Single-Strand Conformation Polymorphism (SSCP) for Diatraea species identification

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Abstract

We propose a Single-Strand Conformation Polymorphism (SSCP) methodology to recognize each species as an effective tool in *Diatraea* species identification.

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Protocol

Step 1.

Dissection and DNA extraction: Abdomens from insects were dissected and maintained in ethanol at 70 % v/v in sterile distilled water to DNA extractions. DNA of male insects were obtained of complete abdomen by using commercial kit (DNeasy Blood & Tissue Kit, QIAGEN) and quantified by spectrophotometry (Nanodrop 1000, Thermo-Fisher).

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DNeasy Blood & Tissue Kit, QIAGEN Cat No./ID: 69504 by Contributed by users

Step 2.

2. COII amplification: In vitro amplifications of CO II fragments were carried out by the Polymerase Chain Reaction method (PCR) in standard conditions (final volume 25 µL) using Taq polymerase (PROMEGA), 50 ng of template and the primers previously described and used in Diatraea: A-298 (5'-ATTGGACATCAATGATATTGA-3') and B-tLYS (5' GTTTAAGAGACCAGTACTTG-3') (Simon et al. 1994; Lange et al. 2004, Palacio et al. 2010). Thermal cycling was performed using the following conditions: 1 cycle at 95 °C for 3 min; 34 cycles at 95 °C for 10 s; 53 °C for 45 s, and 72 °C for 30 s; and one cycle at 72 °C for 5 min. Aliquots of amplification products were resolved in 1% w/v agarose gel electrophoresis and later stained with SYBR-Safe (Invitrogen). Amplicons were used for SSCP methodology.

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Taq polymerase M3001 by Promega

SYBR S33102 by Thermo Fisher Scientific

Step 3.

3. Single-Strand Conformation Polymorphism (SSCP) methodology: The amplicons were prepared for

SSCPs. To this, 1 µL of PCR products amplified using A-298/B-tLYS were mixed with 9 µL of sample buffer (95% v/v Formamide, 20mM EDTA, 0.05% w/v bromophenol blue and 0.05% w/v Xylene-cyanol, in distilled water), denatured at 95 °C for 5 min and immediately stored in ice bath. Then, 1.5 µL of all samples were loaded in 6% non-denaturing polyacrylamide gel (49 acrylamide: 1 bis-acrylamide), and separated by electrophoresis for 4 hours at 600V (BioRad Sequi-Gen GT Nucleic Acid Electrophoresis Cell, 38 x 50 cm). Later, PAGEs were stained using and adapted silver nitrate method. Briefly, gels were fixed for 3 min (10% v/v ethanol, 1% v/v acetic acid in distilled water), oxidized for 3 min (1.5 % v/v nitric acid in distilled water), stained for 20 min (0.1% w/v silver nitrate, 0.045 % v/v formaldehyde in distilled water), revealed for approximately 5 min (3% w/v $NaCO_3$, 0.02 % v/v formaldehyde in distilled water), and the reactions were stopped with an acetic acid solution (5 % v/v in distilled water). Results were documented by digital photography.