LAPORAN AKHIR

Penelitian Unggulan Perguruan Tinggi (M)

IDENTIFIKASI DAN KARAKTERISASI ENZIM PENGURAI SIGNAL ACYL HOMOSERINE LACTONE (AHL) DARI BAKTERI INDIGENOUS TAMBAK

Tahun Ke-1 Dari Rencana 2 Tahun

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ABSTRAK

Tujuan dari penelitian ini adalah untuk mengisolasi dan mengetahui karakter enzim pengurai signal AHL dari Bacillus indigenous tambak udang. Metode identifikasi dilakukan melalui proses bioassay dengan biosensor *Agrobacterium tumefaciens* (ATCC ® BAA-2240™) dan sekuensing nukleotida gen aiiA. Hasil analisa nukleotida dan protein menunjukkan bahwa enzim pengurai AHL pada Bacillus indigenous tambak adalah lactonase dengan struktur yang serupa dengan protein Metallo-Beta-Lactamase superfamily. Hasil karakterisasi menunjukkan bahwa enzim Lactonase dari Bacillus indigenous tambak udang mempunyai aktivitas maksimum pada suhu 30°C dan pH 8,0. Selanjutnya berdasarkan persamaan Michaelis-Menten didapatkan nilai V_{max} dan K_m berturut-turut sebesar 2,67 μM/menit dan 16,76 μM.

Kata kunci : *Bacillus*, quorum sensing, quorum quenching, vibriosis, udang

ABSTRACT

The objective of this research were to isolate and characterize of enzyme which responsible for Acyl Homoserine Lactone (AHL) degradation from shrimp indigenous Bacillus. The identification methods were bioassay with AHL biocensor *Agrobacterium tumefaciens* (ATCC ® BAA-2240™) and aiiA gen sequencing. Research results showed that AHL degradation enzyme responsible for AHL degradation was Lactonase which had a similarity with Metallo-Beta-Lactamase superfamily. The results also showed that the enzyme Lactonase isolated from shrimp ponds indigenous *Bacillus* have maximum activity at 30°C and pH 8.0. Furthermore, the Michaelis - Menten equation revealed that V_{max} and K_m values of *Bacillus* Lactonase were 2.67 μm/ min and 16.76 μM respectively.

Keywords: Bacillus, quorum sensing, quorum quenching, vibriosis, shrimp

RINGKASAN

Strategi biokontrol, melalui proses enzimatik untuk memutus komunikasi antar bakteri sangat menjanjikan karena kemungkinan terjadinya resistansi sangat minimal dan selanjutnya diharapkan dapat meningkatkan produksi tambak. Tujuan penelitian ini ialah mengidentifikasi dan mengkaraterisasi enzim pengurai signal molekul QS AHL pada isolat bakteri dari tambak yang sebelumnya telah diidentifikasi mampu mengurai signal QS AHL dan aplikasinya secara in-vitro dan in-vivo. Hasil penelitian terdahulu menunjukkan garis kekerabatan (16SrRNA) dan kemampuan QQ beberapa isolat Bacillus yang diisolasi dari tambak udang. Pada tahun pertama telah dapat diidentifikasi enzim lactonase (AiiA) yang bertanggung jawab terhadap proses in-aktivasi signal AHL melalui proses bioassay dengan biosensor *Agrobacterium tumefaciens* (ATCC ® BAA-2240™) dan sekuensing nukleotida gen aiiA. Hasil analisa nukleotida dan protein menunjukkan bahwa enzim pengurai AHL pada Bacillus indigenous tambak adalah lactonase dengan struktur yang serupa dengan protein Beta Lactamase superfamily. Hasil karakterisasi menunjukkan bahwa enzim Lactonase dari Bacillus indigenous tambak udang mempunyai aktivitas maksimum pada suhu 30^{o}C dan pH 8,0. Selanjutnya berdasarkan persamaan Michaelis-Menten didapatkan nilai V_{max} dan K_m berturut-turut sebesar 2,67 μM/menit dan 16,76 μM. Pada tahun ke-2 akan dilakukan uji hidrolisis substrat, uji resistansi terhadap protease dan cairan pencernaan, uji aplikasi enzim lactonase indigenous tambak terhadap populasi *V. harveyi* secara in vitro dan in vivo pada udang. .

SUMMARY

Bacterial disease remains an important issue in the shrimp farming industry in Indonesia . In sequence, bacterial diseases such as vibriosis can be triggered the prevalence of viral diseases such as white - spot disease. Due to the missuse of antibiotics in sahrimp farm, antibiotics become increasingly ineffective. Therefore, it is necessary to find another safe and effective biocontrol. One promising way is to break the bacterial communication signal (Quorum Sensing / QS) which is responsible for the bacterial virulence. Termination can be done through the QS signal degradation pathway of signaling molecule (acyl homoserine Lactone / AHL). The overall objective of this research for 2 years is to identify and characterize the degradative enzyme of AHL QS signaling molecules and in- vitro and in – vivo application for shrimp culture. In the first year, it have been identified that enzyme lactonase (Aiia) was responsible for that process by bioassay test using *Agrobacterium tumefaciens* (ATCC ® BAA - 2240TM) and nucleotide sequencing of aiiA gene. The results also showed that the enzyme Lactonase isolated from shrimp ponds indigenous *Bacillus* have maximum activity at 30^{o}C and pH 8.0. Furthermore, the Michaelis - Menten equation revealed that V_{max} and K_m values of *Bacillus* Lactonase were 2.67 μm/ min and 16.76 μM respectively. In the 2nd year, some test will be conducted including specific substrate hydrolysis, resistance test to protease and shrimp digestive enzyme, in-vivo test against *V. harveyi* BB 120 and in vivo biocontrol test in shrimp.

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