DNA-BASED IDENTIFICATION OF *ARMILLARIA* ISOLATES FROM PEACH [*PRUNUS PERSICA* (L.) BATSCH] ORCHARDS IN MÉXICO STATE, MÉXICO

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ABSTRACT

A collaborative project between the Programa de Fitopatología, Colegio de Postgraduados, Texcoco, Edo. de México and the USDA Forest Service-RMRS, Moscow Forest Pathology Laboratory began in 2011 to identify which species of *Armillaria* are causing widespread and severe damage to the peach orchards from México State, México. We are employing a DNA-based approach in which the intergenic spacer 1 region (IGS1) of nuclear rDNA and the translation elongation factor-1 alpha gene will be sequenced and compared to known *Armillaria* species to facilitate species identification.

INTRODUCTION

Peach cultivation constitutes an important crop in México, with a production area of ca. 45,000 ha. Nearly 65 percent of the national production occurs in the highlands of central México (Michoacan, Morelos, and México States; SIAP 2010). In these areas, peach orchards are typically established in areas that were cleared of the native forests (Figure 1). In México State, peach orchards are relatively short-lived, with a production life span of about 10 years. *Armillaria* species are considered the most damaging pathogens of peach trees in this region, where they cause significant annual mortality of orchard-grown peach trees.

Information about *Armillaria* spp. in Méxican fruit orchards is very limited; however, some general information on *Armillaria* species in México is available in reports of edible mushrooms, commercial mushrooms, and ethnobotany (Montoya et al. 2003). Specific information is also found about the identification of *Armillaria* species collected from forests of central México using known haploid tester strains from U.S. (Alvarado-Rosales and Blanchette 1994). Currently, DNA-based diagnostics have not been widely applied to identify *Armillaria* species from México.

Figure 1. Fruit orchards established in areas where native forests are cleared.

OBJECTIVE

The objective of this project is to use DNA–based methods to identify 49 isolates of *Armillaria* collected from peach trees growing in orchards throughout México State for comparisons with *Armillaria* species from native forests. This information will be used to document the distribution of *Armillaria* spp. and help develop species-specific *Armillaria*-resistant peach rootstocks.

METHODS

The *Armillaria* isolates (n = 49) were collected from 15 peach orchards throughout México State. For each of these isolates the intergenic spacer 1 (IGS1) region of rDNA was sequenced, and the translation elongation factor 1 alpha (*tef-1α*) was sequenced for 24 isolates.

The protocol of Kim et al. (2006) was used for PCR amplification of IGS1. Template DNA used for *tef-1α* PCR was obtained by the protocol of Zhang et al. (2010). PCR was performed using the methods of Ross-Davis et al. (2012) except that primers were replaced by ARMEFF (5 ft. CGT GAY TTY ATC AAG AAC ATG AT 3 ft.) and ARMEFR (5 ft. TAC CCG TTC GGC GAT CAA TCT 3 ft.) designed by J.W. Hanna (USDA Forest Service, RMRS). PCR products were sequenced on an ABI3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, U.S.) at the University of Wisconsin Biotechnology Center (Madison, WI, U.S.). The sequences were ed. with BioEdit (ver. 7.1.3; Ibis Biosciences, Inc.). For phylogenetic analysis, parsimony analyses were performed with PAUP (4.0b10) to determine phylogenetic relationships among representative *Armillaria* isolates from species found in Méxican peach orchards and the other North American *Armillaria* species based on *tef-1α* sequence.
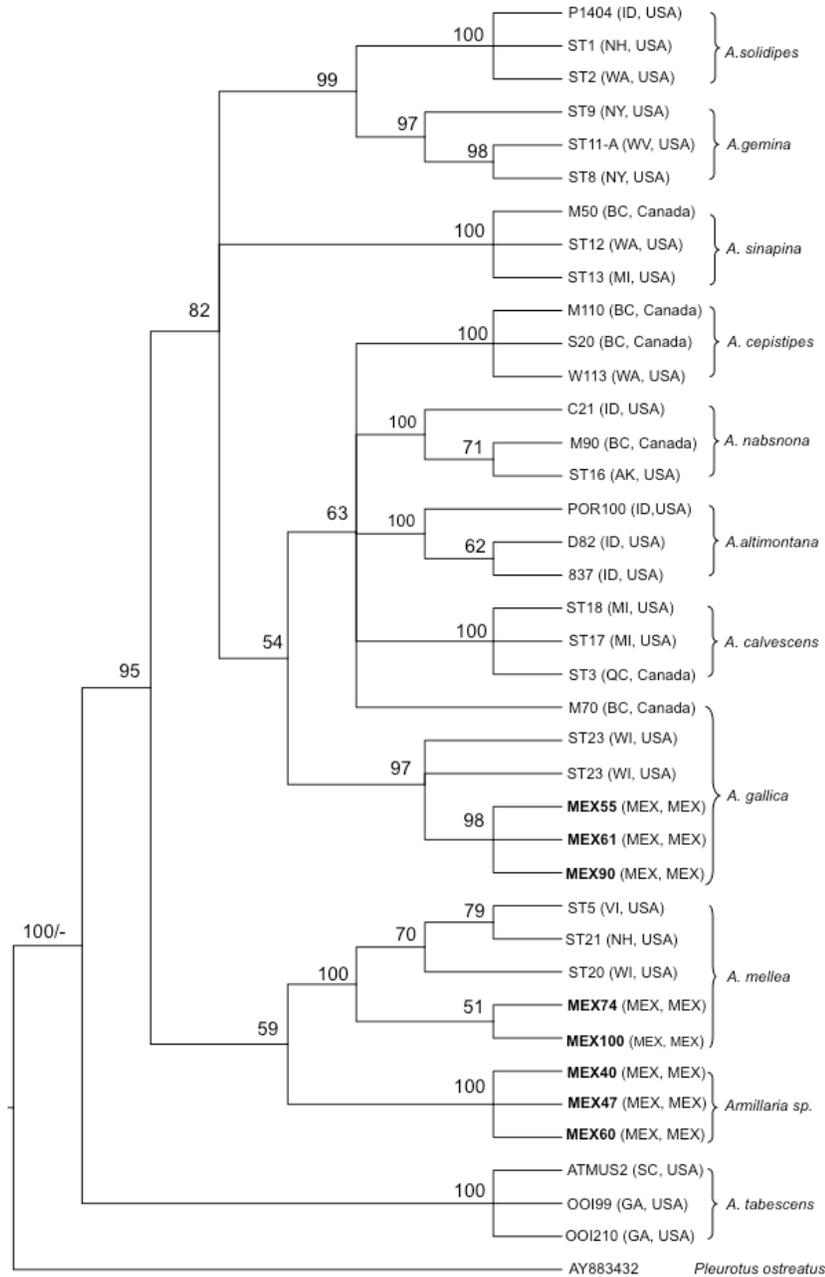
Figure 2. A 50 percent majority-rule bootstrap-consensus tree from the parsimony analysis of the translation elongation factor 1-alpha gene (*tef-1α*). Bootstrap supports are indicated above branches based on 1000 bootstrap replicates.

RESULTS AND DISCUSSION

Based on IGS1 sequences, all *Armillaria* isolates from infected peach trees could be assigned to three different taxa: Five of the 49 isolates were classified as *A. mellea*, eight isolates belonged to a single clade within the *A. gallica* complex, and the remaining 36 isolates were similar to each other, but distinct from other *Armillaria* species for which IGS1 sequences were available. Parsimony analysis of *tef-1α* sequences revealed the presence the *A. mellea*, *A. gallica*, and a unique clade that likely represents an undescribed species (Figure 2).

Figure 3. Mushrooms (basidiomata) of an undescribed *Armillaria* species frequently isolated from infected peach trees in México State.

The undescribed *Armillaria* sp. (Figure 3) is quite distinct from species typically found in association with Armillaria root disease of peach trees in the southeastern U.S. (*A. tabescens*) and México (*A. gallica*). Work is underway to formally describe this undescribed *Armillaria* species. Because this undescribed *Armillaria* sp. is quite damaging to peach production, it is important to document its distribution so appropriate disease management practices can be implemented. In addition, the development of specialized rootstock is perhaps required for resistance to this undetermined *Armillaria* sp. (Schnabel et al. 2005).

Understanding the distribution of *Armillaria* species in México is also critical to predict potentially invasive *Armillaria* species for other States in México. This information also lays a foundation for predicting potential influences of climate change on Armillaria root disease.

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