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CANKER PATHOGENS OF *POPULUS EUPHRATICA* OLIV. IN THE TARIM RIVER BASIN IN XINJIANG

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Abstract

Cryptosphaeria pullmanensis and *Cytospora chrysosperma* were isolated from the infected branches and twigs of *Populus euphratica,* naturally distributed at the edge of Tarim River in Xinjiang. Pathogens were identified based on morphological characteristics and molecular analysis. Both the organisms were found to be pathogenic.

Populus euphratica Oliv. is one of the oldest and most important tree naturally distributed at the edge of the Tarim River in Xinjiang. It plays a crucial role in protecting the vulnerable ecological balance and ensuring the production of oasis agriculture in the Tarim River basin (Pei *et al.* 2013). Anthropogenic activities, pests, diseases and for other reasons, the ecology and environment of *Populus euphratica* were deteriorated and destroyed for long time. Canker symptoms canker were observed on *P. euphratica* in 2015 in the Tarim River basin. The canker pathogens discolored the sapwood. The canker pathogens of *P. euphratica* were *Cytospora chrysosperma, C. germanica, C. atrocirrhata, C. fugax, C. translucens* (Zhuang 2005, Zhang *et al.* 2013, Wang *et al.* 2015). The purpose of the present study was to isolate and identify canker pathogens of *P. euphratica* in southern Xinjiang.

Canker symptoms of *P. euphratica* were collected from natural reserves in Xinjiang and was carried to the laboratory for isolation and identification of pathogen in summer 2015. A total of 14 isolates were isolated from surface-sterilized fragments of bark samples. The isolates were maintained on PDA medium at 4°C. The characteristics of the pycnidium and conidia of canker pathogens were observed under light microscope (Carl Zeiss Axio Imager M2, Germany).

Total genomic DNA of canker pathogens was extracted from PDA-grown 7-day-old mycelium using a standard hexadecyl trimethyl-ammonium bromide method according to the manufacturer's instructions (Doyle and Doyle 1987). The ribosomal DNA internal transcribed spacer (ITS) region was amplified using primers ITS1 and ITS4 (White *et al.* 1990). The β-tubulin gene was amplified using primers bt-2a and bt-2b (Choudhury *et al.* 2013). PCR amplification systems included 25 µl of 2× Power *Taq* PCR MasterMix, 1 µl of 20 µM each primer, 2 µl (100 ng) of fungal genomic DNA, and 21 µl of sterile ddH_2O in a total volume of 50 µl. The PCR amplification was performed on Biometra Thermoblock (Biometra® GmbH, Gőttingen, Germany) using the following thermal profile: initial denaturation 94°C for 5 min, followed by 30 cycles consisting of denaturation at 94°C for 30 sec, annealing 55°C/58°C for 30 sec (ITS annealing is 55°C, β-tubulin gene annealing is 58°C), and extension at 72°C f or 40 sec, and a final extension

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step at 72°C for 10 min was done at the end of the amplification. PCR amplification products were purified and were detected in agarose gel at 1% by electrophoresis, and sent to Beijing Liuhe Huada Gene Technology Co., Ltd. for sequencing. The phylogenetic analysis of sequencing was conducted using the MEGA5.0 program. The phylogenetic relationships of six selected isolates with closely related species were analyzed using the Neighbor-Joining method. Phylogeny was assessed using bootstrap analysis with 1000 replicates.

Pathogenicity of the isolates were confirmed by inoculating surface-sterilized 2-year-old *P. euphratica* twigs. Mycelial PDA plugs of each fungus were inoculated onto five twigs and maintained in a growth chamber at 27°C and 70 - 80% relative humidity. Controls were maintained with non-colonized PDA plugs.

A. *Cytospora chrysosperma* (teleomorph *Valsa sordida*) (Adams *et al.* 2005, Adams *et al.* 2006, Abbasi *et al.* 2015): Anamorph in culture. Fungal colonies pale white to faint yellow and regular margins on the surface. Reverse colour was dark orange yellow to brown. Colony texture was felty, slightly raised, copious, dark pycnidia developed on PDA. Conidiomata had white to yellowish white surfaces. Conidiomatal stromata immersed in bark, discoid, erumpent, circinately arranged, labyrinthine cytosporoid, 1 - 2 mm diam. Conidiomata nearly flat, white to grey, circular to ovoid, 0.4 - 0.5 mm diam., one ostiole per conidiomata. Ostioles dark grey or brown, at the same level as the disc surface. Locules multi-chambered, sub-divided frequently by invaginations into irregular chambers, sharing common walls. Conidiophores hyaline, conidia eguttulate, hyaline, one-celled, slightly curved, (3.5 ~ 5.0) μm × (0.9 ~ 1.4) μm. Teleomorph not seen.

B. *Cytosporina pullmanensis* (teleomorph *Cryptosphaeria pullmanensis*) (Glawe 2014, Ma *et al.* 2016, Mehrabi *et al.* 2017): Anamorph in culture. Fungal colonies on PDA medium were faint yellow fading to white-gray masses and irregular margins with age; reverse coloration bright yellow, fading to white-gray at margin. Colonies cover plates in 6 - 10 d, producing pray or black conidia in 14 - 16 d. Colony texture felty, protrusions with age. Conidiomata had white to brown surfaces during development. Conidiomata immersed in bark, cupulate, stromatic, multi-chambered, interwoven hyphae, emptying into common ostiole. Conidia produced on colonies were allantoid, with flattened bases, tapering toward apices, hyaline, (6.8 - 8.4) × (1.2 - 1.8) μm. Teleomorph not seen.

Comparisons with DNA sequences of canker pathogens of NCBI's Genbank (http://www.ncbi.nlm.nih.gov/) were compared with the sequences reported using BLASTN search method. Fourteen isolates from *P. euphratica* were identified by morphological features of culture, 18S rDNA-ITS and β-tubulin gene sequences analysis, out of which 7 representative strains were assigned to the genus level based on ITS sequence and β-tubulin sequence results (Fig. 1).

The resulting ITS sequence and β-tubulin gene sequence of representative strain of *C. chrysosperma* and *C.pullmanensis* were deposited in the database of NCBI's Genbank. Species identification of the pathogens were verified by its high sequence similarity (99.2%) of the complete internal transcribed spacer (ITS) sequence of 16 - 1 with the *C. pullmanensis* CFCC (KM588264) and high sequence similarity (100%) of the β-tubulin gene sequences of 16 - 1 with the *C. pullmanensis* CFCC89940(KM593249) (Fig. 2).

The DNA sequence data of the strains sets for the ITS and β-tubulin were analysed in combination, based on the phylogenetic analyses for 5.8S rDNA and β-tubulin, as well as the morphological characteristics, the fungus clearly confirmed that the identification results were consistent.

Pathogenicity of typical strains showed sunken lesion, brown or gray spot and inner bark discoloration after 7 days. Conidia were produced in late growth, all these were similar to those observed in forest and lateral discrete ostiolar beaks of conidiomata were visible on bark of twigs

(Fig. 1). The fungus was re-isolated from typical symptomatic twigs. Controlled twigs remained disease-free. Thus, the pathogenicity assay confirmed that *C. pullmanensis* and *C.chrysosperma* were the pathogens causing canker disease.

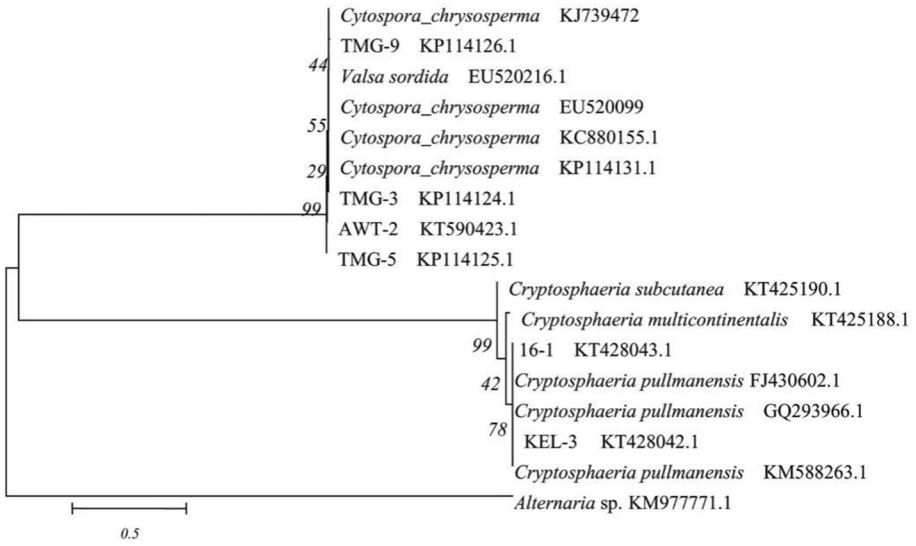

Fig. 1. Phylogenetic relationships among isolates generated through NJ analysis of internal transcribed spacer (ITS) rDNA sequences.

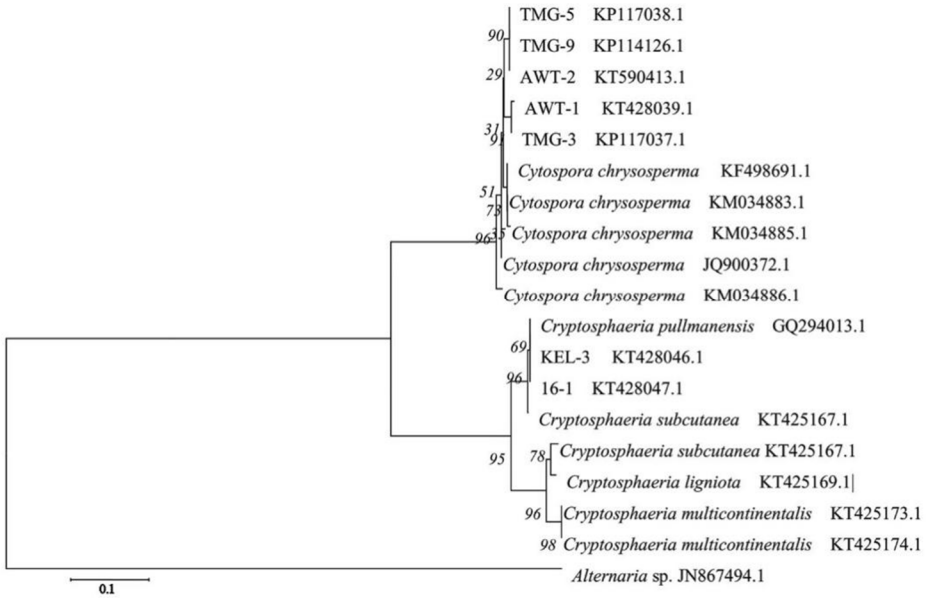

Fig. 2. Phylogenetic relationships among isolates generated through NJ analysis of β-tubulin gene sequences.

Species of *Cytospora* cause cankers and dieback on many genera of hardwoods. *Cytospora chrysosperma* is one of the main species to cause trunk diseases of willow and poplar (Adams *et al.* 2006, Abbasi *et al.* 2015, Wang and Wang 2020). Out of 14 isolates 12 were identified as *C. chrysosperma*, and the rest two were *C. pullmanensis.*

The anamorph of *C. pullmanensis* also resembles the form genus *C. chrysosperma* with respect to the multi-chambered conidiomata, separately emerging ostioles and allantoid conidia, Morphological characteristics were difficult to identify the fungi under the microscope (Sutton *et al.* 1980, Glawe 1984, Rappaz 1987). However, *C. pullmanensis* can be distinguished from species of *Cytospora* having some subtle differences by annellides, sympodulate, longer and larger conidia (Glawe 1984, Trouillas and Gubler 2015).

Cytosporina pullmanensis was less reported to cause canker on *Salix alba* and *Populus alba* in the world, especially in *P. euphratica* (Glawe 1984, Trouillas *et al.* 2011, Ma *et al.* 2016). Canker disease is one of the main diseases of Xinjiang Shelterbelt (Guo *et al.* 2018). The present study confirmed that the pathogens of canker on *P. euphratica* were *C.* p*ullmanensis* and *C. chrysosperma* in Southern Xinjiang.

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