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Molecular Characterization of Two Thermoplastic Biopolymer-Degrading Fungi Utilizing rRNA-Based Technology

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Abstract : Out of 30 fungal isolates, 2 new isolates were proven to degrade poly-β-hydroxybutyrate (PHB). Enzyme assay for these isolates indicated the optimal environmental conditions required for depolymerase enzyme to induce the highest level of biopolymer degradation. The two isolates were basically characterized at the morphological level as Trichoderma asperellum (isolate S1), and Aspergillus fumigates (isolate S2) using standard approaches. The aim of the present study was to characterize these two isolates at the molecular level based on the highly diverged rRNA gene(s). Within this gene, two domains of the ribosome large subunit (LSU) namely internal transcribed spacer (ITS) and 26S were utilized in the analysis. The first domain comprises the ITS1/5.8S/ITS2 regions (> 500 bp), while the second domain comprises the D1/D2/D3 regions (> 1200 bp). Sanger sequencing was conducted at Macrogen (Inc.) for the two isolates using primers ITS1/ITS4 for the first domain, while primers LROR/LR7 for the second domain. Sizes of the first domain ranged between 594-602 bp for S1 isolate and 581-594 bp for S2 isolate, while those of the second domain ranged between 1228-1238 bp for S1 isolate and 1156-1291 for S2 isolate. BLAST analysis indicated 99% identities of the first domain of S1 isolate with T. asperellum isolates XP22 (ID: KX664456.1), CTCCSJ-G-HB40564 (ID: KY750349.1), CTCCSJ-F-ZY40590 (ID: KY750362.1) and TV (ID: KU341015.1). BLAST of the first domain of S2 isolate indicated 100% identities with A. fumigatus isolate YNCA0338 (ID: KP068684.1) and strain MEF-Cr-6 (ID: KU597198.1), while 99% identities with A. fumigatus isolate CCA101 (ID: KT877346.1) and strain CD1621 (ID: JX092088.1). Large numbers of other T. asperellum and A. fumigatus isolates and strains showed high level of identities with S1 and S2 isolates, respectively, based on the diversity of the first domain. BLAST of the second domain of S1 isolate indicated 99 and 100% identities with only two strains of T. asperellum namely TR 3 (ID: HM466685.1) and G (ID: KF723005.1), respectively. However, other T. species (ex., atroviride, hamatum, deliquescens, harzianum, etc.) also showed high level of identities. BLAST of the second domain of S2 isolate indicated 100% identities with A. fumigatus isolate YNCA0338 (ID: KP068684.1) and strain MEF-Cr-6 (ID: KU597198.1), while 99% identities with A. fumigatus isolate CCA101 (ID: KT877346.1) and strain CD1621 (ID: JX092088.1). Large numbers of other A. fumigatus isolates and strains showed high level of identities with S2 isolate. Overall, the results of molecular characterization based on rRNA diversity for the two isolates of T. asperellum and A. fumigatus matched those obtained by morphological characterization. In addition, ITS domain proved to be more sensitive than 26S domain in diversity profiling of fungi at the species level.

Keywords : Aspergillus fumigates, Trichoderma asperellum, PHB, degradation, BLAST, ITS, 26S, rRNA
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