Isolasi, identifikasi molekuler dan analisis keanekaragaman bakteri kelas ktedonobacteria dari tanah hutan di sekitar geiser Cisolok, Jawa Barat = Isolation, molecular identification and bacterial diversity analysis of class ktedonobacteria from forest soil around Cisolok Geyser, West Java

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Abstrak

Penelitian bertujuan mengetahui keanekaragaman bakteri Ktedonobacteria dari sampel tanah hutan di sekitar Geiser Cisolok, Jawa Barat dengan metode culture-dependent dan metode culture-independent. Isolasi bakteri menggunakan medium Reasoner's 2A (10%) dengan penambahan 2% gellan gum, cycloheximide, dan sodium azide. Inkubasi dilakukan pada suhu 30 oC selama 3 minggu. Amplifikasi gen 16S rRNA isolat bakteri menggunakan primer spesifik Ktedonobacteria (primer 161F dan 941R), dan primer universal bakteri (9F dan 1510R). Identitas isolat bakteri diperoleh berdasarkan data full sequence gen 16S rRNA melalui pencarian homologi pada EZBioCloud (www.ezbiocloud.net). Analisis filogenetik menggunakan metode Neighbour Joining, Maximum Evolution, dan Maximum Likelihood. Analisis keanekaragaman bakteri Ktedonobacteria menggunakan Next Generation Sequencing berdasarkan data partial sequence (daerah variabel V1--V3) dari gen 16S rRNA. Analisis data komposisi taksonomi bakteri dan indeks keanekaragaman menggunakan software QIIME2. Empat isolat Ktedonobacteria dengan kode K17-1, K17-2, K42, dan K44 berhasil diperoleh. Analisis filogenetik menunjukkan bahwa keseluruhan isolat merupakan anggota kelas Ktedonobacteria dan berada dalam satu grup dengan type strain Dictyobacter aurantiacus S-27T. Namun demikian, persentase homologi sequence gen 16S rRNA keempat isolat menunjukkan nilai yang rendah terhadap type strain Dictyobacter aurantiacus S-27T, yaitu 97.16 -- 98.02%. Berdasarkan nilai tersebut, keempat isolat yang diperoleh diduga merupakan spesies baru. Hasil analisis dengan software QIIME2 menunjukkan bahwa sampel tanah yang digunakan memiliki nilai indeks keanekaragaman bakteri yang tinggi, dengan nilai sebagai berikut: 6,49 (Shannon-Winner); 0,98 (Simpson); 177 (Chao1); dan 117 (Ace). Filum Acidobacteria, Proteobacteria dan Bacteriodetes, merupakan tiga filum dengan persentase paling besar pada sampel tanah, dengan nilai persentase masing-masing 44%, 25%, dan 9%. Kelas Ktedonobacteria pada filum Chloroflexi memiliki persentase yang sangat rendah, yaitu 1,89%. Namun demikian, analisis filogenetik data amplikon (culture-independent) menunjukkan bahwa Ktedonobacteria yang terdapat pada sampel tanah tersebar dalam 5 grup, yang seluruhnya mengindikasikan taksa baru. Penelitian ini menunjukkan bahwa metode culture-dependent hanya berhasil menemukan satu dari lima grup Ktedonobacteria yang berhasil dideteksi menggunakan metode culture-independent.

.....The study aims to determine the diversity of Ktedonobacteria from forest soil samples around the Cisolok Geiser, West Java with culture-dependent and culture-independent methods. Bacterial isolation using Reasoner's 2A (10%) medium with 2% gellan gum, cycloheximide, and sodium azide. Incubation was carried out at 30 oC for three weeks. Amplification of 16S rRNA gene of bacterial isolates performed using Ktedonobacteria specific primers (primers 161F and 941R), and universal bacterial primers (9F and 1510R). The identity of bacterial isolates was obtained based on full 16S rRNA gene sequence data through a

homology search on EZBioCloud (www.ezbiocloud.net). The phylogenetic analysis was performed by Neighbor-Joining, Maximum Evolution, and Maximum Likelihood methods. Analysis of Ktedonobacteria diversity using Next-Generation Sequencing based on partial sequence data (variable regions V1 -- V3) of the 16S rRNA gene. Analysis of bacterial taxonomy composition data and diversity index was conducted using QIIME2 software. Four isolates of Ktedonobacteria, namely K17-1, K17-2, K42, and K44, were successfully obtained. Phylogenetic analysis showed that all isolates were members of the class Ktedonobacteria and were in the same group as Dictyobacter aurantiacus S-27T. However, the percentage of homology of the 16S rRNA gene sequence of the four isolates showed a low value on the type strain of Dictyobacter aurantiacus S-27T, which accounted for 97.16 -- 98.02%. Based on these values, the four isolates obtained probably belonged to the new species. The results of the analysis with QIIME2 software showed that the soil samples had high bacterial diversity index values, with the following values: 6,49 (Shannon-Winner); 0,98 (Simpson); 177 (Chao1); and 117 (Ace). Phylum Acidobacteria, Proteobacteria, and Bacteriodetes are the three phyla with the largest percentage in soil samples, with percentage values of 44%, 25%, and 9%, respectively. Whereas the class Ktedonobacteria in the phylum Chloroflexi has a very low percentage, which is 1.89%. However, phylogenetic analysis of the amplicon data (culture-independent) showed that Ktedonobacteria found in soil samples distributed into five groups, indicating new taxa. In this study, culture-dependent methods found only one of the five groups of Ktedonobacteria that detected using the culture-independent method.