

Molecular characterization of endophytes isolated from *Saccharum* spp based on esterase and ribosomal DNA (ITS1-5.8S-ITS2) analyses

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ABSTRACT. This study used esterases and ribosomal DNA (rDNA) markers to determine endophytic variability in order to better understand endophyte-host interactions. Polyacrylamide gel electrophoresis and esterase isoenzymes (EST; EC 3.1.1.3), with α -naphthyl acetate and β -naphthyl acetate as substrates, were used to assess relationships among endophytes. ITS1-5.8S-ITS2 sequencing data were used as rDNA markers. Thirty-two esterases were obtained from 37 isolates of *Saccharum* spp, which clustered into five endophyte groups. Esterase EST-06 was observed with the highest frequency, being present in 22 of the 37 isolates analyzed, followed by esterase EST-11, which was present in 20 isolates. The esterases EST-10 and EST-14 were present in 19 isolates and EST-09 was present in 18 isolates. The esterase EST-01 was unique to isolate 33 and can, therefore, be used as a marker for this isolate. None of the esterases identified were common to all isolates tested. Similarly, phylogenetic analysis, based on rDNA sequence data, classified the isolates into 5 genus groups: 1) *Curvularia* with a 100% bootstrap value (BP), 2) *Alternaria* with 100% BP, 3) *Epicoccum* with 60% BP, 4) *Phoma* with 89% BP, and 5)

Saccharicola with 100% BP. This polyphyletic analysis based on several markers, therefore, proved to be a valuable approach in determining the relationship between variation in endophytes and their associated host plants. Furthermore, both the esterase and rDNA analyses obtained similar results and were equally effective in resolving relationships.

Key words: Sugarcane; Endophytic fungi; Isoenzymes; Esterases; Molecular markers; rDNA