Title	Amplification and Partial Sequencing of the Intergenic Regions (IGS) of Ribosomial DNA from *P. digitatum*, *P. ulaiense* and *P. italicum*
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Abstract

The early identification and quantification of *Penicillium digitatum*, *P. ulaiense* and *P. italicum*, responsible of the green, blue and whisker moulds, respectively, is particularly important for citrus in which pathogen and non-pathogen species could be found on the fruit surface. These species are difficult to differentiate using morphological methods and the use of molecular techniques is challenged by the presence of a high number of phylogenetically related species. Moreover, *P. digitatum*, *P. ulaiense* and *P. italicum* have almost identical Internal Transcribed Spacer (ITS) regions i.c. the most widely utilized targets to develop molecular markets. The IGS1 and IGS2 regions have great potential since, like the ITS regions, they are multicopy (up to 100 copies per haploid genome) and their length (3000-4000 bp) provides considerable scope for species-specific primer development. However, their utilization as targets to develop specific molecular markers has been limited mainly because of the difficulties related to the amplification of a long fragment and the lack of effective universal primers. In the present work, new universal primers were designed by comparing IGS flaking regions (28S and 18S genes) from a large number of fungi and utilized to amplify the complete IGS regions of *P. ulaiense*, *P. digitatum* and *P. italicum*. Amplicons were of approximately 4000 bp for *P. ulaiense* and 3500 bp for *P. digitatum* and *P. italicum*. Amplified regions were cloned in pCR®-XL-TOPO® Kit and sequenced for approximately 1000 nucleotide at both 3' and 5' sides for *P. ulaiense* and *P. italicum*. As opposed, only a very short fragment was effectively sequenced from *P. italicum*. Alignment of IGS regions form *P. digitatum* and *P. ulaiense* with other GenBank available IGS sequences and BLAST analyses evidenced very high levels of polymorphisms which are valuable for the development of highly specific molecular markers. Further studies are in progress to complete sequencing and to evaluate intraspecific variation that could seriously compromise the suitability of species-specific primer sets.