



First report of walnut blight canker on walnut tree (*Juglans regia*) by *Pseudomonas flavescens* in South Korea

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Received: 12 May 2019 / Accepted: 1 February 2020 / Published online: 20 February 2020
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Keywords Walnut tree · *Pseudomonas flavescens* · Walnut blight canker

Severely infected walnut trees were observed in six regions of South Korea via a survey of tree diseases that was carried out to identify tree pathogens between 2016 and 2017. Disease symptoms included shoot blight, canker on the branches or twigs and one to several black lesions that appeared on catkins, leaves and nuts. Over 300 bacterial colonies were isolated from infected tissues using nutrient agar medium. Of those, isolates that had rod-shaped colonies, yellow pigments on NA medium and fluorescent pigments produced under UV when grown in King's B agar medium, which is typical characteristics of *Pseudomonas* species, were selected (King et al. 1954). Six representative *Pseudomonas* species, representing each geographical region, were randomly selected and sequenced. All the sequences generated were deposited to the NCBI database (accession Nos. MH709127, MH714738–42). To ensure the identity of the *Pseudomonas* species, three house-keeping genes and 16S rRNA gene region were combined (Hilario et al. 2004), aligned and subsequently used to construct a phylogenetic tree based on a Maximum likelihood analysis. The phylogenetic tree resulted in a supported placement of the *Pseudomonas* strains obtained in this study (strain NIFOS2) with authenticated isolates of *P. flavescens* retrieved from GenBank, confirming its identification as *P. flavescens*. To confirm the pathogenicity, the bacterial suspension made from the strain NIFOS2 was inoculated to the leaves and fruits wounded by a sterile needle. The tested leaves and fruits were placed at 28 °C for 7 days. Necrotic lesions and black spots were observed in 7 days after the inoculation, but not on the controls treated with sterile water. Re-isolations were

successfully made from the inoculated leaves and fruits, fulfilling the Koch's postulates. To our knowledge, this is the first report of walnut blight canker on walnut trees (*J. regia*) caused by *P. flavescens* in South Korea.

Acknowledgements The authors acknowledge the financial support from the National Institute of Forest Science, 'Survey on occurrences of forest pathogens and pests program, no. FE0703-2016-01'.

Compliance with ethical standards

Ethical statements This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Hilario E, Buckley TR, Young JM (2004) Improved resolution on the phylogenetic relationships among *Pseudomonas* by the combined analysis of atp D, car A, rec A and 16S rDNA. *Antonie Van Leeuwenhoek* 86:51–64
- King EO, Ward MK, Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescin. *J Lab Clin Med* 44:301–307

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