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Multilocus Phylogenetic Approach Reveals Informative DNA Barcodes for Studying Evolution and Taxonomy of Fusarium Fungi

Authors : Alexander A. Stakheev, Larisa V. Samokhvalova, Sergey K. Zavriev

Abstract : Fusarium fungi are among the most devastating plant pathogens distributed all over the world. Significant reduction of grain yield and quality caused by Fusarium leads to multi-billion dollar annual losses to the world agricultural production. These organisms can also cause infections in immunocompromised persons and produce the wide range of mycotoxins, such as trichothecenes, fumonisins, and zearalenone, which are hazardous to human and animal health. Identification of Fusarium fungi based on the morphology of spores and spore-forming structures, colony color and appearance on specific culture media is often very complicated due to the high similarity of these features for closely related species. Modern Fusarium taxonomy increasingly uses data of crossing experiments (biological species concept) and genetic polymorphism analysis (phylogenetic species concept). A number of novel Fusarium sibling species has been established using DNA barcoding techniques. Species recognition is best made with the combined phylogeny of intron-rich protein coding genes and ribosomal DNA sequences. However, the internal transcribed spacer of (ITS), which is considered to be universal DNA barcode for Fungi, is not suitable for genus Fusarium, because of its insufficient variability between closely related species and the presence of non-orthologous copies in the genome. Nowadays, the translation elongation factor 1 alpha (TEF1α) gene is the "gold standard" of Fusarium taxonomy, but the search for novel informative markers is still needed. In this study, we used two novel DNA markers, frataxin (FXN) and heat shock protein 90 (HSP90) to discover phylogenetic relationships between Fusarium species. Multilocus phylogenetic analysis based on partial sequences of TEF1α, FXN, HSP90, as well as intergenic spacer of ribosomal DNA (IGS), beta-tubulin (β-TUB) and phosphate permease (PHO) genes has been conducted for 120 isolates of 19 Fusarium species from different climatic zones of Russia and neighboring countries using maximum likelihood (ML) and maximum parsimony (MP) algorithms. Our analyses revealed that FXN and HSP90 genes could be considered as informative phylogenetic markers, suitable for evolutionary and taxonomic studies of Fusarium genus. It has been shown that PHO gene possesses more variable (22 %) and parsimony informative (19 %) characters than other markers, including TEF1α (12 % and 9 %, correspondingly) when used for elucidating phylogenetic relationships between F. avenaceum and its closest relatives – F. tricinctum, F. acuminatum, F. torulosum. Application of novel DNA barcodes confirmed the fact that F. arthrosporioides do not represent a separate species but only a subspecies of F. avenaceum. Phylogeny based on partial PHO and FXN sequences revealed the presence of separate cluster of four F. avenaceum strains which were closer to F. torulosum than to major F. avenaceum clade. The strain F-846 from Moldova, morphologically identified as F. poae, formed a separate lineage in all the constructed dendrograms, and could potentially be considered as a separate species, but more information is needed to confirm this conclusion. Variable sites in PHO sequences were used for the first-time development of specific qPCR-based diagnostic assays for F. acuminatum and F. torulosum. This work was supported by Russian Foundation for Basic Research (grant № 15-29-02527).

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