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The character of PA3235 virulence factors of *Pseudomonas aeruginosa* PAO1 – a preliminary study



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ABSTRACT

Introduction: Many virulence factors of *Pseudomonas aeruginosa* PAO1 are regulated by temperature and host conditions upon infection. Based on microarray data, the PA3235 gene is one of the upregulated genes during cell growth at 37 °C. Until now, no information about its role in PAO1 pathogenicity.

Methods: The PA01 Δ PA3235 strain was constructed by overlapping polymerase chain reaction (PCR) method and through biparental mating. The deletion was confirmed by PCR and restriction analyses. The virulence factors of both *P. aeruginosa* PA01 wild type and Δ PA3235 mutant strains were examined, which consisted of the amount of pyocyanin and pyoverdine, swarming, swimming, and twitching motility, biofilm formation, 3-oxo-dodecanoyl-homoserine lactone concentration, and growth curve profile. Data were analyzed using Student's t-tests to determine differences between treatments. P-value < 0.05 were considered significant.

Results: The ΔPA3235 strain was successfully constructed. At 37 °C, the mutant produced less pyocyanin (p-value 0.0004), pyoverdine (p-value 0.0009), and swarm area than the wild-type. The dendrites pattern of both strains was similar. The mutant and parental strains showed no differences in swimming and twitching motility when incubated at 22 °C and 37 °C. The mutant produced more biofilm compared to the wild-type strain (p-value 0.0013). The AHL was higher in the mutant than in wild type strain (p-value 0.0095) after 24 h incubation. Both the wild type and mutant strains exhibited similar growth patterns in LB broth. The mutant colonies also showed the same morphology as the wild type on the LB plate (not shown here).

Conclusion: The deletion of the PA3235 gene from the *Pseudomonas aeruginosa* genome caused some changes in virulence factors production, as the bacterium grew at body temperature 37°C. We predicted that the PA3235 gene might function to transport molecules involved in the early infection of this bacterium to humans.

Keywords: pathogenicity, pyocyanin, pyoverdine, swarming, swimming, twitching. **Cite This Article:** Wahjudi, M., Widodo, S.S., Artadana, I.B.M., Antonius, Y. 2023. The character of PA3235 virulence factors of *Pseudomonas aeruginosa* PA01 – a preliminary study. *Bali Medical Journal* 12(2): 1368-1376. DOI: 10.15562/bmj.v12i2.4364

> and adaptability properties, as well as a broad metabolic capacity.^{4,5} *P. aeruginosa* that colonizes patients persistently usually has unique regulatory gene features by producing manifold receptors for adherence to the target cells or by expression of adaptable genes which escort the cell growth in diverse habitats, including in blood.^{1,6-9}

> *P. aeruginosa* PAO1 and *P. aeruginosa* isolated from patients express the virulence genes at 37 °C.¹⁰ Some virulence factors, including those involved in quorum sensing, are temperature dependent.^{6,10,11} One of the genes regulated by temperature is the PA3235 gene. PA3235 is a locus tag on *P. aeruginosa* PAO1 genome. The PA3235 genes are predicted to encode a

hypothetical membrane protein, *yjcH*.^{12,13}

Information about the roles of the PA3235 gene in nature or the pathogenicity of P. aeruginosa cells is scarce. Currently, information about this gene is based on the similarities of the nucleotide and protein sequences to other known sequences, and from transcriptomic studies. Microarray data from Barbier et al. exhibited that this gene belongs to temperature and quorum-sensing-dependent genes.10 When the cells were cultured in the whole blood of human volunteers, the gene was expressed 16 times higher compared to cells grown in the common medium Luria Bertani Broth.⁸ All those studies indicate that the PA3235 gene might be involved in P. aeruginosa's pathogenicity against

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INTRODUCTION

Pseudomonas aeruginosa PAO1 is a Gramnegative bacterium that is commonly used as a model microorganism for studying biofilm, quorum sensing and pathogenesis. Although commonly found in soils and aquatic habitats, it can occupy a wide range of hosts, including humans, plants, and animals.¹⁻³ *P. aeruginosa* is a major human nosocomial pathogen, responsible for acute or chronic infections in immunecompromised patients, especially in cystic fibrosis patients.

The ability of *P. aeruginosa* to colonize and grow under a wide range of habitats relies on a few factors, such as its large genome size which provides complexity mammals or when the bacterium infects the human body.

To investigate the involvement of the gene in the pathogenicity of *P. aeruginosa* at 37 °C, we constructed a deleted PA3235 (Δ PA3235) mutant from the parental strain. The Δ PA3235 mutant was subjected to some assays to understand the roles of the gene. In this study, we proved that the PA3235 gene might be involved in some virulence factors level of *P. aeruginosa* during cell growth at 37 °C, the human body temperature.

MATERIALS AND METHODS

Bacterial strains and growth

Bacterial strains and plasmids used in this study are listed in Table 1. Otherwise mention else, cells were grown in Luria Bertani (LB) broth or LB agar (Merck). Gentamicin (Sigma) was added to the medium at a final concentration of 10 mg.mL⁻¹ for Escherichia coli or 30 mg.mL⁻¹ for *P. aeruginosa* cultures.

Construction of PAO1 DPA3235 strain

The upstream region of PA3235 was first amplified using ForA and RevA primers, whereas the downstream region was amplified using ForB and RevB primers pair (Table 2). Both amplicons were purified and ligated using T4 DNA Ligase (Promega), forming a flanking fusion

region without the PA3235 gene. The fusion fragment was further amplified using RevA and ForB primers pair and digested using SacI and EcoRI (Thermo Scientific). The purified fragment was then cloned into pEX18Gm plasmid and transformed into E. coli S17-1λpir cell.^{14,15} The pEX- Δ PA3235 recombinant plasmid was transferred into P. aeruginosa through biparental mating. Overnight culture of P. aeruginosa PAO1 (diluted 10-fold) was incubated at 42 °C prior to mating. The mixture of donor and recipient cells (3:1) was incubated on an LB agar plate for 16 h at 30 °C. The selection of transconjugants was done on an LB plate containing 60 mg of L⁻¹ gentamicin. After the allelic exchange mechanism, transconjugants were counter-selected in an LB agar plate supplemented with 10 % sucrose. The deletion was confirmed by PCR using RevA and ForB primers pair and by restriction analyses.

Pyocyanin production

Pyocyanin was extracted using the method described by Essar et al.¹⁶ Overnight culture was first adjusted to OD_{600} value of 1.0 in LB broth and diluted 100-fold in 25 mL LB. The culture was then incubated at 37 °C or 22 °C for 24 h with 200 rpm shaking. The pyocyanin was extracted from 5 mL supernatant of the culture using 3 mL chloroform. Two milliliters of

 Table 1.
 Bacterial strains and plasmids used in the study.

Strains	Relevant characteristic	Source or reference
P. aeruginosa PAO1	Wild-type	Holloway collection
P. aeruginosa PAO1∆PA3235	Derivative of PAO1, PA3235 deleted	This study
E. coli S17-1 λpir	Δara-leu7697 ΔlacX74 ΔphoAPvuII phoR araD139 ahpC galE galK rpsL F'[lac ⁺ (lacI ^q) pro] gor522::Tn10 (Tc ^R) trxB::kan	15
<i>E. coli</i> JM109 pSB1075	Biosensor for sensing 3O-C12-HSL	20
Plasmid		
pEX18Gm	Gm^R , $oriT^+$ sac B^+ , vector for gene replacement	14
рЕХ- <i>Дра3235</i>	pEX18Gm holding flanking region of PA3235 gene	This study

the organic layer were transferred to a new tube and extracted again using 1 mL 0.2 M HCl. The re-extracted pyocyanin was measured at 520 nm. The concentration of pyocyanin (μ g.mL⁻¹) was determined as (OD₅₂₀ x 17.072/OD₆₀₀). The assay was done in triplicates.

Pyoverdine concentration measurement

Overnight culture of wild-type and Δ PA3235 mutant strain was adjusted to OD₆₀₀ of 1.0 and diluted 100-fold in 25 mL LB. After 24 h incubation at 37 °C or 22 °C, cell-free supernatant containing pyoverdine was measured at 405 nm.

Motility Assay

Swarming, swimming, and twitching motility assays were evaluated for both wild-type and $\triangle PA3235$ mutant strains to see whether the deletion affects bacterial motility. A swarming medium was made by mixing 8 g.L⁻¹ Nutrient Broth (Merck) and 0.5 % agar. The pH of the medium was adjusted to 7.5. The medium was supplemented with 5 g.L⁻¹ filter-sterilized glucose solution added separately after autoclaving. As much as 2 µL culture (OD₆₀₀ of 0.5) was inoculated on the surface of the medium and let dry for several minutes. The plates were incubated upside-down for 24 h.¹⁷ Swimming medium was made of 10 g.L-1 tryptone and 5 g.L-1 NaCl, mixed with 0.3 % agar. Both strains were inoculated as 1 μ L culture (OD₆₀₀ of 0.5) in LB broth on the surface of the medium. The halo diameter was measured after 24 h incubation at 22 °C and 37 °C.¹⁸ Twitching motility was done using LB medium mixed with 1 % agar. The overnight culture of both strains in LB broth was adjusted to OD₆₀₀ 0.5 and inoculated by stabbing into the LB agar. After 24 h incubation at 22 °C and 37 °C, the medium was stained using Coomassie Brilliant Blue (CBB) R-250 and the diameter of the stained area was recorded.

Table 2.Primers used in this study.

Primers Name	Sequence (5'→3')	Position (NC_002516)	Target
ForA	AATACTGCAGCCTCAAGGAGGCGCAGCAATGAT	3623178-3623156	Upstream of
RevA EcoRI	CCTA <u>GAATTC</u> GACATGGTAGTTGAGGCCGAACAG	3622608-3622632	PA3235 region
ForB SacI	TGCC <u>GAGCTC</u> CTGGATTCCGCACTGGATGTTC	3623962-3623941	Downstream of
RevB	CGGCAAGCTTCGCTGGTAGATGCTGTCGTTCA	3623446-3623467	PA3235 region

Crystal violet biofilm assay

Biofilm formation was measured using crystal violet staining as described by Merritt et al. (2005) with modifications.¹⁹ Overnight culture of both wild-type and $\Delta PA3235$ mutant was adjusted to OD_{600} of 0.001 in LB broth. The culture was then inoculated in 96-well polystyrene microtiter as much as 100 µL per well and incubated at 22 °C and 37 °C for 24 h. Biofilm was washed using NaCl 0.9 % three times and fixated with methanol. Staining was done using 1 % crystal violet solution for 30 minutes. The rest of the crystal violet was washed using 0.9 % NaCl solution. Stained biofilm was diluted in 100 uL of 33 % acetic acid and the absorbance was measured at 595 nm.

Growth curve profiling

Cells were refreshed overnight on LB broth at 37 °C. The overnight culture was diluted and used as inoculum to a final OD_{600} of 0.01 in LB broth. The cell suspensions were taken every hour and the cell density was measured at OD_{600} .

Acyl-homoserine lacton (AHL) measurement

The production of 3-oxo-dodecanoyllactone (3O-C12-HSL), homoserine one of the AHLs, was evaluated using E. coli harbouring pSB1075 plasmid as a biosensor.²⁰ Overnight culture of wild-type and ΔPA3235 mutant strain was adjusted to OD600 of 1.0 and diluted 100-fold in 10 mL LB. After 5 h and 24 h incubation at 37 °C, cell-free supernatant was acidified using 1 M HCl (9:1). AHL was extracted from 1 mL acidified supernatant using two times 250 µL of chloroform. The organic phase was moved to a fresh tube and allowed to evaporate. The AHL was then diluted in 100 µL methanol. As much as 2 µL AHL solution was added to a 96-well microtiter plate. Then, two hundred microliters of the 10-fold diluted overnight biosensor were then added. Bioluminescence was measured using a microplate reader (Omega Star).²¹

Data analysis

Data were analyzed using Student's t-tests to determine differences between treatments. P-value < 0.05 were considered significant.



Figure 1. Production of pyocyanin by ΔPA3235 mutant (grey bar) and parental strain (black bar).



Figure 2. Pyoverdine production by Δ PA3235 mutant (grey bar) and parental strain (black bar).

RESULTS

To understand the gene's involvement in the virulence factor production, several assays such as pyocyanin and pyoverdine production, swarming, swimming, twitching motilities, and biofilm formation were performed. Deletion of the PA3235 gene from the PAO1 genome is a good way to study the role of PA3235 in the pathogenicity of this bacterium. In this study, an incubation temperature of 22 °C was used to observe the phenotypes of P. aeruginosa PAO1 at room temperature, while 37 °C represents the human body temperature, as stated by Barbier.¹⁰

Pyocyanin production

The result showed that both wild-type and mutant strains demonstrate no statistical differences in pyocyanin production at 22 °C. A significant difference was observed in 37 °C cultures, where the mutant produced less pyocyanin (p-value 0.0004) (Figure 1).

Pyoverdine concentration measurement

Pyoverdine is an important protein for the iron acquisition of *P. aeruginosa* during infection in the hosts, such as the human body. In comparison to the wild-type strain, the pyoverdine production was



Figure 3. Swarming motility of \triangle PA3235 mutant and parental strain.

lower in the Δ PA3235 mutant when it was grown at 37 °C (p-value 0.0009). No significant difference was observed in the 22 °C cultures (Figure 2).

Effects of PA3235 deletion on *P. aeruginosa* PAO1 motility

Three types of motilities were observed in this study: swarming, swimming, and twitching motilities. Incubation at 22 °C did not promote any swarming motility for both strains. The swarm area of Δ PA3235 mutant was narrower compared to PAO1 when incubated at 37 °C for 24 h (Figure 3). The dendrites pattern of both strains was similar. Both the mutant and parental strains showed no differences in swimming and twitching motility when incubated at 22 °C and 37 °C (Figure 4).

Deletion of PA3235 increased biofilm formation

Biofilm formation of *P. aeruginosa* was determined as the A_{590} of de-stained crystal violet. Interestingly, Δ PA3235 mutant produced more biofilm on the microtiter wall, compared to the wild-type strain (p-value 0.0013). A lower quantity of biofilm was observed after 24 h incubation at 22 °C in both strains (Figure 5).

AHL level is altered at 37 °C in the absence of PA3235

Virulence determinants are known to be regulated by signal molecules. To confirm whether the differences in virulence factor production were influenced by AHL production from the strains at 37 °C, 3-Oxo-C12-HSL was quantified using a biosensor system. The result demonstrated no differences in 3-O-C12-HSL quantity between mutant and wild-type strains after 5 h incubation. However, after 24 h incubation, the AHL was higher in the mutant than in wild type strain (p-value 0.0095) (Figure 6). This may indicate that the reduced or increased virulence factors were not due to the increased level of AHL in $\triangle PA3235$ strain.

The mutation did not change the growth profile of *P. aeruginosa*

To investigate whether the mutation on PA3235 gene affects cell growth, the cells were grown in LB broth at 37 °C with 250 rpm shaking. Both the parental and mutant strains exhibited similar growth patterns in LB broth (Figure 7). The mutant colonies also showed the same morphology as the wild type on the LB plate (not shown here).

DISCUSSIONS

Many genes of P. aeruginosa cells are upregulated or downregulated when the cell is grown at the human body temperature, 37 °C.⁶ Since all these factors are modulated by the quorum sensing system, then signal molecule expression is also important for the pathogenicity of this bacterium. Many of the quorum sensing and virulence determinants genes are modulated by the lasI-rhlR quorum sending system and other regulons.^{22,23} Based on microarray data, the *lasI* gene, one of the signal-molecules synthesizing genes, was upregulated in PAO1 strain during cell growth at human body temperature.¹⁰ The *lasI* gene from rhizospheres isolates also increases at the temperature.²⁴ The PA3235 transcript was increased during cell growth at human body temperature.^{6,10,11}

Although many genes are influenced by temperature, however the result showed that there were differences in phenotypes between wild type and the mutant. The $\Delta PA3235$ mutant showed an increase in biofilm (Figure 5) and the level of 3O-C12-HSL (Figure 6) compared to the PAO1 wild type. The twitching and swimming motility was unchanged (Figure 4), whilst pyocyanin, pyoverdine, and swarming motility were less in this mutant than in the wild type (Figures 1, 2, and 3). These differences were not caused by differences in the growth phases since the wild type and mutant exhibited the same growth patterns in LB broth at 37 °C (Figure 7).

Pyocyanin is partly modulated by the lasI-rhlR quorum sensing system,25 and mostly by pseudomonas quinolone signal (pqs) signal and a complex circuit.²⁶ The presence of iron also influences pyocyanin production levels.⁶ Several enzymes are involved in the biosynthesis and secretion of this phenazine, such as those encoded by phzA-G, phzM and phzS genes.²⁷ Pyocyanin is produced at a higher level at 37 °C by PAO1 and Pseudomonas sp, isolated from the rhizosphere.²⁸ As the PAO1 grows at 37 °C, the phzA-G, phzM and *phzS* biosynthesis genes and type 3 secretion systems are upregulated.6 Conversely, Barbier's microarray data revealed that not all of those pyocyanin transcripts were upregulated, only phzS





was increased and *phzH* was decreased.¹⁰ It is likely that PA3235 is important for pyocyanin production. Although the 3-OC12-HSL level of the mutant was higher than the wild type, however pyocyanin concentration was lower in Δ PA3235 mutant than in wild-type strains at 37 °C (Figure 1). By looking at the prediction that PA3235 protein is a membrane protein, it may be true that this protein is involved in the exports of molecules crucial for phenazine metabolism.

Like pyocyanin, pyoverdine was also produced in a lower quantity in Δ PA3235 than in the wild-type strains (Figure 2). Pyoverdine is expressed under iron-limiting conditions,²⁹ not by signal molecules,²⁵ nor body temperature.¹⁰ It is interesting to note that the deletion of PA3235 caused reducing of pyoverdine in the mutant, although it grew in rich, noniron limited, medium, and under higher 3-O-C12-HSL concentration (Figure 6).

Motility is important for virulence, and biofilm attachment, development,

maturation, and dispersion.³⁰ Swimming and swarming motility rely on flagella whilst twitching on motility type IV pili. In this research, swimming and twitching motility are unaffected by the mutation. The swarming motility of P. aeruginosa PAO1 is modulated by quorum sensing.³¹ Interestingly, in this study, we found that the 3O-C₁₂-HSL level was significantly increased in $\triangle PA3235$ strain although the swarming motility was reduced (Figure 3 and Figure 6). Based on microarray data, the PA3235 does not belong to genes involved in swarming motility,³² and the transcript was downregulated in tendrils compared to non-swarming cells.33 The swarming motility is also regulated by multifactorial determinants, for instance rhamnolipid, nitrogen starvation as well as particular amino acids,³¹⁻³⁴ and nutrition availability in the environment, especially iron.^{29,31} The decreased swarming area exhibited by the mutant might be attributed to the lessen of pyoverdine production which in turn causes less iron uptake necessary for PAO1 cells to swarm.

Although the PA3235 gene was known to be expressed less in biofilm compared to planktonic cells (E-GEOD-10030)35, however the result of this study revealed that deletion of this gene caused increasing biofilm quantity. Based on microarray data, the PA3235 belongs to the genes that are downregulated in biofilm,25 and expressed less in biofilm compared to the planktonic cells (E-GEOD-10030).35 During the growth of P. aeruginosa in LB Broth, the PA3235 gene was upregulated at the early stationary phase and then decreased at the stationary phase on static incubation (E-GEOD-4026).²⁶ The result of this study revealed that deletion of this gene caused increasing in biofilm quantity. It seems that the PA3235 gene might involve in the early stage of biofilm formation. Initiations of biofilm formation are driven by complex factors, such as antimicrobials, motility, and iron.³⁶⁻³⁹ Other studies have proven that swarm behaviour and biofilm formation are inversely regulated in P. aeruginosa cells.40 Swarming motility is important for early biofilm development, defect in swarming motility impairs biofilm formation.^{36,41} This was confirmed in this study that the biofilm of $\triangle PA3235$ was increased at 37 °C, whilst swarming was decreased (Figure 5 and Figure 3).

incubation and then the signal molecule was less in the extracellular environment

after the culture reached the stationary



stage. The 3-O-C12-HSL was produced constitutively in small amounts, and deliberately from the cell. When this molecule reaches a certain threshold amount in the environment, it is taken back to the cell by facilitated diffusion. We predicted that PA3235 is important for transporting this AHL and certain nutrients into the cell. There are four main reasons. First, the PA3235 protein is predicted as a transmembrane protein according to TMHMM-2.0 analyses.42 Until now, no information about the transport protein of the AHL, nor the real role of the PA3235 protein. Second, no AHL synthesis genes were detected in the stationary phase.²⁵ The PA3235 gene was categorized as a quorum sensing modulated gene;^{25,43} which expression was upregulated at the early stationary phase and decreased at the stationary phase (E-GEOD-4026).²⁶ This means that this protein might be involved in transporting the AHL into the cells. So that, the absence of PA3235 protein might cause problems in the uptake of 3-O-C12-HSL into the cell and the molecule getting abundant in the extracellular medium. Third, the lower amount of 3-O-C12-HSL inside the cell might cause changes in a few phenotypes. Fourth, the availability of certain nutrients is important for the phenotypic traits of the colony. For example, iron impacted the pyoverdine level of cells, pyocyanin, swarming and biofilm. The presence of amino acid and other nutrients are also important for swarming motility. This means that the protein might involve in the metabolism or transportation of certain molecules. PA3235 is predicted to encode a

hypothetical membrane protein with an unknown function. It is comprised of 312 nucleotides, and encodes a protein with a domain similar to E. coli K12 YjcH, an inner membrane protein.13 Analysis of the genome structures showed that this protein is highly conserved amongst other P. aeruginosa strains.44 This gene is not known as a protein related to efflux pump, siderophore or pyochelin. However, during the growth of P. aeruginosa in iron limiting medium, the PA3235

-PAO1 wild type

• ΔPA3235

10

11

12

genes.



0.5

0

1

2

3

The increased level of AHL in $\Delta PA3235$ quorum-sensing dependence might be responsible for the modulation The mutant showed the same amount of many virulence factors which are of 3-O-C12-HSL as the PAO1, until 5 h

5

6

Incubation time (hour)

7

8

9

gene was expressed at the logarithmic phase (E-GEOD-2885).45 Furthermore, microarray data revealed that the expression was downregulated in response siderophore/β-lactam conjugate to addition (E-MTAB-1381,46 and the PA3235 expression is not regulated by Fe in iron depletion condition.⁴⁵ This hypothesis was supported by the evidence that pyoverdine and swarming motility were reduced in Δ PA3235 strain, whereas the biofilm was increased compared to the wild type. Low iron concentration in the environment impedes biofilm formation and lowers the swarming motility.^{47,39} However, high iron concentration can disrupt the biofilm.48 The abundant quantity of ferric ions reduces rhamnolipid production which in turn represses swarming motility. A study by Schmidberger et al. (2014) has proven that excess ferric ion decreases the expression of the genes involved in rhamnolipid production, such as rhlA, and rhlC.49 Rhamnolipid was known to be crucial in surface motility, and biofilm formation.⁵⁰ PvdQ, that is important for pyoverdine synthesis, is also expressed in iron-limiting condition, which then related to swarming motility and biofilm development. Looking at the previous evidence, we believe that the deletion of PA3235 enables cells to uptake more Fe3+ ions from the medium than the wild type, resulting in less pyoverdine production and swarming motility as the formation of thicker biofilm. The association between this gene and iron uptake still needs to be confirmed. We predicted that PA3235 might contribute to the uptake and release of molecules.

Furthermore, the PA3235 might be involved in the early pathogenesis of this bacterium in host cells. When the cells were harvested at the early stationary phase, the gene was upregulated at 37 °C compared to 22 °C,10 and then downregulated at the stationary growth phase.6 The results of microarray data indicate that this gene might be involved in the early infection stage of P. aeruginosa in mammalian host cells. The PA3235 was expressed in response to epithelial cells (GDS2502)⁵¹ and in human whole blood cells,⁸ mimicking the condition when the bacterium infects humans, such as cystic fibrosis patients. However, the expression

of this gene was downregulated when this bacterium was grown on mouse tumors, cystic fibrosis lung and burned wound mouse,¹¹ or from infected human, such as sputum and burn wound.⁵² Until now, no information about factors which may inhibit its expression in these cells.

Based on this evidence, we proposed that the PA3235 might involve in the pathogenesis of P. aeruginosa during infecting the human body. It is highly likely that the PA3235 is modulated by quorum sensing and involved in the transport of molecules out or into the P. aeruginosa cells, which are important for early pathogenesis. Future works are needed to find the precise molecules transported by the PA3235 protein and to identify the exact roles of this gene in the pathogenicity of the bacterium during infection in mammalian and human cells. Given that the gene has not been previously characterized and the influence of the gene on the increase of biofilm and 3O-C12-HSL, we hypothesize that the modulation of this gene might be used to control P. aeruginosa pathogenicity when this bacterium infects the warm-blood hosts.53 Biofilm is the persistent form of PAO1 against antibiotics and immune cells.^{22,54-56}

Infections by P. aeruginosa are still an issue until now. However, the bacterium has been known as an opportunistic pathogen however infected patients are rarely survived. The severe effects are caused by these factors: virulence factors, resistance towards antibiotics, emerging of multidrug resistance antibiotics. The finding of anti-infectives against this bacterium is important. In this study, we prove that the PA3235 gene influences increasing biofilm and 3O-C12-HSL, lowering pyocyanin, pyoverdine, and swarming motility. Biofilm is the persistent form of PAO1 against antibiotics and immune cells. Several reported microarray data gave the impression that this gene might be involved in the early pathogenesis of P. aeruginosa, given the facts that PA3235 gene has not been previously characterized and its influence on several virulence factors, we hypothesize that the modulation of this gene might be used to control P. aeruginosa pathogenicity in human.

CONCLUSIONS

The Δ PA3235 mutant showed some differences when grown at 37 °C compared to the wild type. Both strains showed no differences in growth patterns, twitching or swimming motilities. We predicted that the PA3235 gene might play a role in the pathogenesis of PAO1 to the human body by facilitating the transport of molecules out or into PAO1 cells.

ETHICAL CLEARANCE

This research received ethical approval from Universitas Surabaya, with certificate number 33A/KE/VIII/2018.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

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AUTHOR'S CONTRIBUTION

Mariana Wahjudi is involved in conceptualization, funding acquisition, analysis and interpretation of results, and manuscript preparation and finalization. Samuel Stefanus Widodo: study conception, design, and data collection. Ida Bagus Made Artadana: data analysis and interpretation of results. Yulanda Antonius: manuscript finalization and review

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