

ANALISIS GENOM BAKTERI *Lysinibacillus sphaericus* PATOGENIK TERHADAP LARVA NYAMUK *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae)

Afiannisa Viersanova
19/447326/PBI/01605

INTISARI

Lysinibacillus sphaericus merupakan bakteri entomopatogen yang spesifik terhadap nyamuk vektor terutama *Culex* dan *Anopheles* spp., sehingga banyak digunakan sebagai bioinsektisida untuk mengendalikan vektor ini. *L. sphaericus* memiliki variasi efisiensi toksisitas yang beragam, hal ini menyebabkan terus dilakukannya eksplorasi terhadap isolat baru dengan toksisitas tinggi dan toksin baru yang dapat mengatasi masalah resistensi. Penelitian ini bertujuan untuk mengidentifikasi dan menganalisis hubungan kekerabatan bakteri yang secara morfologis merupakan *L. sphaericus*, yakni isolat 15.4, 229C, 6.2, 4D21, dan 6B4 berdasarkan gen 16S rRNA, serta mengidentifikasi karakteristik genom dan karakteristik toksin kelima isolat bakteri *L. sphaericus* tersebut berdasarkan hasil analisis sekuen genom. Isolat koleksi ini didapatkan sebelumnya dari sampel tanah rizosfer dan kotoran burung dari berbagai area di Indonesia

Identifikasi molekuler dilakukan dengan gen 16SrRNA dan hubungan kekerabatan dianalisis dengan rekonstruksi pohon filogenetik dengan metode *Neighbor-Joining*. Untuk menganalisis genom dan toksin, teknik *NGS* dilakukan kemudian analisis dilakukan dengan menggunakan *bioinformatics tools* yang didapatkan secara bebas. Hasil identifikasi molekuler 16S rRNA menunjukkan bahwa spesies terkait terdekat dari isolat 6.2 dan 4D21 ialah *L. fusiformis*, sementara isolat 229C ialah *L. sphaericus*, dan 6B4 ialah *L. macroides*. Namun rekonstruksi pohon filogenetik tidak menunjukkan pembentukan kelompok sesuai dengan penunjuk spesiesnya. Analisis genom menunjukkan bahwa 15.4 memiliki kualitas rendah sehingga tidak dilakukan analisis lanjutan dan 6B4 mengalami kontaminasi, namun keempat isolat selain isolat 15.4 menunjukkan kualitas sekuensing yang tergolong median hingga sangat baik, dengan ukuran genom 4,64 – 16 Mbp dan G+C konten 36-49%. Analisis gen penyandi toksin menunjukkan keempat isolat tidak memiliki binary toxin (Bin protein), crystal toxin (Cry48/ Cry49 protein), maupun Sphaericolysin. Namun, terdapat gen *mosquitocidal toxin* (Mtx protein), s-layer protein, hemolysin, dan *chitin binding protein* yang teridentifikasi, di mana gen protein ini diketahui berkaitan dengan toksisitas *L. sphaericus* terhadap nyamuk dan menjadi jawaban atas permasalahan resistensi terhadap binary toksin.

Kata Kunci: Analisis genom, *Culex quinquefasciatus*, entomopatogen, *Lysinibacillus sphaericus*.

GENOME ANALYSIS OF *Lysinibacillus sphaericus* PATHOGENIC TO *Culex quinquefasciatus* SAY, 1823 (Diptera: Culicidae)

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ABSTRACT

Lysinibacillus sphaericus is an entomopathogenic bacteria is known to be specific to vector mosquitoes, especially *Culex* and *Anopheles* spp., so it has been widely used as bio insecticide to control these vectors. *L. sphaericus* has a wide variation of toxicity, which have led to continuous exploration of new isolates with higher toxicity and a new toxin to deal with resistance problems. This study aimed to identify and analyze the genome of morphologically identified *L. sphaericus* bacteria isolates, namely isolates 15.4, 229C, 6.2, 4D21, and 6B4 based on the 16S rRNA gene, as well as to identify genomic characteristics and toxin genes of the five *L. sphaericus* isolates using the whole genome sequence analysis. These isolates were previously obtained from soils rhizosphere and bird droppings from different area of Indonesia.

Molecular identification was carried out with the 16S rRNA gene and the relationship was analysed by reconstructing the phylogenetic tree using *Neighbours-Joining*. To analyse the genome and toxins, the *NGS* technique was used and then the analysis was carried out using a couple of freely available bioinformatics tools. The result of the 16SrRNA gene blasting showed that the closest related species of 6.2 and 4D21 is *L. fusiformis*, 229C is *L. sphaericus*, and 6B4 is *L. macroides*. However, the reconstruction of the phylogenetic tree did not show formation of clusters aligned to the species. Genomic analysis showed that 15.4 sequencing results had low quality, so this isolate was not analysed any further. Eventhough the isolate 6B4 was contaminated, but all the four isolates showed sequencing quality that was classified as median to very good, with a genome size ranging from 4.64 to 16 Mbp and a G+C content of 36 to 49%. Toxin gene analysis indicate that the four isolates did not have any binary toxin (Bin protein), crystal toxin (Cry48/ Cry49 protein), or Sphaericolysin, but there were *Mosquitocidal toxin* (Mtx protein), s-layer protein, hemolysin, and chitin binding protein gene identified, all of which are known to be associated with the toxicity of *L. sphaericus* to mosquito and could be the solution to the resistance problem to binary toxins of *L. sphaericus*.

Keywords: *Culex quinquefasciatus*, entomopathogen, genome analysis, *Lysinibacillus sphaericus*