Re-identifikasi lima strain rhizopus oryzae went prinsen geerligs koleksi UICC berdasarkan data sequence daerah internal transcribed spacers ribosomal DNA = Reidentification of five UICC strains of rhizopus oryzae went prinsen geerligs based on sequence data of internal transcribed spacers region of ribosomal DNA

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Abstrak

Universitas Indonesia Culture Collection (UICC) memiliki koleksi strain-strain Rhizopus oryzae Went dan Prinsen Geerligs yang diisolasi dari tempe dan telah diidentifikasi berdasarkan karakter fenotipik (morfologi dan fisiologi). Tujuan penelitian adalah melakukan re-identifikasi lima strain R. oryzae (UICC 10, UICC 85, UICC 119, UICC 120, dan UICC 135) berdasarkan data sequence daerah internal transcribed spacers ribosomal DNA dan analisis filogenetik untuk memperoleh identitas spesies yang akurat.

Amplifikasi dan sequencing daerah ITS rDNA dilakukan menggunakan primer forward ITS 5 dan primer reverse ITS 4. Data sequence dikirim ke database DNA GenBank untuk pencarian homologi dengan program BLAST. Sequence alignment dilakukan menggunakan program Clustal X. Konstruksi pohon filogenetik dilakukan menggunakan metode Neighbor Joining, model dua parameter Kimura, dan nilai bootstrap 1000 kali pengulangan. Karakterisasi morfologi dan pengujian kemampuan tumbuh pada variasi suhu dilakukan untuk mendukung hasil re-identifikasi dan melengkapi deskripsi strain masing-masing.

Hasil amplifikasi daerah ITS rDNA menunjukkan bahwa kelima strain R. oryzae UICC memiliki panjang fragmen dengan ukuran 600--700 pb. Hasil pencarian homologi BLAST menunjukkan bahwa kelima strain memiliki homologi 99,8% terhadap type strain R. oryzae CBS 112.07T. Hasil analisis filogenetik menunjukkan bahwa kelima strain berada dalam satu grup yang monofiletik dengan strain tipe R. oryzae CBS 112.07T dan strain-strain R. oryzae dari database DNA GenBank, dengan dukungan nilai bootstrap yang tinggi (84%). Re-identifikasi lima strain R. oryzae UICC secara molekuler menghasilkan identitas spesies yang sama, yaitu sebagai R. oryzae, dan didukung oleh karakter morfologi dan fisiologi.

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Universitas Indonesia Culture Collection (UICC) has collection of Rhizopus oryzae Went and Prinsen Geerligs strains, which were isolated from tempeh. These strains were identified based on phenotypic characters (morphology and physiology). The aim of this study was to re-identify five strains of R. oryzae (UICC 10, UICC 85, UICC 119, UICC 120, and UICC 135), based on internal transcribed spacers (ITS) region of ribosomal DNA sequence data to obtain accurate identification at species level.

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 DNA database for homology search using BLAST. Sequence alignment was carried out using Clustal X. Construction of phylogenetic tree was performed using Neighbor Joining method, Kimura's two parameter model and bootstrap values of 1000 iterations. Characterization of the five strains based on morphology and growth ability at temperature variations were carried out to support the description of each strain.

Amplification result showed that the strains have fragment length of ITS region of rDNA about 600--700 bp. Results of BLAST homology search of the strains showed a very high similarity (99.8%) to the type strain

R. oryzae CBS 112.07T. Phylogenetic tree showed that the five strains were clustered together in a monophyletic group with the type strain R. oryzae CBS 112.07T and other R. oryzae strains from GenBank DNA database, and supported by high bootstrap value (84%). Re-identification of five strains of R. oryzae UICC based on molecular method showed that they were identified as R. oryzae, similar to previous identification, and supported by morphological and physiological characterization.