

## 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.