Mining endophytic bacteria and fungi for polyketide biosynthetic genes

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Endophytic microorganisms are a group of bacteria and fungi that habits in symbiotic manner the inner tissue of a plant, through all or part of the life cycle, without causing any apparent damage. They have emerged as a novel target for the screening of new source of bioactive compounds. An endophytic culture collection formed with isolates from industrial crops such as sugar cane, citrus, cotton, eucalyptus and grass was selected to be searched for the presence of polyketide biosynthetic genes by means of degenerated PCR approach. More than seven hundred isolates between bacteria and fungi from sugar cane were screened for genes involved in the biosynthesis of polyketides types PKSI (fungi), PKSII (actinobacteria) and PKSIII (actinobacteria). For PKSI there were found 26 sequences with the expected size of about 750pb with primers that identify the KS domain (ketosynthase); in which 16 amplified with the LC1/2c primers, showing an identity between 83 and 99% with related PKS sequences. Ten sequences amplified with the KS3/4c primers, showing an identity between 65 and 92%; and ten sequences with the Cmet1/3c (carboxymethyl) primers with the expected DNA size of about 350 pb, showing an identity between 54 and 92%. Putative introns were detected in 12 nucleotide sequences: seven introns in sequences obtained with the primers KS3/4c, four introns with the primers Cmet1/3c, and one in a sequence obtained from primers LC1/2c. A multiple alignment of the deduced amino acid sequence from the KS domain, ranging from 154 to 217 amino acids, confirmed a conserved cystein residue responsible for the ketosynthase activity. A highly level of conservation between the regions encompass this active site, compared with known KS sequences of producers of non reduced polyketides (YWA1 from *Emericella nidulans*, CAA46695.2 and PKS1 of *C. leginarium,* BAA18956.1), and reduced polyketides (LNKS, AAD39830.1 and LDKS, AAD34559.1) from *Aspergillus terreus*. The fungal reducing PKSs (R-PKS), non reducing PKSs (NR-PKS) and partial reducing (PR-PKS) clades were reliable in a phylogenetic tree. The investigation for PKSII genes was based in the amplification of KSα, a subunit of the conserved gene β-ketosynthase. Forty isolates were positive, 16S rRNA identified the bacteria as members of *Streptomyces, Nocardia* and *Nocardiopsis.* Thirty isolates were also positive for ketosynthase of the PKSI. Seven *Streptomyces* amplified for PKSIII genes showing homologies with those of enzymes PhlD, RppB, RppA and THNS related to CHS (chalcone synthase) that is involved in flavonoids biosynthesis in plants.