PRELIMINARY STUDY OF *pa0305* EXPRESSION DURING *Pseudomonas aeruginosa* CELL GROWTH PHASES AND ITS VIRULENCE FACTORS MEASUREMENT

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ABSTRACT

Pseudomonas aeruginosa PAO1 was shown to have three acyl homoserine lactone acylases encoded by *pvdQ, quiP* and *pa0305*. One additional gene, *pa1893*, still remains to be proved to be another penicillin acylase protein. Initial studies on PAO1 *pa0305* deletion strains suggested that triple mutant (Δ*pvdQ,* Δ*quiP,* Δ*pa0305*) showed a higher 3-oxo-C_{12}-HSL accumulation compared to the double mutant PAO1 (Δ*pvdQ,* Δ*quiP*). When PA0305 was checked for its activities, the activity towards 3-oxo-C_{12}-HSL was recorded with k_{cat}/K_m $7.8x10^{-4}$ $M^{-1}s^{-1}$. However, when the *pa0305* was over expressed in PAO1, it did not show quenching activity as high as when it was done *in vitro*. mRNA quantification showed that *pa0305* was expressed at low amounts during the late logarithmic phase from the growth curve of PAO1 and Δ*pa0305*. It suggested the existence of a regulation controlling the *pa0305* expression in cells. In order to check when the *pa0305* was expressed, a plasmid containing the interregional sequence (between *pa0305* and *pa0306*) was introduced into PAO1 and Δ*pa0305*. This interregional sequence was cloned upstream the *lacZ* gene reporter. Every two hours along the growth each culture was analyzed for the β-galactosidase and LasA elastase activities and the pyocyanin accumulation. The results showed that the interregional activated during the early logarithmic phase, followed by the *de novo* production of pyocyanin and *de novo* activity of LasA elastase. Compared to the wild type, the absence of *pa0305* in the PAO1 chromosome led to a greater accumulation of pyocyanin and to a stronger activity of LasA elastase. The increase was occurred during the log phase where the increase of virulence factors at late stationary phase was doubled compared to the wild type. These results showed that the activation of putative promoter of *pa0305* in PAO1chromosome lead to the decrease of virulence factors.

Keyword : *pa0305*, PAO1, AHL acylase, β-galactosidase, pyocyanin, LasA elastase

STUDI PENDAHULUAN EKSPRESI *pa0305* DALAM BERBAGAI TAHAPAN PERTUMBUHAN SEL *Pseudomonas aeruginosa* DAN PENGUKURAN FAKTOR-FAKTOR VIRULENSI

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ABSTRAK

Pseudomonas aeruginosa PAO1 telah terbukti memiliki tigamacam asil homoserin lakton asilase yang dikode oleh *pvdQ, quiP* dan *pa0305*. Sementara gen lainnya, *pa1893*, diprediksi mengode protein penisilin asilase. Studi sebelumnya tentang strain PAO1 dengan delesi pada *pa0305* menunjukkan bahwa *triple* mutan (Δ*pvdQ,* Δ*quiP,* Δ*pa0305*) menunjukkan akumulasi 3-okso-C_{12}-HSL yang lebih tinggi dibandingkan PAO1 *double* mutan (Δ*pvdQ,* Δ*quiP*). Saat PA0305 diuji aktivitasnya terhadap bermacam-macam AHL, protein tersebut menunjukkan aktivitas terhadap 3-okso-C_{12}-HSL dengan k_{cat}/K_m $7.8x10^{-4}$ $M^{-1}s^{-1}$. Namun saat *pa0305* dioverekspresi dalam PAO1, protein tersebut tidak menunjukkan aktivitas sebesar saat PA0305 diuji secara *in vitro*. Kuantifikasi mRNA menunjukkan bahwa *pa0305* terekspresi pada akhir fase logaritmik dari kurva pertumbuhan PAO1 dan Δ*pa0305*. Hal tersebut menunjukkan bahwa terdapat proses regulasi yang mengontrol ekspresi *pa0305* di sel. Untuk mengetahui kapan gen *pa0305* terekspresi, plasmid yang mengandung daerah inter regional (di antara *pa0305* dan *pa0306*) dimasukkan ke PAO1 dan Δ*pa0305*. Sekuen inter regional tersebut diklon di daerah upstream dari gen reporter *lacZ*. Setiap 2 jam selama fase pertumbuhan sel, tiap kultur dianalisa aktivitas β-galaktosidase dan LasA elastase serta akumulasi piocianin. Hasil penelitian menunjukkan bahwa *putative* promoter *pa0305* diaktifkan di awal fase logaritmik dan terus aktif sepanjang fase stasioner. Aktivasi *putative* promoter tersebut diikuti dengan produksi awal dari piocianin dan LasA elastase. Dibandingkan dengan *wild type*, absennya *pa0305* pada kromosom PAO1 menyebabkan tingginya akumulasi piocianin dan aktivitas LasA elastase. Peningkatan paling besar terjadi saat fase log di mana peningkatan jumlah faktor virulensi pada Δ*pa0305* besarnya mencapai 2 kali jumlah faktor virulensi pada PAO1. Hasil tersebut menunjukkan bahwa teraktivasinya *putative* promoter tersebut pada kromosom PAO1 menyebabkan produksi faktor-faktor virulensi lebih rendah

Kata kunci : *pa0305*, PAO1, AHL asilase, β-galaktosidase, piocianin, LasA elastase