Molecular-based identification of six strains of entomopathogenic fungi

Isra' Al_qadi, Iba' Farrah, and Naim Iraki

UNESCO Biotechnology Educational and Research Center, Bethlehem University, Bethlehem, Palestine

The genes coding for the ribosomal RNA (rRNA) are arranged in a tandem array with transcribed spacers separating each gene from the other. These spacer sequences include; internal transcribed spacer 1 (ITSI), which separates the 18S rRNA gene from the 5.8S gene and the ITSII, which separates the latter gene from the 28S rRNA gene. These three genes together with the two internal spacers constitute one unit that is flanked by two sequences known as external transcribed spacers (ETS). Each unit of rRNA genes and internal and external transcribed spacers is separated from another repeating unit by a sequence known as intergenic spacer (IGS) (Hillis and Dixon 1991, Charlesworth et al 1994). The coding regions of the functional rDNA is highly conserved. In contrast, the sequences of the non functional ITS and ETS regions are variable.

The sequence variation in ITS I, ITS II and ETS regions is utilized for molecular taxonomy in eukaryotes (Sansour et al 2003, Tymon et al 2004). This sequence variation could be useful for discrimination between two species and in some cases even between two strains of the same species (Tymon et al 2004). Sequence analysis and the probable detection of sequence variation among strains of the same species, would allow construction of specific primers for PCR-based diagnosis of the concerned strain. This diagnosis technique could be useful for studying the infectivity of several strains applied simultaneously to the same host.

We analyzed the 5.8S, ITS1 and ITS2 sequences of six strains of EPF, two of which are Beauveria bassiana strains; PAL-B01 and PAL-B02 from Palestine, and four Metarhizium anaisoalpiae strains; PAL-M01 and PAL-M02 from Palestine, and M7 and PPRC from Israel. There was a clear molecular differentiation between Beauveria and Metarhizium species. This differentiation is more informative and reliable in ITSI compared to that in ITSII. The polymorphism can be summarized as follows:

1-The two Beauveria bassiana Palestinian strains; PAL-B01, PAL-B02 were identical in the whole sequenced region (ITS I, 5.8S, and ITSII) with no difference in any gap or single nucleotide polymorphism (SNP) between the two isolates.

2-The sequence of n either one of the two Beauveria bassiana strains showed a 100% match with any sequence in the gene bank. This indicates that they are new strains and their sequence should be deposited in the gene bank.

3-The sequences of the two Palestinian Metarhizium strains; PAL-M01 and PAL-M02 were different in one SNP and one gap. This difference could be used for developing specific primers for use in diagnosis.

4-The sequence of the Metarhizium strain PAL-M01 differs from that of PAL-M02 and from that of the Israeli Metarhizium strain M7. However, the sequences of the latter two strains were identical.

In conclusion, the sequence analysis of the ITS regions succeeded to document differences between the two fungal species as well as between two Metarhizium strains. Application of additional molecular-based techniques (e.g. RAPD and RFLP) is required to differentiate between the two isolates of the Beauveria b. species.

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References

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