The Molecular Identification of the Wood-Rotting Basidiomycetes from the Georgian Forests

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Abstract

The development of a universal approach to the identification of fungi from the environment is impeded by the limited number and narrow phylogenetic range of the named internal transcribed spacer DNA sequences available on GenBank. The main goal of the presented research was an assessment of the potential impact of systematic DNA sequencing from a fungal herbarium collection. Within this context, 21 strains of wood-rotting *Basidiomycetes* were selected for molecular investigations. All strains have been collected and isolated in Georgia from different eco-geographical niches. Initial identification was performed on the basis of morphological and ecological characters. In addition, they were cultivated on various plant wastes used as nutritional substrates, and all of them showed promising hydrolase or oxidase activity.

The cell morphology was evaluated by an epifluorescence optical microscope (OLYMPUS BH2) combined with a digital camera (SONY). The analysed samples were prepared with DAPI solution. Images were captured using Simple PCI imaging software (Hamamatsu) and processed with Adobe Photoshop CS.

All selected strains have undergone genomic DNA extraction, PCR, and Internal Transcribed Spacer (ITS) sequencing. After PCR, DNAs were analysed with FlashGel™ DNA Cassettes, which enable both estimation of DNA size and quantity. Amplification of the ITS fragment was performed according to the CIRM-CF protocol using two universal primers, ITS 1 (TCC GTA GGT GAA CCT GCG G) and ITS 4 (TCC TCC GCT TAT TGA TAT GC).

Among 21 fungal strains, 16 strains were pure, and molecular identification was performed by using NCBI BLAST with the counting as the query sequence. Sequences similar to the query sequence were searched using the NCBI Basic Local Alignment Search Tool (BLAST). For all investigated fungal strains, the following species have been identified: *Trametes versicolor* (5 strains); *Fomes fomentarius* (1 strain); *Bjerkandera adusta* (1 strain); *Trametes hirsuta* (2 strains); *Phellinus tuberculosus* (2 strains); *Lenzites betulina* (3 strains); *Trametespubescens=T. velutina*(1 strain). For 2 strains, Blast results did not allow discrimination between two closely related species: the sequence corresponds either to *Coriolopsis trogii (Trametes trogii)* or to *Coriolopsis gallica*. Nomenclatural update (current name) was made according to the Index Fungorum (http://www.indexfungorum.org/) and MycoBank (http://fr.mycobank.org/). Final identification was made by comparing the molecular identification with the identification based on morphological data.

Acknowledgment

This work was supported by the SRNSF of Georgia (Grant No.FR-22-1612).