Isolasi dan Karakterisasi Hypolithic Rare-Actinomycetes dari Sampel S3.5.3 di Kawasan Geotermal Cisolok = Isolation and Characterization of Hypolithic Rare-Actinomycetes from S3.5.3 Sample in Cisolok Geothermal Area

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Abstrak

Rare-actinomycetes tersebar di berbagai habitat terutama di habitat ekstrem seperti kawasan geotermal. Penelitian mengenai rare-actinomycetes dilakukan terkait potensinya sebagai penghasil senyawa bioaktif baru yang bermanfaat dalam bidang kesehatan, industri dan farmasi. Tujuan dari penelitian ini adalah mengisolasi dan mengkarakterisasi secara fenotip dan genotip rare-actinomycetes dari sampel tanah di bawah batuan kuarsa (S3.5.3) di hutan kawasan geotermal Cisolok. Metode pengayaan sampel tanah dilakukan menggunakan medium 10% R2A (Reasoner’s 2A) cair dengan penambahan cycloheximide 100 ppm dan sodium azide 60 ppm, kemudian diinkubasi pada suhu 30°C selama 30 hari. Isolasi rare actinomycetes dilakukan dengan metode membran filter dan spread pada medium 10% R2A gellan gum yang diinkubasi pada suhu 45°C. Karakterisasi dilakukan secara genotipik (berdasarkan data sequence gen 16S rRNA, analisis homologi sequence, dan rekonstruksi pohon filogenetik dengan metode Neighbour-Joining, Maximum Likelihood, dan Minimum Evolution); dan karakterisasi fenotipik (morfologi, fisiologi, dan biokimia). Sebanyak 26 isolat diperoleh dari sampel S3.5.3. Lima isolat dengan karakter morfologi actinomycetes yang diisolasi dari suhu 45°C dengan membran filter, dipilih untuk diidentifikasi. Hasil analisis sequence gen 16S rRNA dari lima isolat menunjukkan persentase homologi sebesar 95,46-99,56% terhadap Micromonospora yasonensis DS3186T. Berdasarkan hasil analisis filogenetik dengan metode Neighbour-Joining, kelima isolat memiliki hubungan kekerabatan terdekat dengan Micromonospora yasonensis DS3186T. Kelima isolat merupakan anggota class Actinomycetes, ordo Micromonosporales, family Micromonosporaceae. Karakter fenotipik kelima isolat sesuai dengan Micromonospora yasonensis sebagai spesies terdekatnya. Kelima isolat merupakan bakteri termotoleran (tumbuh pada suhu 30-45°C dan suhu optimum 40°C), aerobik, Gram positif, menghasilkan miselium substrat tanpa adanya miselium aerial, positif katalase, dan menghasilkan soluble pigment. Penelitian ini mengungkapkan bahwa Micromonospora yasonensis dapat ditemukan di kawasan geotermal dan berasosiasi dengan batuan kuarsa.
.....Rare-actinomycetes are distributed in various habitats, particularly in extreme environments such as geothermal areas. Research on rare-actinomycetes focuses on their potential as producers of new bioactive compounds beneficial in health, industrial, and pharmaceutical fields. The aims of this study were to isolate and characterize rare-actinomycetes from soil samples beneath quartz rocks (S3.5.3) in the geothermal forest of Cisolok. Soil sample enrichment was performed using 10% R2A (Reasoner’s 2A) liquid medium supplemented with 100 ppm cycloheximide and 60 ppm sodium azide, incubated at 30°C for 30 days. Rare actinomycetes isolation was carried out using the membrane filter method and spread on 10% R2A agar with gellan gum, incubated at 45°C. Characterization included genotypic analysis based on the sequence of 16S rRNA gene, supported by phenotypic characterization (morphology, physiology, and biochemistry). A total of 26 isolates were obtained from sample S3.5.3. Five isolates with actinomycetes morphology isolated at 45°C using the membrane filter method were selected for characterization. Analysis of the 16S rRNA

gene sequences from these five isolates showed homology levels of 95.46-99.56% to Micromonospora yasonensis DS3186T. Based on phylogenetic analysis using the Neighbour-Joining method, the five isolates were most closely related to Micromonospora yasonensis DS3186T. These isolates belong to the class Actinomycetes, order Micromonosporales, and family Micromonosporaceae. Phenotypic characteristics of the five isolates were consistent with Micromonospora yasonensis as their closest species. These isolates are thermotolerant bacteria (growing at temperatures of 30-45°C; optimum temperature 40°C), aerobic, Gram-positive, produce substrate mycelium without aerial mycelium, positive for catalase, and produce soluble pigment. This study reveals that Micromonospora yasonensis can be found in geothermal areas associated with quartz rocks.