Analysis of the Effectiveness of *Chrysomya sp.* Maggot Extract in Inhibiting the icaA and icaD Genes Regulator *Staphylococcus epidermidis* Biofilm

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Keywords: Biofilm, Polysaccharide Intercellular Adhesion, icaAD Gene, Maggot, PCR

Abstract: Maggots have proven to be one of the significant ingredients in degrading biofilms by destroying the polysaccharide intercellular adhesion (PIA) produced by the enzyme encoded by the icaADBC gene. This experimental study aims to prove the action target of maggot *Chrysomya sp.* extract on icaAD biofilm regulatory genes. Biofilm DNA extraction was carried out against bacterial cultures of *S. epidermidis* ATCC 35984 and *S. epidermidis* TCC 35983 incubated with *Chrysomya sp.* maggot extract, using Microtiter plate (MTP) method. The *Chrysomya sp.* maggot extract concentration was 0%, 20%, 40%, 60%, 80%, and 100% with an incubation period of 3 and 24 hours. PCR analyzed gene expression with the primer of icaAD genes. The qualitative test was carried out by 2% agarose gel electrophoresis. IcaAD genes of both *S. epidermidis* strains were detected in all treatments. The icaA band size of ± 980 bp and the icaD gene size of ± 380 bp can be observed either after the intervention of *Chrysomya sp.* maggot extract in various concentration (0%, 20%, 40%, 60%, 80%, and 100%) or after the incubation period of 3 and 24 hours. *Chrysomya sp.* maggot extract does not affect the icaAD biofilm regulatory genes of *Staphylococcus epidermidis*.

1 INTRODUCTION

The formation of biofilms is influenced by bacteria and external factors that produce extracellular polysaccharide adhesin, called polysaccharide intercellular adhesin (PIA) or polymeric N-acetyl-glucosamine (PNAG). PIA is also affected by the ica-operon regulatory (icaR) enzyme: an operon containing the icaADBC gene, a known regulator of biofilm formation in Staphylococcus (O'Gara, 2007).

S. aureus and *S. epidermidis* contain the intercellular adhesion operon (ica), which is responsible for the production of PIA. The icaA and icaD genes play the most important role among other ica genes in biofilm formation. The icaA gene encodes N-acetylglucosaminyltransferase, an enzyme involved in the synthesis of PIA. Furthermore, the icaD gene plays an essential role in N-acetylglucosaminyltransferase's maximal expression, leading to complete phenotypic expression of capsular polysaccharides (Nasr et al., 2012). Several studies have shown that the formation of biofilms by Staphylococcus in some invasive medical devices causing the nosocomial infection is associated with the presence of both icaA and icaD genes as essential virulence factors of these bacteria (Arciola et al., 2001; Ghasemian et al., 2015; Nasr et al., 2012).

Maggots (larvae) of green flies affect biofilms and their virulence factors (Anjarwati et al., 2017; Anjarwati and Hapsari, 2014; Bohova et al., 2014; van der Plas et al., 2007). Maggot extract has different effectiveness against different bacterial species. Insects, including flies, can produce antimicrobial peptides (AMP). AMP has a good effect on Gram-positive, Gram-negative, and fungal infections. The

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Tamalsir, D., Darmawan, A. and Anjarwati, D.
Analysis of the Effectiveness of Chrysomya sp. Maggot Extract in Inhibiting the icaA and icaD Genes Regulator Staphylococcus epidermidis Biofilm.
DOI: 10.5220/0010488801210125
In *Proceedings of the 1st Jenderal Soedirman International Medical Conference in conjunction with the 5th Annual Scientific Meeting (Temilnas) Consortium of Biomedical Science Indonesia (JIMC 2020)*, pages 121-125
ISBN: 978-989-758-499-2

antimicrobial activity by peptides on the defensin group affects the permeabilization of the target membrane. This influence is related to inhibition of RNA, DNA, protein synthesis, and reducing bacteria's viability. Therefore, the icaA and icaD genes' expression is expected to be inhibited (Parnés and Lagan, 2007).

Maggot extract can break down the biofilms of various bacteria(Cazander et al., 2009). The results obtained in previous studies concluded that the maggot extract of Chloroprocta sp. at different concentrations and incubation times had antibacterial activity against planktonic bacteria and *S. epidermidis* biofilms. The extract can break down the cell aggregation by destroying the PIA produced by the enzyme encoded by the icaADBC or Aap genes in the accumulation phase of biofilm formation (Anjarwati et al., 2017). This study aims to prove the action target of maggot *Chrysomya sp.* extract on icaAD biofilm regulatory genes.

2 METHODS

This research is a laboratory experiment with a factorial pattern and a completely randomized design. The aim was to analyze *Chrysomya sp.* maggot extract's ability to inhibit the icaA & icaD, a gene regulator for the biofilm *Staphylococcus epidermidis*. This study's bacterial strains were the strong biofilm producer *S. epidermidis* ATCC 35984 and the intermediate biofilm producer *S. epidermidis* ATCC 35983.

This study's number of maggots was 1 g/ml PBS, with one gram of maggots equivalent to ±20 maggots. In this study, the flies were the green flies *Chrysomya sp.* originating from the genus of Chrysomya, family Challiphoridae, Order Diptera, class Insecta, Arthropoda division, kingdom Animalia, which is a type of greenfly that is widely found in the Purwokerto area(Anjarwati et al., 2017; Hidayati et al., 2020).

The *Chrysomya sp.* maggot extract tested was at concentrations of 0%, 20%, 40%, 60%, 80%, and 100% with an incubation period of 3 hours and 24 hours Microtiter plate (MTP) method. Furthermore, DNA isolation was first carried out by extracting both *S. epidermidis* biofilm by destroying the bacterial cell walls (Quick DNA Fungal/Bacteria Mini-Prep Kit, Zymo Research Corp). After obtaining the DNA isolation sample, Polymerase Chain Reaction (PCR) optimization was carried out to get optimal PCR results. The qualitative test was carried out by 2% agarose gel electrophoresis (Mahardhika et al., 2020).

The primers (Invitrogen Custom DNA Oligos and design tools) used as follows: Forward icaA primer: 5'CCTAACTAACGAAAGGAG3 ', reverse icaA primer: 5'AAGATATAGCGATAAGTG3', forward primer icaD: 5'AAACGTAAGAGGTG3 ', primer reverse icaD: 5'AGCAATATGATCAAGATAC3', carried out with a denaturation step for 50 seconds at 94 °C, annealing primer at 49 ° C for both icaA and icaD genes and a polymerization step at 72 °C during 1 minute. The polymerization was concluded with an elongation period of 10 minutes at 72 °C. The amplified gene was then poured into 2% agarose gel to undergo the electrophoresis process (Mahardhika et al., 2020).

3 RESULTS

The results of the PCR examination of the icaA and icaD biofilm regulatory genes of the two S.epidermidis strains ATCC 35984 and ATCC 35983 have been given *Chrysomya sp.* maggot extract as the following figure.

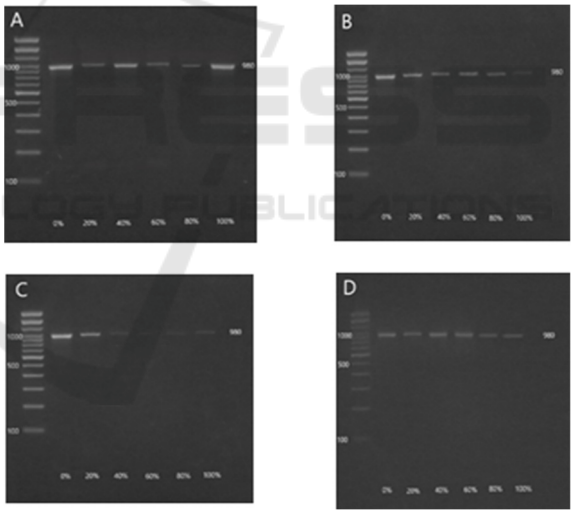

Figure 1: PCR examination results of the icaA gene on the ATCC35983 S.epidermidis and ATCC35984 S.epidermidis biofilm samples on maggot extract administration in different concentrations and incubation times, A. S.epidermidis ATCC35983, 3 hours; B. S.epidermidis ATCC35983, 24 hours; C. S.epidermidis ATCC35984, 3 hours; D. S.epidermidis ATCC35984, 24 hours. The icaA gene appears at 980 bp.

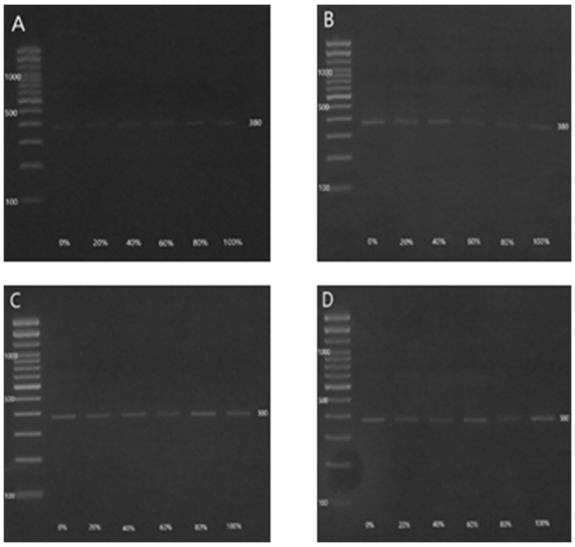

Figure 2: PCR examination results of icaD genes in the ATCC35983 S.epidermidis and ATCC35984 S.epidermidis biofilm samples on maggot extract administration in different concentrations and incubation times, A. S.epidermidis ATCC35983, 3 hours; B. S.epidermidis ATCC35983, 24 hours; C. S.epidermidis ATCC35984, 3 hours; D. S.epidermidis ATCC35984, 24 hours. The icaD gene appears at 380 bp.

4 DISCUSSION

The genetic and molecular basis for *S. epidermidis* biofilm formation is quite varied. The broadest theory in biofilm formation is the involvement of adhesive polysaccharide capsules (PSA) and adhesin intracellular polysaccharides (PIA) or polymeric N-acetyl-glucosamine (PNAG) (Rachmawati et al., 2020). *S. epidermidis* contains the intercellular adhesion operon (ica) responsible for the production of PIA. This operon contains the icaADBC gene and the icaR gene that regulates PIA production1. Both icaA and icaD are prominent supporters of the biofilm formation mechanism in the S.epidermidis (Zhou et al., 2013). Figures 1 and 2 described the icaA gene at 980 bp and the icaD gene at 380 bp in the ATCC35983 S.epidermidis and ATCC35984 S.epidermidis biofilm with/without maggot extract administration in different concentrations and incubation times.

Maggot extract has different effectiveness against different bacterial species. Several studies have shown that maggot extract can reduce bacterial biofilms from different bacterial strains through other mechanisms. Maggot extract is more effective in preventing biofilm formation and damaging mature biofilms in E. cloacae bacteria than S. aureus bacteria. The same study concluded that maggot extracts significantly affected cell viability in E. cloacae biofilms while it failed in S. aureus bacteria (Bohova et al., 2014). Other studies conducted on Pseudomonas performed that maggots have a low antimicrobial effect in inhibiting the formation of pseudomonas biofilms and not inhibiting the growth of these bacteria (Anders S. Andersen Dorthe Sandvang et al., 2010; Bexfield et al., 2004). The *Lucilia sericata* maggot extract showed no direct bactericidal or bacteriostatic activity against planktonic organisms of several different bacteria types in vitro. However, *L.sericata* influenced clinical observations of maggot therapy (Cazander et al., 2009).

Maggot extract can break down the biofilms of various bacteria (Cazander et al., 2009). In the present study, *Chrysomya sp.* maggot extract does not affect the icaA & icaD genes regulator biofilm *Staphylococcus epidermidis*. The possibility was maggot extract can only damage the biofilm structure by damaging PIA in the accumulation process of biofilms so that it does not directly affect the icaA & icaD genes in biofilm formation. This result is in line with the previous research, which concluded that the extract could break down cell aggregation by destroying the PIA produced by the enzyme encoded by the icaADBC or Aap genes in the accumulation phase of biofilm formation. The underlying mechanism is maggots' protease activity or glucosaminidase damaging the polysaccharide structure (PIA) of the biofilm (Anjarwati et al., 2017).

Downregulation of the icaA gene from the icaADBC operon can decrease PIA/PNAG production, leading to a reduction in biofilm formation. Interestingly, icaA appears to rise during the Mid-Logarithmic (ML) growth phase but decreases in the Stationary phase in RT-PCR when given CCG-2979, a low molecular weight compound derived from HTS(Ma et al., 2012). For the record, some genes also play an essential role in the virulence of Staphylococcus, for example, icaADBC, SigB, Agr, RNAIII, CodY genes. Changes in the profile of some genes can cause damage biofilm formation at different stages and lead to decreased virulence. The CodY gene is a gene that can suppress the Agr operon and icaADBC. CodY inhibition can have other effects on biofilm formation. Activating CodY can increase biofilm formation in the aureus strain SA564 but reduces biofilm formation in high biofilm-producing S30 isolates (Majerczyk et al., 2008; Tu Quoc et al., 2007), so further research to see the role of maggot extract on biofilm operon genes, in particular

regarding the role of other genes in the biofilm formation process, needs to be done.

The antimicrobial peptide (AMP), which can be produced by some insects, including greenfly larvae, has various mechanisms of action against pathogenic bacteria, such as permeabilization of cell membranes, identification of specific protein targets, inhibition of RNA and DNA. Bacteria can develop resistance to AMP. Components that can cause bacterial resistance to AMP include A. Secreted bacterial proteases, for example, lipopolysaccharide in the outer membrane of gram-negative bacteria, teichoic wall acid and lipoteichoic acid in gram-positive bacterial cell walls, B. Multidrug efflux pumps., And C. Extracellular biofilm matrix (Bechinger and Gorr, 2017). This study's weakness is that the extract used is a crude extract, so further research is needed to see the content of *Chrysomya sp.* Maggot extract and its mechanism of action on genes that play a role in biofilm formation.

5 CONCLUSION

Chrysomya sp. maggot extract could not inhibit the icaA & icaD gene regulator of ATCC35983 *S. epidermidis* and ATCC35984 *S. epidermidis* biofilm.

ACKNOWLEDGEMENTS

Many people supported this paper. Therefore, we are grateful to Ari Asnani, S.Si., M.Sc., Ph.D., Dr. Ir. Sri Rahayu., M.Si, and Dr. dr. Lantip Rudjito, M.Si. Med for the advice to make this paper better. The Funding was from Jenderal Soedirman University and Directorate General of Research and Development of the Ministry of Research, Technology, and Higher Education, Republic of Indonesia.

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