EIDE, JOHN D., GARRY A. SMITH and JOHN J. WEILAND. USDA, Agricultural Research Service, 1307 North 18th St., Northern Crop Science Laboratory, Fargo, ND 58105-5677. **Use of PCR to detect the sugarbeet root maggot biocontrol fungus *Metarhizium anisopliae*.**

ABSTRACT

Current methods for detection and identification of entomopathogenic fungi are laborious and time consuming, and identification of different strains of the same fungal species is even more difficult. Attempts at the genetic characterization of *Metarhizium anisopliae* (Metschnikoff) Sorokin have included the use of randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism analysis (RFLP) analysis, and others . These studies have met with limited success. *M. anisopliae* have been collected for studying genetic polymorphisms using rRNA analysis and mtDNA RFLP. The objective of this study was to prepare PCR (polymerase chain reaction) primers specific for the detection of strains of *M. anisopliae* that are pathogenic to the sugarbeet root maggot.

The entomopathogenic fungi examined in this study included *Beauveria bassiana, Cordyceps militaris, Hirsutella thompsonii, M. anisopliae, M. flavoviride, Syngliocladium tetanopsis* and *Verticillium lecanii*. In addition, the following ATCC strains of *Metarhizium* were used: ARS-T1 (fungi re-isolated from third instar sugarbeet root maggots inoculated with *M. anisopliae* 22099), 16085, 38630, 56096, 62176, 60335 and 32969. All fungal cultures were grown in 50 ml of 1% peptone, 2% dextrose broth. The DNA was extracted from each and PCR was carried out using standard procedures in a Perkin Elmer thermocycler. The PCR products were separated in 1% agarose gels. The DNA fragments were cloned into an Invitrogen plasmid pCR2.1 and the resulting plasmid transformed into *Escherichia coli* TOP10F's using the manufacturer instructions. Plasmid DNA was isolated using the alkaline lysis PEG 8000 precipitation method. DNA was sequenced at the Iowa State University DNA Sequencing facility.

PCR primers specific for the 5' end (5FWDACT) and 3' end (MIDREVACT) of the actin gene coding sequence were synthesized. These primers were used in the PCR to amplify a 1.3-kb DNA fragment in *M. anisopliae* ARS-T1 and five other *M. anisopliae* strains. These same primers detected a 1.2-kb fragment in the entomopathogenic fungi *B. bassiana, C. militaris, H. thompsonii,* and *V. lecanii*. The *M. anisopliae* fragments were cloned and both strands sequenced. In order to obtain the complete nucleotide sequence two primers internal to the 1.3-kb actin fragment were synthesized. These primers were used in the PCR with *M. anisopliae* DNA and amplified a 450-bp fragment that was cloned and sequenced. The intron sequences are being examined for unique sequences specific for *M. anisopliae*. The rRNA genes of these fungi also are being examined for the presence of distinguishing sequence characteristics. Two primers, ITS1 and ITS4 specific for the ITS (Internal transcribed spacers) region of the nuclear rRNA gene were synthesized. Use of these primers in the PCR with *M. anisopliae* DNA produced a 600-bp fragment. We have also synthesized two primers E24 and PN29 for use in amplification of the 28S rDNA. The primers amplified a 1.1-kb fragment from DNA of *M. anisopliae* in all strains tested except ATCC 38630. These primers amplified larger fragments of approximately 2 kpb in *C. militaris, B. bassiana* and *V. lecanii*. *S. tetanopsis* produces a 500 bp fragment with the E24 and PN29 primers. This fragment contains group I introns which have been useful for differentiating between strains of entomopathogenic fungi. Use of PCR with the above sets of primers will help differentiate entomopathogenic fungal species.