Penapisan aktivitas selulolitik dan karakterisasi actinobacteria termofilik dari tanah di Kawasan Geotermal Cisolok, Jawa Barat = Cellulolytic activity screening and characterization of thermophilic actinobacteria from the soil of Cisolok Geothermal Area, West Java

Putri Pratiwi Setyaningsih, author

Abstrak

Tujuan dari penelitian ini adalah untuk memperoleh isolat 'Actinobacteria' termofilik dari tanah di sekitar geiser Cisolok, Jawa Barat yang memiliki aktivitas selulolitik pada suhu tinggi serta mengetahui posisi filogenetik isolat terpilih terhadap spesies-spesies terdekatnya berdasarkan gen 16S rRNA. Penapisan kemampuan degradasi selulosa 17 isolat dilakukan secara kualitatif pada 'Minimal medium' (Mm) padat yang ditambahkan substrat yaitu 'carboxymethyl cellulose' (CMC) 1% (b/v) atau 'microcrystalline cellulose' (MCC) 1% (b/v) kemudian diinkubasi selama 7 hari. Pengamatan dilakukan dengan pewarnaan 'Congo red' 0,2% (b/v) dan zona bening pada sekitar koloni mengindikasikan degradasi substrat. Hasil penapisan menunjukkan bahwa 15 isolat mendegradasi CMC 1% dan 12 isolat mendegradasi MCC 1% pada suhu 45 ^oC, 14 isolat mendegradasi CMC 1% dan MCC 1% pada suhu 50 ^oC, 4 isolat mendegradasi CMC 1% dan MCC 1% pada suhu 55 ^oC, dan 3 isolat mendegradasi CMC 1% dan MCC 1% pada suhu 60 ^oC. Tiga isolat (SL1-2-R-2, SL1-2-R-3, dan SL1-2-R-4) yang mendegradasi CMC 1% dan MCC 1% hingga 60 ^oC merupakan isolat terpilih. Identifikasi dan karakterisasi telah dilakukan pada penelitian sebelumnya dan melaporkan tiga isolat terpilih memiliki kekerabatan terdekat dengan 'Actinomadura keratinilytica' WCC-2665^T(=NBRC 105837^T). Hasil pengujian menunjukkan 'type strain' NBRC 105837^T mendegradasi CMC 1% dan MCC 1% pada medium Mm padat dengan suhu 45, 50, 55, dan 60 ^oC setelah inkubasi 7 hari. 'Crude enzyme' dari tiga isolat potensial dan 'type strain' NBRC 105837^T menunjukkan aktivitas selulolitik pada medium Mm padat yang ditambahkan CMC 1% atau MCC 1% pada suhu 45, 50, 55, dan 60 ^oC. Analisis filogenetik tiga isolat terpilih berdasarkan gen 16S rRNA menggunakan metode 'Neighbor-Joining' (NJ), 'Minimum Evolution' (ME), dan 'Maximum Likelihood' (ML) menunjukkan bahwa tiga isolat terpilih berada pada satu 'clade' monofiletik dengan 'Actinomadura' 'keratinilytica' WCC-2665^T. Analisis filogenetik juga menunjukkan dua kelompok yang terpisah berdasarkan kemampuan menghasilkan selulase pada anggota famili 'Thermomonosporaceae'.

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The aims of this study were to obtained thermophilic 'Actinobacteria' isolates from soil around Cisolok geyser, West Java with the ability to degrade cellulose at high temperatures and to analyze the phylogenetic position based on 16S rRNA gene of the selected isolates compared to closely related species. Cellulose degradation screening was performed on Minimal (Mm) medium with the addition of 1% (w/v) carboxymethyl cellulose (CMC) or 1% (w/v) microcrystalline cellulose (MCC) as substrate then incubated for 7 days. Cellulose degradations were observed by staining the plates with 0,2% (w/v) Congo red and clear zone formation around the bacterial colony would indicate the cellulose degradation. The results showed that 15 isolates were able to degrade 1% CMC and 12 isolates were able to degrade 1% MCC at 45

^oC, 14 isolates were able to degrade 1% CMC and 1% MCC at 50 ^oC, 4 isolates were able to degrade 1% CMC and 1% MCC at 55 ^oC, and 3 isolates were able to degrade 1% CMC and 1% MCC at 60 ^oC. Three isolates (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) were selected due to their CMC and MCC degrading ability at 60 ^oC. Molecular identification based on 16S rRNA gene and characterization in previous study showed that the three selected isolates are closely related to 'Actinomadura keratinilytica' WCC-2665^T(=NBRC 105837^T). The assay showed that type strain NBRC 105837^T was able to degrade 1% CMC and 1% MCC at 45, 50, 55, and 60 ^oC after 7 days of incubation. Cellulolytic activity show that the crude enzymes of the three selected isolates and type strain were able to degrade 1% CMC and 1% MCC at 45, 50, 55, and 60 ^oC. Phylogenetic analysis using Neighbour-Joining (NJ), Minimum Evolution (ME), and Maximum Likelihood (ML) methods showed that the three selected isolates were clustered together in monophyletic clade with 'Actinomadura keratinilytica' WCC-2265^T with 100% bootstrap value. Phylogenetic analysis also showed that cellulase producers and non-cellulase producers in 'Thermomonosporaceae' were grouped into different clades.