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DNA extraction, PCR amplification, and sequencing of multiple loci of Colletotrichum species from an ancient herbarium specimen V.2

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We use this protocol and it's working

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Abstract

This protocol was developed for DNA extraction, PCR amplification, and sequencing of the ITS2 region and multiple genes of Colletotrichum species from an ancient herbarium specimen.

Troubleshooting

Ancient herbarium specimen

1 An isotype specimen of C. humuli (MSC0211749, diseased leaf of H. lupulus) was received from the Michigan State University Herbarium (MSC); it was collected in Manhattan, Kansas, USA, on June 2, 1890.

DNA extraction

2 Total genomic DNA from the herbarium specimen was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) with a modification.

2.1 Approximately 5 mg of symptomatic tissue bearing Colletotrichum conidiomata was collected from the specimen using a sterile scalpel under a SteREO Discovery.V12 microscope (Carl Zeiss, Göttingen, Germany).

2.2 The tissue was transferred to a 2-mL Lysing Matrix E tube (MP Biomedicals, Eschwege, Germany) containing 0.1 mm silica spheres, 1.4 mm ceramic spheres, and one 4 mm glass bead.

2.3 After adding 400 µL AP1 buffer from the kit and 4 µL RNase, the tissue was disrupted using a FastPrep-24TM instrument (MP Biomedicals, Eschwege, Germany).

2.4 The tube was incubated overnight at 65 °C.

2.5 The subsequent steps were performed according to the manufacturer's instructions.

PCR amplification and sequencing

3 To amplify the ITS2 region, a newly designed forward primer ITSCF1: 5′-GTAATGTGAATTGCAGAATTCAGTG-3′ and the reverse primer ITS4 were used.

4 A 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene and partial sequences of the chitin synthase 1 (CHS-1), histone 3 (HIS3), and actin (ACT) genes were amplified using the primer pairs GDF1/GDR1, CHS-79F/CHS-345R, CYLH3F/CYLH3R, and ACT-512F/ACT-783R, respectively.

5 PCR reactions were performed in a total volume of 25 µL.

5.1 PCR master mix (2X): 12.5 µL

5.2 Nuclease-free water: 8.5 µL

5.3 Forward primer: 1 µL (4.5 pmol)

5.4 Reverse primer: 1 µL (4.5 pmol)

5.5 DNA template (100 ng/µL): 2 µL

6 The PCR conditions for all primer pairs: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min.

7 PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and checked by gel electrophoresis before being sent to Macrogen (Seoul, South Korea) for sequencing. All primers used for PCR amplification were also used for sequencing.