (tooth infection), caries dental, lung infections, urinary tract infections, postoperative infections and bacteraemia, also nosocomial infections associated with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *K. pneumoniae*, and *E. coli* microbes [2][3].

Escherichia coli is a gram-negative bacterium that has the ability to secrete biofilm. Physiological properties of biofilm able to increase the tolerance of *E. coli* to environmental stress, immune system of the host, and even biocides (including antibiotics) [4]. Harper et al., [1] reported that bacteria in biofilm showed a higher resistance to antibiotics up to 1000 times when compared to bacteria that live freely or planktonic. Lebeaux et al.,[5] also added that bacterial cells that have formed a biofilm or sessile are more difficult to be treated by antibiotics due to the exopolysaccharide and extracellular DNA (eDNA) of the biofilm matrix make antibiotic molecules difficult to penetrate it. Therefore, an antibiofilm agent is needed to treat *E. coli* infection. One of the antibiofilm agents that have the potential are bacteriophage.

Bacteriophages are viruses that infect bacteria. There are two types of bacteriophages, lysogenic bacteriophages that infect bacteria by entering the bacterial genome and killing the bacteria; and lytic bacteriophages that can replicate within the host bacterial cell and produce new individual phage that can infect more bacteria. These lysogenic and lytic mechanism that helps bacteriophages infect bacteria in the biofilm. Apart from being natural predators, bacteriophages are also not affected by antibiotic resistance.

Bacteriophages are also known to produce enzymes that can degrade the extracellular matrix. In addition, bacteriophages can also infect persister cells, i.e. cells that are in the dormant phase. When the cell is in a dormant state, the phage will also be in a dormant phase. However, when the cell is metabolically active again, the phage also becomes active again [1]. In this scientific paper, we will discuss bacteriophage as an antibiofilm agent for *Escherichia coli*.

2 RESULT

2.1 *Escherichia Coli*

Escherichia coli (***Figure. 1***) is a gram-negative bacterium in the form of a bacillus with a length of 1-3 µm and a diameter of 0.5 µm. This bacterium was first isolated from the feces of small children in 1885 by a pediatrician named Theodor Escherich. *E. coli* itself is a microbiome flora in the digestive system of humans and mammals. *E. coli* can also be found in soil and water, which makes this bacterium as a bioindicator of water quality. Apart from being a microbiome flora, several strains of *E. coli* can also act as a pathogen that infects the digestive system and other body parts [6] [7].

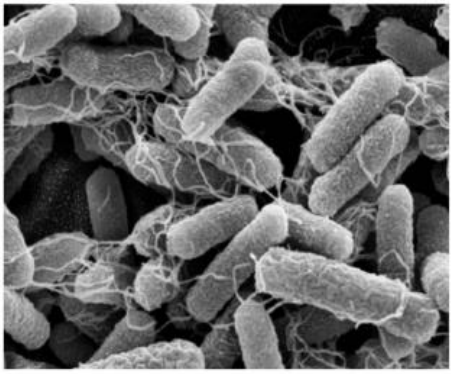

Figure 1. *Scanning Electron Micrograph of an E. coli. [8]*

Pathogenic *E. coli* are classified into two categories, intestinal *E. coli* (InPEC (Intestinal Pathogenic E. *coli*)) and extraintestinal *E. coli* (ExPEC (Extraintestinal Pathogenic E. *coli*)). *E. coli* InPEC consists of

E. coli that produce toxins associated with Crohn's disease, diarrhea, and bleeding. While *E. coli* ExPEC is associated with urinary tract infections, neonatal sepsis (blood infection in newborns), meningitis, mastitis, etc [9]. *E. coli* is also known to cause infections in medical devices such as prosthetic grafts and joints, shunts, and catheters [10]. Usually, *E. coli* infection is treated with antibiotic, but it is known that *E. coli* has the ability to be resistant to antibiotics which leads to problems to in treating infectious diseases caused by *E. coli*.

According to Poirel et al., [11], *E. coli* is a bacterium that can resist with many groups of antibiotics. Below is ***Table. 1*** that shows the resistance of *E. coli* to various types of antibiotics:

Table 1. *Antibiotic Resistance in Escherichia coli.*

No.	Antibiotic	References
1.	Ampicilin, amoxicillin, clavulanic acid, norfloxacin,cefuroxime, ceftriaxone, dan co-trimoxazole.	[12]
2.	Tetracycline, phenicol, sulphonamide, trimetoprim, and fosfomycin	[11]
3.	Ciprofloxacin, beta-lactam, quinolone, aminoglycoside, sulphonamide, dan fosfomycin.	[13]

E. coli is resistant to many antibiotics such as ampicillin, norfloxacin, tetracycline, ciprofloxacin, etc (***Table. 1***). Olorunmola et al., [14] reported, from 137 isolates of *E. coli* that cause urinary tract infection, some of them had resistance to the following antibiotics: ofloxacin (51.1%), ciprofloxacin (65.7%), nalidixic acid (67.2%), gentamicin (82.5%), tmp (85.4%), Augmentin (88.3%), norfloxacin (86.9%), erythromycin (93.4%), amoxicillin (94.2%), and tetracycline (96.4%).

The antibiotics resistance can be caused by extrinsic and intrinsic factors. Extrinsic factors are related to over-used and over-prescribed of antibiotics [15] [13]. Intrinsic factors include the structure of the outer membrane of *E. coli* as a gram-negative bacteria that is impermeable to molecules that enter the cell [13], horizontal gene transfer

mechanism i.e., the ability of bacteria to transfer plasmids containing antibiotic resistance genes to the strains or to the bacteria of other species [15] [11], and the ability of *E. coli* in biofilm formation [16].

2.2 *Biofilm*

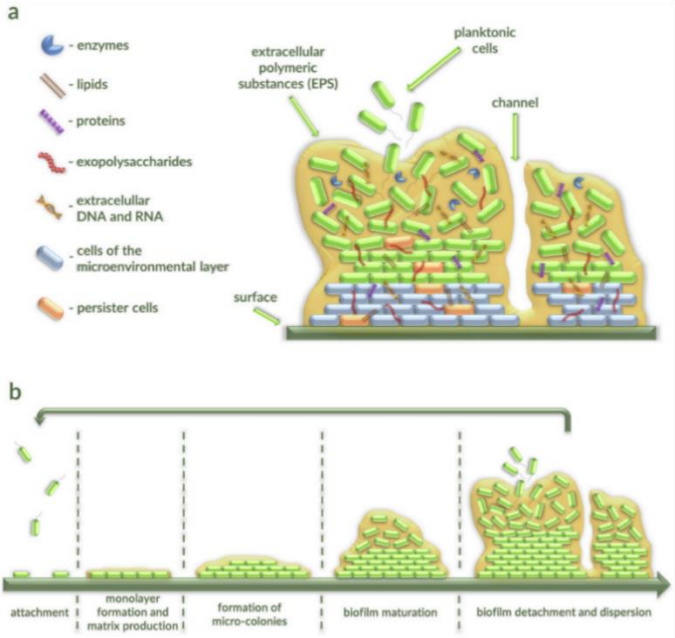

Figure 2. *Composition of biofilm (a) and biofilm formation (b)* [17].

Biofilm is a group of microbes that live in the matrix secretions of extracellular polymeric substance (EPS) or exopolysaccharides. EPS is a biopolymer which usually has size 10-30 nm and thickness around 0.2 – 1µm. 5-35% of the biofilm's volume is microbe and the rest is matrix. This matrix generally consists of ~1% DNA, ~1% RNA, 1-60% structural proteins, 1-40% lipids, enzymes, 40-95% extracellular polysaccharides, also 2-35% microbes which consist of bacterial cells on the surface of microecosystems that is initiate biofilm formation (cells of the microenvironmental layer) and dormant persister or bacterial cells (***Figure. 2a)*** [17]. The EPS matrix also contains

extracellular deoxyribonucleic acid (e-DNA) [18] [1] [19]. In biofilm, there are also channels where water, air, or nutrients needed by bacteria flow [17]. Biofilm formed through quorum sensing mechanism and transcription of different genes [19].

Quorum sensing is the regulation of gene expression in response to an increase in the bacterial population. Bacteria communicate through quorum sensing by secreting chemical molecules called autoinducers. As the bacterial population increases, the concentration of autoinducers also increase. Quorum sensing is used as a regulation of the physiological activities of bacteria, for example, symbiosis, virulence, motility, conjugation, as well as biofilm formation. Biofilm formation has several stages including, attachment, transition, formation and maturation, and the detachment or dispersion stage (***Figure. 2b***) [20].

The attachment stage begins when environmental signals induce bacterial planktonic cells. This signal can be a change in nutrient concentration, pH, temperature, oxygen, or osmolarity. Bacteria respond to these signals by attaching to surfaces. Attachment can be done by flagella, pili, or lipopolysaccharide. Hydrophobicity or the hydrophobic nature of a surface can also affect the attachment of bacteria. It is known that bacteria tend to form biofilm on hydrophobic surfaces. At this early stage, bacterial cells can still easily be separated from the surface or reversible [20] [21] [19].

The next stage is the transition where the reversible attachment turns into irreversible, thus the bacteria are not easily separated from the surface. At this stage the bacteria will divide and form microcolonies. Bacteria in the microcolonies can communicate with each other through the quorum sensing mechanism. Quorum sensing causes bacteria to secrete exopolysaccharides (EPS). EPS will be thickened so that bacterial motility will be reduced. Bacteria also lose their flagella, so that planktonic bacteria turn into sessile bacteria [19]–[21].

The next stage is formation and maturation. Bacterial cells continue to communicate through quorum sensing so that EPS will continue to be produced until it becomes a biofilm with a three-dimensional structure.

Bacteria also continue to divide until they reach maximum density. In this stage the formation of channels, pores, and other structures required by the biofilm occurs. This formation needed for facilitates the circulation of water and nutrients as excretory channels for microcolonies in the biofilm [19]–[21].

The last stage is dispersion stage. This stage is characterized by a degraded biofilm matrix. Some bacteria can secrete enzymes that help degrade the biofilm matrix, one of them is *E. coli* that secretes N-acetyl-heparosan lyase. After the matrix is degraded, the bacteria will secrete proteins related to the formation of flagella, thus restore the motility of the bacteria. This causes the sessile bacteria return to planktonic bacteria [19]–[21].

Biofilm provide advantages for bacteria such as increasing environmental stress tolerance, protection from physical stress, nutritional deficiencies, and enzymes retention [22]. Planktonic bacteria and sessile bacteria has difference advantages in terms of survivability. Lea et al., [22] reported, sessile bacteria in biofilm colonies has ability to upregulate genes related to environmental stress response. Moreover, sessile bacteria that live in biofilm have a higher infection rate. Other than that, bacteria within biofilm more resistant with antibiotic 10-1000 times higher than the planktonic bacteria [16], [23], [24]. The resistance of bacteria with antibiotics occurs due to several factors. The first factor is the structure of the polymer matrix which makes antibiotic molecules difficult to penetrate [25]. The matrix of biofilm acts as a barrier for molecules that diffuse into the biofilm hence difficult for antibiotic molecules to reach bacteria within the biofilm [26]. Biofilm can also inactivate antibiotic molecules that enter them through diffusion-reaction inhibition. The inhibitory reaction occur due to chelation mechanism or complex formations or degradation using enzymes reaction [16].

Furthermore, bacteria that live in biofilm are known to be in a stationary phase where bacteria do not reproduce i.e., bacteria experience a non-growth phase. Some bacteria in this phase also experience dormancy (persister) which means the bacteria reduce

their metabolic activity so that antibiotics become ineffective at killing bacteria [16], [25], [27]. According to Stewart [28], usually antibiotics works by killing bacteria that metabolically active. Metabolically active bacteria have the ability to synthesize macromolecules which are the target molecules of antibiotics in recognizing bacteria. In contrast, inactive or dormant bacteria will not produce macromolecules.

Another factor that causes biofilm resistance to antibiotics is horizontal gene transfer. The high population density in the biofilm allows bacteria to carry out symbiosis. The symbiosis that occurs between bacteria causes gene transfer. Bacteria that have plasmids with resistance genes can transfer these genes through a horizontal gene transfer mechanism[11], [25]. Horizontal gene transfer is more effective in bacteria that live in biofilm compared to planktonic bacteria. Krol et al., [29] reported, the gene transfer ability in biofilm is more effective 7-700 times. Besides, the structure of the biofilm increases the stability of the plasmid and the mobility of the transferred genes. The density of bacteria population in the biofilm increases the mobile genetic elements (MGEs). The structure of the biofilm matrix also maintains the quality of the conjugative pili [30]. This also showed in a study by Krol et al., [29] that said, the gene transfer mechanism happened if bacteria close with each other which occur if the density of the bacterial population is high. Living in biofilm causes bacteria to be close to each other, this facilitates the transfer of plasmids [29].

Based on the explanation above, it can be concluded that biofilm resistance to antibiotics occur due to various factors such as biofilm physical and physiological factors, and factors related to genetic material [25]. Therefore, biological agent to treat biofilm is needed and one that has potential is bacteriophage.

2.3 *Bacteriophage*

Bacteriophage or also called phage are virus that infect bacteria (***Figure. 3a***). The name bacteriophage comes from the word's bacteria and phage in which in Greek means bacteria-eater. As a natural predator of bacteria, phage can be found wherever bacteria exist with a ratio about 10 to 1. Approximately there are more than

1031 phages in the sea, freshwater, and on the land [31]; [32]. Like virus, bacteriophage also have genetic material in the form of DNA or RNA that wrapped by a capsid made of protein. This capsid is attached to a fibrous tail. The tail fiber will attach to receptors on the bacterial cell surface for identify the bacteria (***Figure. 3b***) [33] [34]).

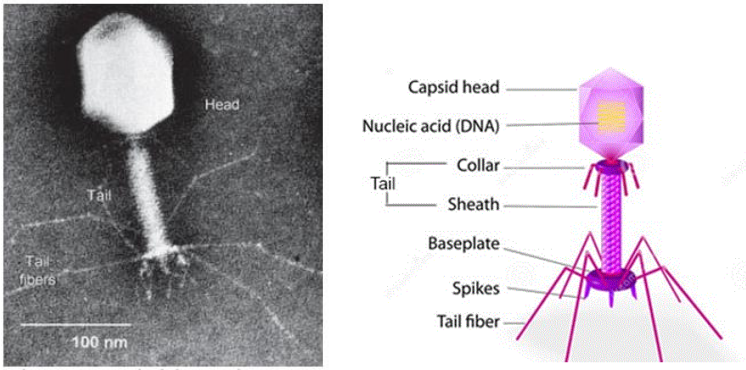

Figure 3. *Scanning electron micrograph of a bacteriophage (a) and general structure of a bacteriophage (b) [35] [36]*

When a phage attaches to a bacterial cell receptor, it will replicate by either lytic or lysogenic mechanisms. In the lytic mechanism, the phage will inject its genetic material into the bacterial cell. After the genetic material enters the cell, phage will use the host's ribosomes to replicate its body parts e.g., capsid proteins, tail fibres, and genetic material. The body parts then will arrange to become a phage. Then phage make the host cell secrete the enzyme lysozyme which destroys the bacterial cell wall. Bacterial cells will rupture and release approximately 200 phage particles. Meanwhile, in the lysogenic mechanism, the genetic material of the phage that injected into the bacterial cell will integrate into the bacterial chromosome to become a prophage. When a bacterial cell replicates, the prophage is passed on to daughter cells without killing the cell. Prophage can initiate lytic mechanisms if there is a change in environmental conditions [33], [37].

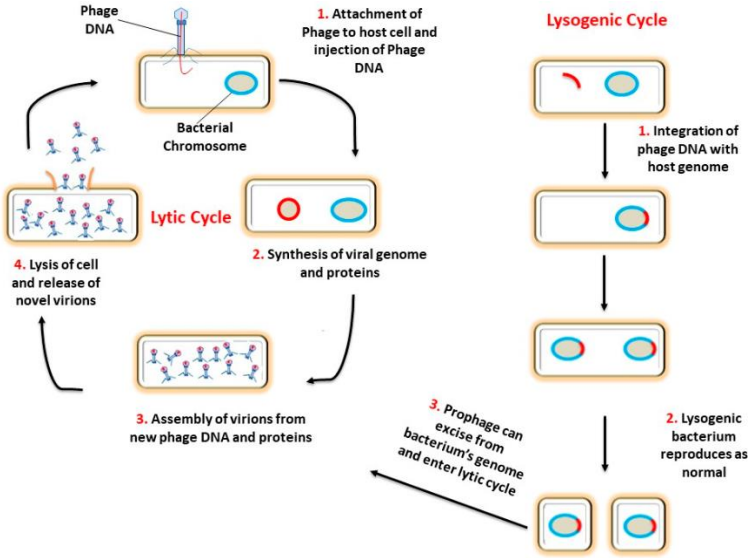

Figure 4. *Lytic and lysogenic mechanism.* [38]

Studies about phage as an alternative treatment have been carried out for a long time. Phage is considered to be the alternative for bacterial infection treatment because of several reasons such as phage is a natural predator of bacteria that is specific, do not affected by the ability of bacterial resistance, has ability to produce enzymes that lead to biofilm degradation and infect bacteria in it, and can infect persister cells or dormant bacteria [34][1], [39].

Phages are obligate intracellular parasites which need a host to replicate. Without bacteria as hosts, phages cannot reproduce or metabolize. Therefore, phages adapt to effectively infect bacteria [33], [40]. According to [41], phages have a co-evolutionary mechanism against bacteria that means when bacteria mutate and have mechanisms to defend from phages, the phages will also adapt to be able to infect bacteria. On the other hand, antibiotics doesn't have the ability to adapt with bacteria resistance. With this co-evolutionary ability, the phage certainly has the advantage to infect pathogenic bacteria.

The next characteristic of phage that is considered potential is phage usually specific. Phage are known to have a narrow host ratio i.e. phages can only infect one bacterial species or strain [33] [34]. Phage have a certain host ratio and this ratio depends on the presence of an antifage mechanism in a strain. It also depends on the generalization or specialization of the host to the phage receptor. The specific characteristics of phages make phages do not infect other bacteria i.e., microbiome flora. Phage also cannot infect other cell beside bacteria i.e., animal or plant cells. In contrast, antibiotics are broad-spectrum antibacterial agents which means antibiotic do not have a specific target, thus they can kill many bacteria of various species [39]. Therefore, antibiotic therapy not only killing pathogenic bacteria, but often infected microbiome flora also. This make the microbiome flora imbalance and causes the host to become more sensitive to infection [42], [43].

Another characteristic of phages that are considered to be potential as antibiofilm is the ability of phages to penetrate to EPS biofilm matrix which is impermeable to antibiotic molecules (***Figure. 5***). Biofilm can be physically destroyed or chemically degraded using enzymes. One of the enzymes that can be used is EPS depolymerase which is secreted by phages [39], [41], [40]. EPS Depolymerase showed ability to degrade polysaccharide bonds, exopolysaccharides, and O-polysaccharides of biofilm lipopolysaccharides, also peptidoglycan in the bacterial cell wall thus phages can infect bacteria within the biofilm [41]. However, not all bacteriophages can secrete depolymerase enzymes [1]. Even so, a study by Doolittle et al., [44] showed, bacteriophage *E. coli* T4 can still penetrate efficiently into the biofilm even though it does not have the ability to code gene that can secrete depolymerase.

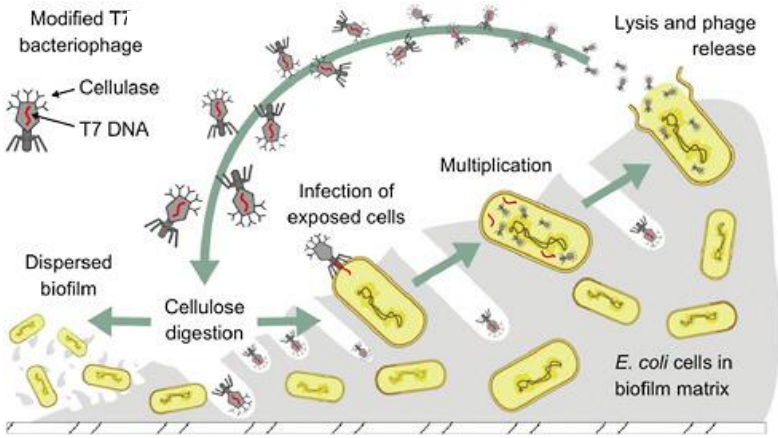

Figure 5. *Phage mechanism to infect bacteria within biofilm. (Collins, 2010)*

Furthermore, the characteristics of phages that are considered to be potential as an antibiofilm agents are phage can infect persister cells. As mentioned above, antibiotics are not effective against metabolically inactive bacteria or bacteria that are dormant or persister. On the other hand, phages have the ability to infect persister bacteria[1]. A study by Pearl et al.,[45] showed, persister bacteria are protected from phage lysogenic mechanisms where the phage genetic material cannot combine with the bacterial chromosome to form a prophage. However, persister bacteria cannot avoid the lytic mechanism of the phage. Quantitative analysis of gene expression showed that gene expression related to the lytic mechanism would be suppressed during bacterial dormancy. But when the bacteria are metabolically active again and reproduce, the phage will re-express the gene and continue the lysis mechanism [45].

Based on that, the bacteriophage can be alternative that has potential to be antibiofilm agent for *E. coli*. Bumunang et al., [46] in his study reported, the bacteriophage strain SA21RB could reduce the formation of biofilm *E. coli* strain O154:H10 and O113:H21 after 24 hours. In this study, the assay was carried out using the microplate phage virulence assay method where biofilm were formed on stainless

steel plates then incubated for 24 hours, 48 hours, and 72 hours. After incubation, the stainless steel plate was transferred to a petri dish containing 5 mL of phage suspension with a concentration of 1013 PFU/mL for 3 hours, then the result showed that 1013 PFU/mL phage suspension could reduce the biofilm by 2.5 and 2.1 log10 CFU/cm2 in the biofilm that incubated for 24 hours.

Another study by Gonzales-Gomez et al., [47] also showed that bacteriophage treatment PL-01, GB-02, and GB-03 could reduce *E. coli* biofilm MGA-EC-27, MGA-EC25, and MGA-EC21. In this study, screening was carried out on strains of *E. coli* bacteria that has ability to formed biofilm. Then the best strains were obtained, MGA-EC-27, MGA-EC25, and MGA-EC-21 which could form colonies in the biofilm up to 8 log10 CFU/ cm2 with an incubation time of 24-120 hours. Biofilm was formed on a stainless-steel plate and treated by adding phage with a concentration of 108 or 109 PFU/mL for 1 hour. After that, the population density in the biofilm was measured and the results showed that there was a reduction of 0.95 log10 CFU/mL-6.70 log10 CFU/mL and the best concentration was at phage 109 PFU/mL. Another study by Triana [48] also showed that the bacteriophage EC RTH 04 showed 50-150% antibiofilm activity against *E. coli* biofilm. Triana [48] tested phage activity against *E. coli* biofilm EC RTH4 using the microplate flat bottom 96 wells assay method. This method begins *E. coli* were cultured on a microplate and incubated thus biofilm can be formed, then the biofilm is treated by adding a phage suspension that has been diluted with serial dilution method until 108. The biofilm activity was tested by 3 methods, prevention, inhibition, and degradation. The result showed, the bacteriophage EC RTH 04 that had isolated from toilet water in the Cimande area, Kab. Bogor has activities to prevent, inhibit, and degrade biofilm where the highest activity is degradation activity. In the degradation activity with concentration of 104 reached 152.446%.

3 CONCLUSIONS

From the discussion above, it can be concluded that bacteriophages are considered to have potential as antibiofilm agents for *E. coli* because it has several advantages over antibiotics which are bacteriophage is natural predators of bacteria that is specific, do not affected by the ability of bacterial resistance, has ability to produce enzymes that degrade biofilm and infect bacteria in it and can infect persister or dormant bacteria. These are based on the studies by Bumunang et al., [46] which reported that bacteriophages can reduce the formation of *E. coli* biofilm by 2.5 and 2.1 log10 CFU/cm2; Gonzales-Gomez et al., [47] that reported, bacteriophage can reduce E. *coli* biofilm by 0.95 log10 CFU/mL - 6.70 log10 CFU/mL; and Triana [48] that showed, *E. coli* antibiofilm activity on prevention, inhibition, and degradation activities about 50-150%.

REFERENCES

[1] D. R. Harper *et al.*, "Bacteriophages and Biofilms," *Antibiotics*, vol. 3, no. 3. pp. 270–284, 2014. doi: 10.3390/antibiotics3030270.

[2] L. Wrzosek *et al.*, "Bacteroides thetaiotaomicron and Faecalibacterium prausnitziiinfluence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent," *BMC Biol.*, vol. 11, no. 1, p. 61, 2013, doi: 10.1186/1741-7007-11-61.

[3] S. Miquel, R. Lagrafeuille, B. Souweine, and C. Forestier, "Anti-biofilm activity as a health issue," *Front. Microbiol.*, vol. 7, no. APR, pp. 1–14, 2016, doi: 10.3389/fmicb.2016.00592.

[4] C. Beloin, A. Roux, and J.-M. Ghigo, "Escherichia coli Biofilms BT - Bacterial Biofilms," T. Romeo, Ed. Berlin, Heidelberg: Springer Berlin Heidelberg, 2008, pp. 249–289. doi: 10.1007/978-3-540-75418-3_12.

[5] L. David, G. Jean-Marc, and B. Christophe, "Biofilm-Related Infections: Bridging the Gap between Clinical Management and Fundamental Aspects of Recalcitrance toward Antibiotics," *Microbiol. Mol. Biol. Rev.*, vol. 78, no. 3, pp. 510–543, Sep. 2014, doi: 10.1128/MMBR.00013-14.

[6] R. A. Welch, "The Genus Escherichia BT - The Prokaryotes: A Handbook on the Biology of Bacteria Volume 6: Proteobacteria: Gamma Subclass," M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, Eds. New York, NY: Springer New York, 2006, pp. 60–71. doi: 10.1007/0-387-30746-X_3.

[7] A. C. L., B. Ludmila, van der W. P. W. J. J., and S. C. J., "Survival, Biofilm Formation, and Growth Potential of Environmental and Enteric Escherichia coli Strains in Drinking Water Microcosms," *Appl. Environ. Microbiol.*, vol. 82, no. 17, pp. 5320–5331, Sep. 2016, doi: 10.1128/AEM.01569-16.

[8] Z. D. Blount, "The unexhausted potential of E. coli," *Elife*, vol. 4, p. e05826, 2015, doi: 10.7554/eLife.05826.

[9] P. Vogeleer, Y. D. N. Tremblay, A. A. Mafu, M. Jacques, and J. Harel, "Life on the outside: Role of biofilms in environmental persistence of Shiga-toxin producing Escherichia coli," *Front. Microbiol.*, vol. 5, no. JULY, pp. 1–12, 2014, doi: 10.3389/fmicb.2014.00317.

[10] R. Andreas *et al.*, "Type 1 Fimbriae Contribute to Catheter-Associated Urinary Tract Infections Caused by Escherichia coli," *J. Bacteriol.*, vol. 196, no. 5, pp. 931–939, Mar. 2014, doi: 10.1128/JB.00985-13.

[11] P. Laurent *et al.*, "Antimicrobial Resistance in Escherichia coli," *Microbiol. Spectr.*, vol. 6, no. 4, p. 6.4.14, Jul. 2018, doi: 10.1128/microbiolspec.ARBA-0026-2017.

[12] G. Sharma *et al.*, "Escherichia coli biofilm: development and therapeutic strategies," *J. Appl. Microbiol.*, vol. 121, no. 2, pp. 309–319, 2016, doi: 10.1111/jam.13078.

[13] M. Galindo-Méndez, "Antimicrobial Resistance in Escherichia coli," L. Rodrigo, Ed. Rijeka: IntechOpen, 2020, p. Ch. 9. doi: 10.5772/intechopen.93115.

[14] F. O. Olorunmola, D. O. Kolawole, and A. Lamikanra, "Antibiotic resistance and virulence properties in escherichia coli strains from cases of urinary tract infections," *African J. Infect. Dis.*, vol. 7, no. 1, pp. 1–7, 2013, doi: 10.4314/ajid.v7i1.1.

[15] H. D. Kalter, R. H. Gilman, L. H. Moulton, A. R. Cullotta, L. Cabrera, and B. Velapatiño, "Risk Factors for Antibiotic-Resistant Escherichia coli Carriage in Young Children in Peru:

Community-Based Cross-Sectional Prevalence Study," *Am. Soc. Trop. Med. Hyg.*, vol. 82, no. 5, pp. 879–888, 2010, doi: 10.4269/ajtmh.2010.09-0143.

[16] D. Sharma, L. Misba, and A. U. Khan, "Antibiotics versus biofilm: an emerging battleground in microbial communities," *Antimicrob. Resist. Infect. Control*, vol. 8, no. 1, p. 76, 2019, doi: 10.1186/s13756-019-0533-3.

[17] G. Topka-Bielecka *et al.*, "Bacteriophage-Derived Depolymerases against Bacterial Biofilm," *Antibiotics*, vol. 10, no. 2. 2021. doi: 10.3390/antibiotics10020175.

[18] H. Flemming and B. Centre, "Biofilms," no. January 2002, 2016, doi: 10.1002/9780470015902.a0000342.pub2.

[19] M. Jamal *et al.*, "Bacterial biofilm and associated infections," *J. Chinese Med. Assoc.*, vol. 81, no. 1, 2018, [Online]. Available: https://journals.lww.com/jcma/Fulltext/2018/01000/Bacterial_biofilm_and_associated_infections.3.aspx

[20] R. Van Houdt and C. W. Michiels, "Role of bacterial cell surface structures in Escherichia coli biofilm formation," *Res. Microbiol.*, vol. 156, no. 5, pp. 626–633, 2005, doi: https://doi.org/10.1016/j.resmic.2005.02.005.

[21] M. S. Aparna and S. Yadav, "Biofilms: Microbes and disease," *Brazilian J. Infect. Dis.*, vol. 12, no. 6, pp. 526–530, 2008, doi: 10.1590/S1413-86702008000600016.

[22] B. Lea, S. Clarissa, G. Annelies, G. Anna, and L. Christophe, "Planktonic and Sessile Artificial Colonic Microbiota Harbor Distinct Composition and Reestablish Differently upon Frozen and Freeze-Dried Long-Term Storage," *mSystems*, vol. 5, no. 1, pp. e00521–19, Jan. 2020, doi: 10.1128/mSystems.00521-19.

[23] K. Myszka and K. Czaczyk, "Bacterial Biofilms on Food Contact Surfaces - a Review," *Polish J. Food Nutr. Sci.*, vol. 61, no. 3, pp. 173–180, 2011, doi: 10.2478/v10222-011-0018-4.

[24] K. K. Jefferson, "What drives bacteria to produce a biofilm?," *FEMS Microbiol. Lett.*, vol. 236, no. 2, pp. 163–173, Jul. 2004, doi: 10.1111/j.1574-6968.2004.tb09643.x.

[25] G. M. Abebe, "The Role of Bacterial Biofilm in Antibiotic Resistance and Food Contamination," *Int. J. Microbiol.*, vol.

2020, p. 1705814, 2020, doi: 10.1155/2020/1705814.

[26] E. Drenkard, "Antimicrobial resistance of Pseudomonas aeruginosa biofilms," *Microbes Infect.*, vol. 5, no. 13, pp. 1213–1219, 2003, doi: https://doi.org/10.1016/j.micinf.2003.08.009.

[27] W. T. K., K. S. J., and K. B. W., "Bacterial Persister Cell Formation and Dormancy," *Appl. Environ. Microbiol.*, vol. 79, no. 23, pp. 7116–7121, Dec. 2013, doi: 10.1128/AEM.02636-13.

[28] P. S. Stewart, "Mechanisms of antibiotic resistance in bacterial biofilms," *Int. J. Med. Microbiol.*, vol. 292, no. 2, pp. 107–113, 2002, doi: https://doi.org/10.1078/1438-4221-00196.

[29] J. E. Król *et al.*, "Invasion of E. coli biofilms by antibiotic resistance plasmids," *Plasmid*, vol. 70, no. 1, pp. 110–119, 2013, doi: https://doi.org/10.1016/j.plasmid.2013.03.003.

[30] J. S. Madsen, M. Burmølle, L. H. Hansen, and S. J. Sørensen, "The interconnection between biofilm formation and horizontal gene transfer," *FEMS Immunol. Med. Microbiol.*, vol. 65, no. 2, pp. 183–195, Jul. 2012, doi: 10.1111/j.1574-695X.2012.00960.x.

[31] M. R. J. Clokie, A. D. Millard, A. V Letarov, and S. Heaphy, "Phages in nature," *Bacteriophage*, vol. 1, no. 1, pp. 31–45, Jan. 2011, doi: 10.4161/bact.1.1.14942.

[32] H. LaFee, S., & Buschman, "Novel Phage Therapy Saves Patient with Multidrug-Resistant Bacterial Infection.," *UC San Diego Health*, 2017. https://health.ucsd.edu/news/releases/Pages/2017-04-25-novel-phage-therapy-saves-patient-with-multidrug-resistant-bacterial-infection.aspx

[33] L. G. Campbell, N. A., Reece, J. B., Mitchell, *Biologi*. Jakarta: Erlangga, 2002.

[34] I. U. Haq, W. N. Chaudhry, M. N. Akhtar, S. Andleeb, and I. Qadri, "Bacteriophages and their implications on future biotechnology: a review," *Virol. J.*, vol. 9, no. 1, p. 9, 2012, doi: 10.1186/1743-422X-9-9.

[35] N. Chemate, S. Z., Wahid, A. A., Inamdar, "Phage therapy: Emergence of a novel therapy to control baterial pathogens," *Inven. Rapid Pharm biotech &microbio*, vol. 2, pp. 1 – 7, 2015.

[36] N. Mansour, "Bacteriophages are natural gift, could we pay further attention!," *J. Food Microbiol.*, vol. 1, p. 22, Sep. 2017.

[37] L. D. Kasman, L. M., Porter, *Bacteriophages*. StatPearls Publishing, 2021.

[38] O. Stone, E., Campbell, K., Grant, I., & McAuliffe, "Understanding and Exploiting Phage-Host Interactions.," *Viruses*, vol. 11 (6), p. 567, 2019, doi: https://doi.org/10.3390/v11060567.

[39] E. Casey, D. Van Sinderen, and J. Mahony, "In Vitro Characteristics of Phages to Guide 'Real Life' Phage Therapy Suitability," *Viruses*, vol. 10, no. 4. 2018. doi: 10.3390/v10040163.

[40] S. T. Abedon, "Ecology of Anti-Biofilm Agents I: Antibiotics versus Bacteriophages," *Pharmaceuticals*, vol. 8, no. 3. pp. 525–558, 2015. doi: 10.3390/ph8030525.

[41] C. Ferriol-González and P. Domingo-Calap, "Phages for Biofilm Removal," *Antibiotics*, vol. 9, no. 5. 2020. doi: 10.3390/antibiotics9050268.

[42] F. Rafii, J. B. Sutherland, and C. E. Cerniglia, "Effects of treatment with antimicrobial agents on the human colonic microflora.," *Ther. Clin. Risk Manag.*, vol. 4, no. 6, pp. 1343–1358, Dec. 2008, doi: 10.2147/tcrm.s4328.

[43] J. Ramirez, F. Guarner, L. Bustos Fernandez, A. Maruy, V. L. Sdepanian, and H. Cohen, "Antibiotics as Major Disruptors of Gut Microbiota," *Front. Cell. Infect. Microbiol.*, vol. 10, no. November, pp. 1–10, 2020, doi: 10.3389/fcimb.2020.572912.

[44] D. E. Doolittle, M. M., Cooney, J. J., Caldwell, "No Title," *J. Ind. Microbiol,* vol. 16, pp. 331 – 341, 1996.

[45] S. Pearl, C. Gabay, R. Kishony, A. Oppenheim, and N. Q. Balaban, "Nongenetic Individuality in the Host–Phage Interaction," *PLOS Biol.*, vol. 6, no. 5, p. e120, May 2008, [Online]. Available: https://doi.org/10.1371/journal.pbio.0060120

[46] E. W. Bumunang, C. N. Ateba, K. Stanford, Y. D. Niu, Y. Wang, and T. A. McAllister, "Activity of Bacteriophage and Complex Tannins against Biofilm-Forming Shiga Toxin-Producing Escherichia coli from Canada and South Africa," *Antibiotics*, vol. 9, no. 5. 2020. doi: 10.3390/antibiotics9050257.

[47] J. P. González-Gómez *et al.*, "Efficacy of Novel Bacteriophages against Escherichia coli Biofilms on Stainless Steel," *Antibiotics*, vol. 10, no. 10. 2021. doi:

10.3390/antibiotics10101150.

[48] E. Triana, "AKTIVITAS ANTIBIOFILM BAKTERI Escherichia coli OLEH BAKTERIOFAG SECARA IN VITRO," *Ber. Biol.*, vol. 17, no. 1, p. 77, 2018, doi: 10.14203/beritabiologi.v17i1.3234.