Monika Kałużna[1], Andjelka Prokić[2], Virginia O. Stockwell[3], Aleksa Obradović[2], Joël F. Pothier[4]

[1] *The National Institute of Horticultural Research, Skierniewice, Poland.*
[2] *University of Belgrade, Faculty of Agriculture, Belgrade, Serbia.*
[3] *United States Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Unit, Corvallis, OR, USA.*
[4] *Environmental Genomics and Systems Biology Research Group, Institute for Natural Resource Sciences, Zurich University of Applied Sciences (ZHAW), Wädenswil, Switzerland*

Specific and sensitive detection systems for *Xanthomonas arboricola* pv. *corylina* - the causal agent of bacterial blight of hazelnut based on comparative genomics

Xanthomonas arboricola pv. *corylina* (*Xac*) is responsible for bacterial blight on hazelnut (*Corylus avellana* L., Kałużna et al., 2021). The disease was first reported in the early 20th century in Oregon. To limit the risk of introduction to other countries especially via planting material, this bacterium was listed by the European Plant Protection Organization (EPPO) as A2 quarantine pathogen in 1978 and as Regulated Non-Quarantine Pest (RNQP) since 2020. *Xac* affects mainly nurseries and young orchards by causing significant plant mortality. However, the disease occurs also very often in production crops, especially on susceptible cultivars. Although the disease is devastating and constitutes a major cause of yield losses, causing even 100% death of young trees and planting material, fast, specific and sensitive detecting systems for *Xac* were not yet developed. In the work presented here, specific primers pairs designed to detect the causal agent of hazelnut bacterial blight *Xac* are described.

Material and methods

Comparative genomics

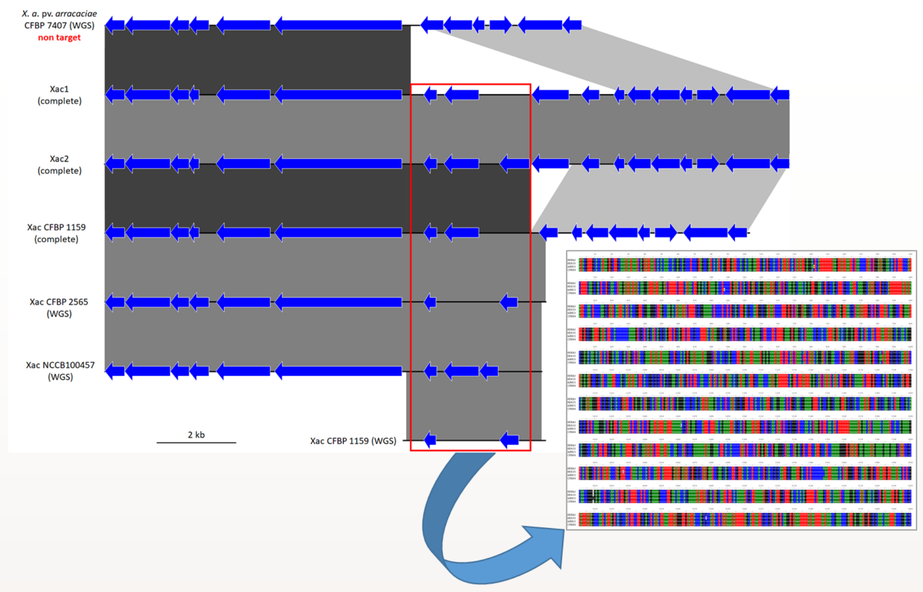
- Conserved and specific molecular markers identified using a **'dual-BLAST' approach**
- Three publicly available WGS genomes used as input/target (NCCB 100457, CFBP 1159^{PT}, CFBP 2565)
- Segmentation into 300 bp length fragments wi. 50 bp overlaps + duplicate removal (*n* = 61'114)
- Highly conserved molecular markers validated against a local *nt* BLAST database (*n* = 17'155)
- Specific molecular markers re-assembled (from *n* = 93 to 63)
- Further specificity analysis against: 1) *X. arboricola* WGS online, 2) *Xanthomonas* WGS online, 3) *nr*/*nt* online, and 4) two in house *Xac* complete genomes (Pothier et al., 2022)
- A 1-kb region identified as highly conserved and *Xac*-specific, extended to 2.4 kb with comparative genomics

Primers

- Conventional PCR - 6 primers pairs (of 8) – 197 bp to 1455 bp
- Real-time PCR SYBR Green I - 4 primers pairs (of 6) – 88 bp to 170 bp
- Real-time PCR TaqMan - 2 primers pairs (of 2) – 155 bp to 198 bp
- Loop-mediated Isothermal Amplification (LAMP) - 3 primers sets

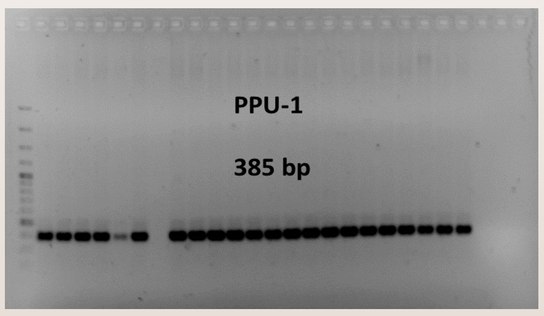
DNA tested (102 in total)

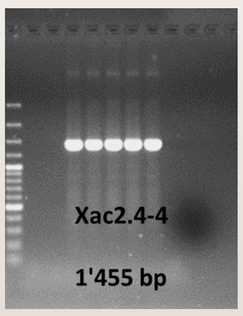
- *Xac* strains including CFBP 1159^{PT}, LMG 688
- *X. a.* pv. *arracaciae* CFBP 7407^{PT}, *X. a.* pv. *guizotiae* CFBP 7408^{PT}
- *X. a.* pv. *zantedeschiae* CFBP 7410^{PT}, *X. a.* pv. *celebensis* CFBP 3523^{PT}
- *X. a.* pv. *fragariae* CFBP 6771^{PT}, *X. a.* pv. *populi* CFBP 3123^{PT}
- *X. a.* pv. *pruni* CFBP 2535^{PT}, *X. a.* pv. *juglandis* CFBP 2528^{T} and CFBP 7179
- *Pseudomonas avellanae* CFBP 4060
- Pathogenic and non pathogenic *Pseudomonas* spp. and other isolates from hazelnut/walnut
- Fungi isolated from diseased hazelnut/walnut
- DNA isolated from visually healthy leaves of five hazelnut cultivars
- Naturally and artificially infected plant material

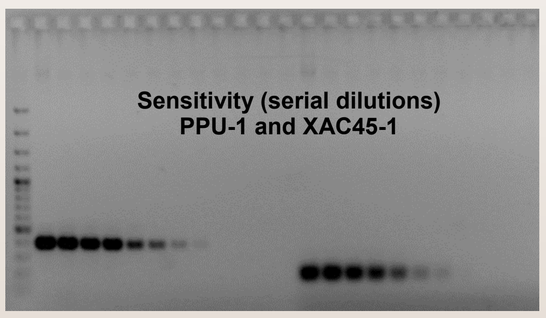


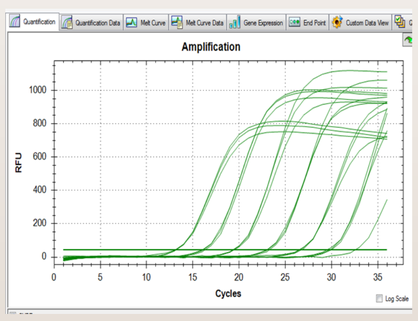
Results

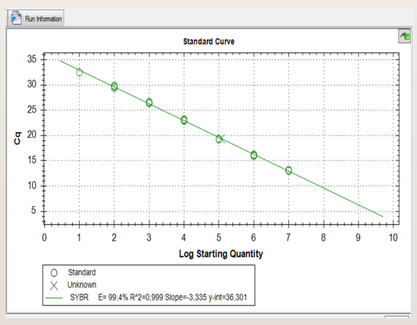
- In the specificity assays with the DNA of bacterial and fungal strains the positive reaction product were observed only when targeted *Xac* DNA was used.
- Analysis of total DNA from plant isolated from of visually healthy leaves excluded potential unspecific products.
- The sensitivity of the *Xac* target DNA were: 100 fg to 10 pg for conventional PCRs, 1 fg to ~ 10 fg for real-time PCRs, 1 pg for LAMP. In case of detection in plant material the limit of detection was 10^1-10^2 x lower.
- All primers pairs allowed for detection of *Xac* in artificially and naturally infected plant material.











Conclusion and outlook

- So far, the first reliable and specific systems targeting the economically relevant bacterial pathogen of hazelnut.
- Primers for different PCR techniques making them more useful for a wide group of researchers according to available lab equipment and skills.
- Specific primers allow for greatly shortening the time required for diagnosis, while highly increasing assay accuracy and lowering detection limit even pg of DNA directly in the plant material.
- Relatively simple and inexpensive, and it does not require the time-consuming step of pre-incubation on microbiological media.

This work was financed by the National Science Centre, Poland, Grant UMO- 2017/26/M/NZ9/01024 “The threat of crop plants by *Xanthomonadaceae* with particular emphasis on the invasive species *Xanthomonas arboricola* and *Xylella fastidiosa* and vectors“. Based upon work from COST Action CA16107 EuroXanth supported by COST (European Cooperation in Science and Technology).
The authors would like to thank the HPC team of the School for Life Sciences and Facility Management at ZHAW for the compute resources and support and Profesor Ewa Zalewska from University of Life Sciences in Lublin, Dr. Bill Weldon from the Oregon Agricultural Research Service, and all producers for providing diseased plant material.