Genotyping and Thermotolerance Characterization of Several Isolates of Entomopathogenic Fungi from Palestine ppu pdf.pdf

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Some of the Entomopathogenic Fungi (EPF) belonging to the genera; Metarhizium and Beauveria are currently used as biocontrol agents and substitute the harmful chemical pesticides. Four EPF isolates were collected from the Midwest area of the West Bank and identified as Metarhizium anisopliae and Beauveria bassiana based on morphological traits of the conidia, colony and conidiophores. Also, the .infectivity of these isolates to mammalian ectoparasites was investigated in other studies

The main objective of the reported work was to apply molecular-based techniques to assess the morphological-based identification and to differentiate between isolates of the same species in order to have diagnostic tools that could be useful for studying the infectivity of several strains applied .simultaneously to the same host

These techniques include sequence analysis of □- tubulin (BT) gene, the Internal Transcribed Spacers (ITS) 1 & 2 of the rDNA, construction of Restriction Fragment Length Polymorphism (RFLP) patterns in an attempt to create an isolate fingerprint, and Inter-Simple Sequence Repeat (ISSR) analysis. Another objective of this work included characterization of the thermotolerance of each Palestinian isolate for the purpose of selecting a proper isolate to control mammalian ectoparasites.

For molecular characterization, sequences of ten isolates of EPF were analyzed for comparison purposes, four of which are B. bassiana: B-Bot, the Israeli isolate B-Bug, the Palestinian isolates B-151 and B-Med, and six isolates of M. anisopliae: the Palestinian isolates M-Th2, M-153, the Israeli isolates Ma-7, M-2004, M-WG and the Ethiopian one PPRC.

Sequencing data of ITS confirmed six isolates of M. anisopliae while the other four were Beauveria bassiana isolates. The ITS sequences of the Palestinian B. bassiana and M. anisopliae isolates were similar to the corresponding sequences of the same species available in the GenBank. Restriction digestion of ITS2 sequences of M. anisopliae isolates was able to produce a fingerprint for M.a.153.

The ITS and BT sequences failed to differentiate between Beauveria isolates except the B-Bot genotype, but ITS discriminated three Metarhizium isolates: Ma-7, M-153, and PPRC. However, ISSR analysis detected high level of polymorphism among Beauveria (80%) and Metarhizium (90%) isolates.

Results of thermotolerance assessment showed that the isolates of the same species varied in their response to elevated temperatures in regard to spore germination and mycelial growth. All Palestinian isolates were capable of growth at temperatures (25 - 32□C) with apparent differences among isolates in their tolerance to the maximal temperature 32□C. At this temperature, the B.b.151 isolate showed high germination rate (99%), and the highest mycelial growth diameter value (18.13 mm + 1.56 SD) that is significantly higher than the isolates; B-Med, M-Th2 and M-153 (U=360.0, 130.5, 0.00, P=.041, P<.001, <.001, respectively). In view of the present results, the isolate B-151 appears to be a good candidate for use as biopesticide taking into account its thermotolerance and virulence as documented in other study.

It is recommended to explore larger region of the BT gene and apply additional molecular-based techniques that may produce more variations. Additional research could be done in exploring naturally occurring thermotolerant isolates from the Jordan Valley, where the temperature is higher.

Also, efforts should be put to test the ability of an isolate to germinate under optimal temperatures upon exposure to heat shock treatment, and to study the expression of the heat shock proteins genes in the different isolates studied.

Key words: Metarhizium anisopliae, Beauveria bassiana, morphological, ITS, RFLP, □-tubulin, ISSR, thermotolerance.

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