

Re-identifikasi lima strain rhizopus arrhizus fischer koleksi UICC berdasarkan data sequence daerah internal transcribed spacers ribosomal dna = Re identification of five strains of rhizopus arrhizus fischer from UICC collection based on internal transcribed spacers region of ribosomal dna sequence data

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Abstrak

Universitas Indonesia Culture collection (UICC) memiliki koleksi strain-strain *Rhizopus arrhizus* Fischer yang diisolasi dari tempe dan telah diidentifikasi berdasarkan karakter morfologi dan fisiologi. Penelitian bertujuan memperoleh identitas yang akurat pada tingkat spesies dari lima strain *R. arrhizus* (UICC 26, UICC 36, UICC 39, UICC 55, dan UICC 121) berdasarkan data sequence daerah internal transcribed spacers ribosomal DNA dan analisis filogenetik. Amplifikasi dan sequencing daerah ITS rDNA dilakukan menggunakan primer forward ITS5 dan primer reverse ITS4. Pencarian homologi sequence dilakukan dengan program BLAST. Sequence alignment dilakukan menggunakan program Clustal X. Konstruksi pohon filogenetik dilakukan menggunakan metode neighbor joining dengan model dua parameter Kimura dan nilai bootstrap 1000 pengulangan. Karakterisasi morfologi dan fisiologi (pengujian pertumbuhan pada variasi suhu) dilakukan untuk melengkapi deskripsi strain. Panjang fragmen daerah ITS rDNA kelima strain *R. arrhizus* sekitar 600--700 pb.

Hasil BLAST menunjukkan kelima strain UICC memiliki homologi dengan type strain *R. oryzae* CBS 112.07T pada kisaran 98,9--99,8%. Pohon filogenetik menunjukkan strain UICC 36 dan strain UICC 55 berada dalam satu grup yang monofiletik dengan type strain *R. oryzae* CBS 112.07T dan neo type *R. arrhizus* NRRL 1469NT. Saat ini *R. arrhizus* merupakan sinonim dari *R. oryzae*, sehingga kedua strain tersebut diidentifikasi sebagai *R. oryzae*. Strain UICC 26, UICC 39, dan UICC 121 berada dalam satu grup yang monofiletik dengan type strain *R. delemar* CBS 120.12T, sehingga ketiga strain diidentifikasi sebagai *R. delemar*. *Rhizopus oryzae* dan *R. delemar* merupakan dua spesies yang berkerabat sangat dekat, sehingga secara morfologi tidak dapat dibedakan. Re-identifikasi lima strain *R. arrhizus* UICC secara molekuler menghasilkan identitas spesies yang berbeda menjadi *R. oryzae* dan *R. delemar*, namun karakter morfologi dan fisiologi lima strain tersebut menunjukkan karakter sebagai *R. oryzae*.

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Universitas Indonesia Culture Collection (UICC) has collection of *Rhizopus arrhizus* Fischer strains, which were isolated from tempeh and were identified based on morphological and physiological characters. The aim of this study was to obtain accurate identification at species level of five strains of *R. arrhizus* (UICC 26, UICC 36, UICC 39, UICC 55, and UICC 121) based on internal transcribed spacers (ITS) region of ribosomal DNA (rDNA) sequence data and phylogenetic analysis. Amplification and sequencing of ITS region of rDNA were performed using forward primer ITS5 and reverse primer ITS4. Sequence homology search was performed 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 morphological features and growth at variation of temperature were carried out to support the description of the strains. The fragment length of ITS region

rDNA from five strains of *R. arrhizus* was about 600--700 bp.

Results of BLAST homology search of the strains showed 98.9--99.8% similarities to the type strain *R. oryzae* CBS 112.07T. Phylogenetic tree showed that UICC 36 and UICC 55 were clustered together in a monophyletic group with the type strain *R. oryzae* CBS 112.07T and neo type strain *R. arrhizus* NRRL 1469NT. Currently, *R. arrhizus* is a synonym of *R. oryzae*, therefore both strains were identified as *R. oryzae*. Strains UICC 26, UICC 39, and UICC 121 were clustered together in a monophyletic group with the type strain *R. delemar* CBS 120.12T, therefore they were identified as *R. delemar*. *Rhizopus oryzae* and *R. delemar* are very closely related species and morphologically similar. Re-identification of five strains *R. arrhizus* UICC based on molecular has difference of species identity as *R. oryzae* and *R. delemar*, but the five strains show similar morphological and physiological characters as *R. oryzae*.