Re-identifikasi lima strain rhizopus oligosporus saito koleksi UICC berdasarkan data sequence daerah internal transcribed spacers ribosomal DNA = Re-identification of five UICC strains of rhizopus oligosporus saito based on sequence data of internal transcribed spacers region of ribosomal DNA

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Abstrak

Universitas Indonesia Culture Collection UICC memiliki koleksi Rhizopus spp. yang diisolasi dari tempe dan telah diidentifikasi berdasarkan karakter morfologi dan fisiologi. Tujuan penelitian adalah re-identifikasi lima strain R. oligosporus koleksi UICC UICC 27, UICC 40, UICC 51, UICC 67, dan UICC 116 berdasarkan data sequence daerah internal transcribed spacers rDNA dan analisis filogenetik. Amplifikasi dan sequencing daerah ITS rDNA menggunakan pasangan primer forward ITS5 dan primer reverse ITS4. Data sequence daerah ITS rDNA Rhizopus koleksi UICC dikirim ke database GenBank untuk pencarian homologi BLAST terhadap spesies terdekat dengan pembatasan pencarian hanya pada type material. Pembuatan pohon filogenetik menggunakan metode neighbor joining dengan model evolusi dua parameter Kimura, serta bootstrapping dilakukan sebanyak 1000 kali pengulangan. Karakterisasi morfologi makroskopik dan mikroskopik dan fisiologi pertumbuhan pada variasi suhu dilakukan untuk menunjang hasil identifikasi molekuler.

Hasil elektroforesis gel produk PCR daerah ITS rDNA menunjukkan kelima strain memiliki ukuran fragmen ITS rDNA pada kisaran 500--700 pb, dan hasil sequencing lengkap daerah ITS rDNA kelima strain menunjukkan panjang berkisar antara 655--658 pb. Hasil BLAST menunjukkan strain UICC 27, UICC 40, UICC 51, dan UICC 67 memiliki homologi 99,2 ; sedangkan strain UICC 116 memiliki homologi 99,8 terhadap type strain R. oryzae CBS 112.07T. Berdasarkan konsep spesies filogenetik, strain UICC 27, UICC 40, UICC 51, dan UICC 67 dapat diidentifikasi sebagai R. delemar karena pada pohon filogenetik berada dalam satu kelompok yang monofiletik dengan type strain R. delemar CBS 120.12T, dengan dukungan nilai bootstrap 90 ; sedangkan strain UICC 116 diidentifikasi sebagai R. oryzae karena pada pohon filogenetik berada dalam satu kelompok yang monofiletik dengan type strain R. oryzae CBS 112.07T , dengan dukungan nilai bootstrap 82 . Hasil penelitian menunjukkan bahwa kedua spesies tidak dapat dibedakan secara morfologi, kelima strain menunjukkan karakter morfologi yang sesuai dengan R. oryzae. Namun demikian, terdapat perbedaan karakter fisiologi antara R. oryzae dan R. delemar, yaitu pertumbuhan pada variasi suhu.

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Universitas Indonesia Culture Collection UICC has strains collection of Rhizopus spp. which were isolated from tempeh and identified based on phenotypic characters morphology and physiology . The aim of this study was to re identify five strains of R. oligosporus UICC 10, UICC 85, UICC 119, UICC 120, and the UICC 135 , based on internal transcribed spacers region of ribosomal DNA sequence data and phylogenetic analysis. Amplification and sequencing of ITS region of rDNA were performed using primer set forward primer ITS 5 and reverse primer ITS 4. The sequence data was sent to GenBank for homology search using basic local alignment search tool BLAST program, restricted only to type materials. Construction of

phylogenetic tree was performed using Neighbor Joining method with Kimura's two parameter evolution model and bootstrapping with 1,000 replicates. Characterization of morphological and physiological growth at variation of temperature data was carried out to support molecular identification results.

Gel electrophoresis showed that five strains have fragment length of ITS region of rDNA, about 500 700 bp. Full sequence data of ITS rDNA of five strains showed the length of their ITS rDNA was ranged 655 658 bp. BLAST homology search results showed that UICC 27, UICC 40, UICC 51, and UICC 67 strain have similarity 99,2 to the type strain of R. oryzae CBS 112.07T, whilst UICC 116 strain showed a higher similarity 99,8 to the type strain of R. oryzae CBS 112.07T. Based on phylogenetic species concept, four strains UICC 27, UICC 40, UICC 51, and UICC 67 were identified as R. delemar. They were clustered with the type strain of R. delemar CBS 120.12T in a monophyletic group, and supported by 90 bootstrap value. Another one strain UICC 116 was identified as R. oryzae. It was clustered with the type strain of R. oryzae CBS 112.07T in a monophyletic group and supported by 82 bootstrap value. Morphological characterization results indicated that the two species are indistinguishable and they showed morphological characters that correspond to R. oryzae. However, the physiological characters i.e. growth at temperature variations differ between of R. delemar and R. oryzae.