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SHORT COMMUNICATION

Genetic diversity of *Erwinia amylovora* isolates from fire blight diseased trees in Central and Eastern Georgia

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Abstract
The genetic diversity of 52 *Erwinia amylovora* isolates from pome fruit trees with fire blight in Central and Eastern Georgia from the years 2020–2022 was examined using specific PCR and genotyping of CRISPR repeat regions 1 and 2. The analysis identified three distinct genotypes: (A, a, α), matching historical Western European strains; (A, z, α), distinctive for Georgia and differing by a three-spacer deletion in CRR2 (2034–2036); as well as novel genotype (A, ä, α), which was not observed previously. Genotypes (A, a, α) and (A, z, α) were found to coexist geographically in all four regions investigated, whereas genotype (A, ä, α) was reported only in one case in the region of Kvemo Kartli. On separate instances, multiple genotypes were detected even within the same orchard or tree, illustrating the complex genetic landscape of *E. amylovora* in the country.

Keywords Apple · Pear · Quince · Genotyping · Caucasus

Fire blight, caused by *Erwinia amylovora*, is a bacterial disease that affects plants in the Rosaceae family, in particular in pome fruit trees (Vanneste 2000). It causes wilted, blackened blossoms, shoots, and branches with a scorched appearance that resemble fire damage (Johnson 2000). The disease can lead to severe economic losses in fruit orchards due to its rapid local spread under favorable conditions, such as warm, humid environments during the flowering period, and through wind and rainfall, potentially leading to the eradication of entire orchards (Van der Zwet and Keil 1979). The long-distance spread of the bacteria is primarily attributed to the trade of plant material (Thomson 2000). Originally indigenous to North America, fire blight is now present in more than 50 countries worldwide (Anonymous 2022). Management strategies often involve a combination of cultural practices, chemical control measures, and the selection of resistant plant varieties (Gusberti, 2015). Timely interventions, such as pruning infected material and the application of preventive measures during high-risk periods, are crucial for minimizing the disease's impact (DuPont et al. 2023).

Georgia's diverse ecosystems, resulting from its varied soils and climates, support a rich local gene pool and autochthonous varieties of pome fruits, especially in the main production region of Shida Kartli, in the center of the country. After the collapse of the Soviet Union, Georgia experienced a prolonged economic and humanitarian crisis that severely impacted its fruit production sector. The resurgence of fruit orchards began with the importation of fruit tree seedlings from various countries and coincided with the initial fire blight outbreak in the Mtskheta-Mtianeti region in 2016 (Gaganidze et al. 2018). Shortly after this first appearance in apple seedlings, fire blight was detected in other pome fruit plants, such as pear and quince, in three other regions of Central-Eastern Georgia (Shida Kartli, Kvemo Kartli, and Kakheti), as well as in the western region of Imereti (Gaganidze et al. 2021). Surveys conducted from 2016 to 2020 reported around 200 different instances of fire blight

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affecting all five aforementioned regions and common pome fruit species such as apples, pear and quince (Gaganidze et al. 2021). Isolates recovered from the affected trees exhibited some phenotypical variability, such as differences in esculin hydrolysis or the ability to utilize certain sugars (Gaganidze et al. 2021; Amashukeli et al. 2023).

E. amylovora is otherwise a remarkably homogeneous species (Smits et al., 2010), both biochemically (Dye 1981; Holt et al. 1994; Paulin 2000) as well as genetically, particularly within the Widely Prevalent (WP) group that in the 20th century was disseminated worldwide from the East Coast of the United States (Parcey et al. 2020). Despite that, genotyping based on hypervariable regions of the genome, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs), enables the identification and differentiation of specific strains within the population (Rezzonico et al. 2011; McGhee and Sundin 2012). Investigating the genetic diversity of *E. amylovora* in Georgia is important to understand the diversity and evolution of the pathogen, as well as its introduction and dissemination patterns, thus laying the foundation for effective containment strategies in the country. Sequencing the CRISPR regions of four Georgian isolates from the years 2016–2018 (GE01-GE04) revealed two distinct genotypes: genotype (A, a, α), matching those of the earliest strains isolated in Europe (Rezzonico et al. 2011; Kurz et al. 2021), and a novel genotype (A, z, α) recognizable by the deletion of three contiguous spacers (2034–2036) next to the leader-proximal region of CRISPR Repeat Region 2 (CRR2) (Gaganidze et al. 2021). Further detections of fire blight foci occurred in the 2020–2022 seasons, with additional 52 *E. amylovora* isolates recovered from infected apple, pear, and quince trees across seven municipalities within four regions of Central and Eastern Georgia: Shida Kartli (Gori, Kareli, Khashuri), Kvemo Kartli (Marneuli), Mtskheta-Mtianeti (Mtskheta), and Kakheti (Sighnaghi, Lagodekhi) (Table 1). Identity of these isolates as *E. amylovora* was confirmed by PCR using the primers pairs A and B as previously described (Bereswill et al. 1992; Amashukeli et al. 2023). A study of their phenotypic characteristics revealed variability in esculin hydrolysis and the utilization of certain sugars and polyols (Amashukeli et al. 2023). The aim of this work was to genotype the two active CRRs of these new isolates using a polyphasic approach combining PCR-based typing (Rezzonico et al. 2011) and DNA sequencing.

Genetic diversity was assessed by PCR length polymorphism of CRR1 and CRR2 utilizing specific primer pairs flanking spacer deletions previously identified in Georgian isolates through sequencing of their CRISPR regions (Gaganidze et al. 2021). Reactions were performed in a total volume of 25 µl, including 12.5 µl of 2x PCR BIO Tag Mix (PCR Biosystems, London, United Kingdom), 1.25 µl of cell lysate from a 1:20-diluted overnight culture in Luria-Bertani medium, and primer pairs C1f04/C1r09 (CRR1) and C2f01/C2r03 (CRR2) at a final concentration of 0.2 µM and 0.4 µM, respectively (Rezzonico et al. 2011). The thermal cycling protocols consisted of initial denaturation of 3 min at 95 °C, followed by 35 cycles of denaturation at 95 °C, annealing at 60 °C, and elongation at 72 °C, with the times for each step being 15 and 30 s for CRR1 and CRR2, respectively. The final elongation consisted of 3 min at 72 °C, followed by cooling at 4 °C (Kurz et al. 2021). PCR products were separated by electrophoresis for 40 min at 80 V on a 1.5% agarose gel in TAE buffer containing ethidium bromide and visualized in a UV transilluminator (Sambrook et al. 2000). 100-bp DNA Ladder (New England Biolabs, Ipswich, USA) was used as size marker.

PCR amplification of CRR1 revealed that all Georgian isolates amplify the 276-bp fragment that is characteristic for the duplication of spacer 1029 in CRR1, which is typical for archetypal genotype A (Fig. 1A) (Kurz et al. 2021), whereas for CRR2 three distinct amplicon sizes (743 bp, 682 bp and 561 bp) were detected (Fig. 1B). Five representative isolates (GE6053, GE6163, GE6931, GE13011, and GE13133) were selected, based on their PCR amplification patterns and geographical origin, for whole genome sequencing using Illumina technology as described elsewhere (Pothier et al. 2022). The spacer composition of their CRRs was extracted using CRISPRfinder (Grissa et al. 2007) and resulted in the characterization of a third CRR2 genotype (named ä) that is present in the country, alongside the two (a and z) that were already described previously (Gaganidze et al. 2021). On the other hand, no variability could be found in the other CRISPR arrays, which matched CRR1 and CRR4 genotypes A and a, respectively (Fig. 2). Genotype (A, a, α), represents the most complete spacer arrays among the WP group of *E. amylovora* and corresponds to the oldest CRISPR genotype ever detected in Europe (Rezzonico et al. 2011). Genotype (A, z, α) differs from (A, a, α) by the deletion of three contiguous spacers (2034–2036) near the leader-proximal region of CRR2 (Rezzonico et al. 2011) and was also previously identified in an earlier work with Georgian isolates from the years 2016–2018 (Gaganidze et al. 2021). A novel genotype denominated (A, ä, α) was found in isolate GE13011 from the municipality of Marneuli in the Kvemo Kartli region and is characterized by the deletion of 5'-terminal spacer 2037 in CRR2, thus allowing for further genotypic diversification within this highly homogeneous species. A list of all the analyzed strains, along with their genotypes, host plants, isolation years, and geographical origins, is presented in Table 1. Fire blight could be detected in all four regions of Eastern Georgia under investigation and the spatio-temporal distribution of the different genotypes is depicted in Fig. 3. In Shida Kartli, the primary fruit-growing region, fire blight

Table 1 List of *Erwinia amylovora* isolates from Georgia used in this work

Isolate	Geographic origin				
	Region	Municipality, village	Host	Year	Genotype
GE01	Shida Kartli	Kareli	Apple	2016	A, z, α
GE02	Kvemo Kartli	Marneuli	Apple	2018	A, z, α
GE03	Kvemo Kartli	Marneuli	Apple	2018	A, a, α
GE04	Shida Kartli	Gori	Apple	2018	A, a, α
GE1012	Mtskheta-Mtianeti	Mtskheta, Ksovrisi	Apple	2020	A, z, α
GE1022		Mtskheta, Ksovrisi	Apple	2020	A, z, α
GE1023		Mtskheta, Ksovrisi	Apple	2020	A, z, α
GE1131		Mtskheta, Ksovrisi	Apple	2020	A, z, α
GE1133		Mtskheta, Ksovrisi	Apple	2020	A, z, α
GE1212	Shida Kartli	Kareli, Kekhijvari	Apple	2020	A, z, α
GE1412		Gori, Karaleti	Apple	2020	A, z, α
GE2763		Khashuri, Osiauri	Pear	2020	A, z, α
GE2772		Khashuri, Osiauri	Pear	2020	A, z, α
GE3833	Kakheti	Lagodekhi, Chabukiani	Quince	2020	A, a, α
GE5831		Sighnaghi, Jugaani	Apple	2021	A, z, α
GE5833		Sighnaghi, Jugaani	Apple	2021	A, z, α
GE5941		Sighnaghi, Jugaani	Apple	2021	A, a, α
GE6052		Sighnaghi, Jugaani	Apple	2021	A, a, α
GE6053		Sighnaghi, Jugaani	Apple	2021	A, a, α
GE6162 *		Sighnaghi, Jugaani	Apple	2021	A, a, α
GE6163 *		Sighnaghi, Jugaani	Apple	2021	A, z, α
GE6821	Shida Kartli	Khashuri, Gomi	Apple	2021	A, z, α
GE6822		Khashuri, Gomi	Apple	2021	A, z, α
GE6931		Khashuri, Gomi	Quince	2021	A, z, α
GE7151		Khashuri, Gomi	Quince	2021	A, z, α
GE7261		Khashuri, Gomi	Quince	2021	A, z, α
GE7371		Khashuri, Gomi	Quince	2021	A, z, α
GE7372		Khashuri, Gomi	Quince	2021	A, z, α
GE7481		Khashuri, Gomi	Apple	2021	A, z, α
GE7591		Khashuri, Gomi	Pear	2021	A, z, α
GE7610		Khashuri, Gomi	Pear	2021	A, z, α
GE8012		Gori, Karaleti	Apple	2021	A, z, α
GE8013		Gori, Karaleti	Apple	2021	A, z, α
GE8121		Gori, Karaleti	Apple	2021	A, a, α
GE8672		Gori, Karaleti	Pear	2021	A, z, α
GE8673		Gori, Karaleti	Pear	2021	A, z, α
GE8782		Gori, Karaleti	Apple	2021	A, z, α
GE8892		Gori, Karaleti	Apple	2021	A, z, α

Table 1 (continued)

Isolate	Geographic origin				
	Region	Municipality, village	Host	Year	Genotype
GE12421	Kvemo Kartli	Marneuli, Kachagani	Apple	2022	A, z, α
GE12431		Marneuli, Kachagani	Apple	2022	A, z, α
GE12521		Marneuli, Kachagani	Apple	2022	A, z, α
GE12621		Marneuli, Kachagani	Apple	2022	A, z, α
GE12631		Marneuli, Kachagani	Apple	2022	A, z, α
GE12711		Marneuli, Kachagani	Apple	2022	A, z, α
GE12721		Marneuli, Kachagani	Apple	2022	A, z, α
GE12731		Marneuli, Kachagani	Apple	2022	A, z, α
GE12821		Marneuli, Kachagani	Apple	2022	A, z, α
GE12831		Marneuli, Kachagani	Apple	2022	A, z, α
GE12931		Marneuli, Kachagani	Apple	2022	A, z, α
GE13011 #,^		Marneuli, Kachagani	Apple	2022	A, ä, α
GE13021 #,^		Marneuli, Kachagani	Apple	2022	A, z, α
GE13131 §,^		Marneuli, Kachagani	Apple	2022	A, z, α
GE13132 §,^		Marneuli, Kachagani	Apple	2022	A, z, α
GE13133 §,^		Marneuli, Kachagani	Apple	2022	A, z, α
GE13134 §,^		Marneuli, Kachagani	Apple	2022	A, a, α
GE14331		Kareli, Tatanaantubani	Apple	2022	A, a, α

Isolates marked with superscripts originate from the same field (* and ^) or tree (# and §). Isolates GE01-GE04 were previously characterized elsewhere (Gaganidze et al. 2021)

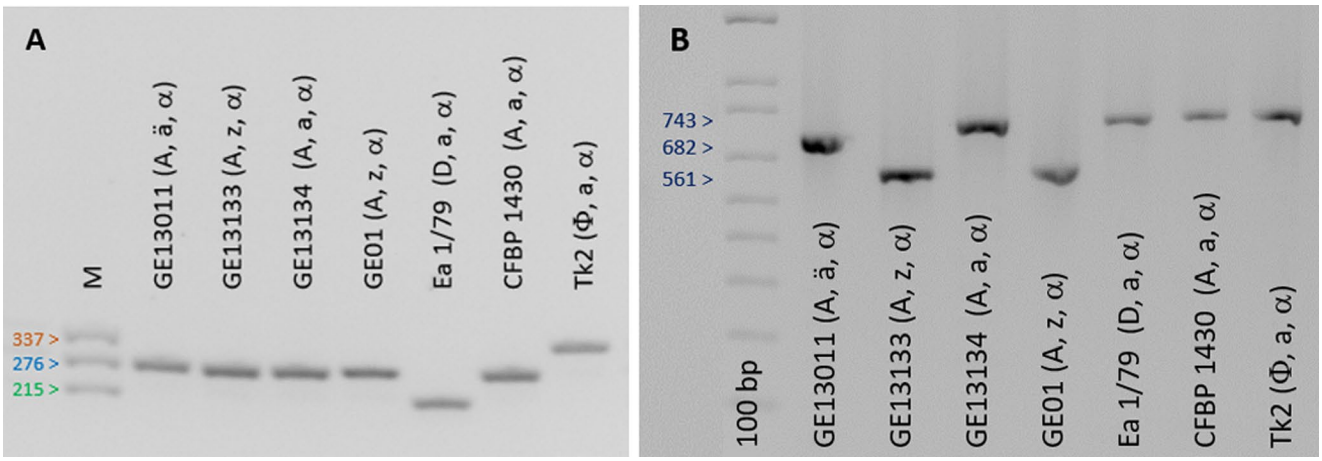

Fig. 1 (**A**) PCR amplification of the CRR1 around spacer 1029 in representative isolates of *E. amylovora* using primers C1f04 and C1r09. All isolates from Georgia were shown to bear two copies of spacer 1029 (A-derived genotype) that resulted in a 276 bp amplicon. Strains Ea 1/79 (215 bp, Germany, 1979), CFBP 1430 (276 bp, France, 1972) and Tk2 (337 bp, Turkey, 2012) with one, two and three copies of spacer 1029 were used as references. (**B**) PCR amplification of the 5'-terminal region of CRR2 using primers C2f01 and C2r03. In the Georgian isolates three disctinct genotypes were observed: beside the complete CRR2 array in *E. amylovora* GE13134 (genotype a), the two other isolates GE13011 and GE13133 displayed shorter amplicon revealing the loss of one (genotype ä) and three spacers (genotype z), respectively. All three reference strains corresponded to the CRR2 genotype a as expected, thus yielding a 743 bp amplicon

was confirmed in three municipalities, whereby two genotypes (A, a, α) and (A, z, α) were detected in Gori and Kareli, while only the latter was spotted in Khashuri. The situation is similar in the Kakheti region, with both genotypes found in the diseased pome fruit trees of the Sighnaghi municipality, while only archetypal genotype (A, a, α) could be detected in Lagodekhi. In the Mtskheta-Mtianeti region, only genotype (A, z, α) was found, while in the Marneuli municipality in Kvemo Kartli all three genotypes (A, a, α), (A, z, α), and (A, ä, α) were present.

After three decades of relative genetic stability (Rezzonico et al. 2011), the emergence of novel CRISPR genotypes in the WP group of *E. amylovora* has been noticed with increasingly frequency in Europe and in Asia, possibly suggesting an ongoing adaptation process (Doolotkeldieva et al. 2021; Kurz et al. 2021). Although this process prevalently occurs through spacer(s) deletions within the

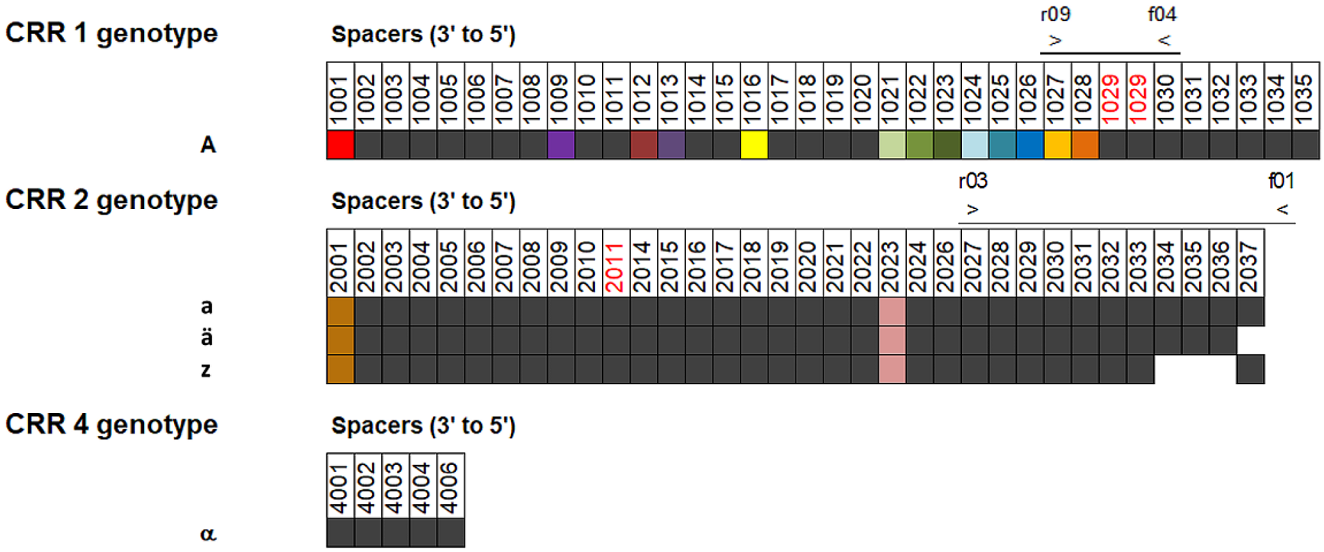

Fig. 2 Spacer composition of the CRISPR Repeat Regions (CRRs) genotypes found in Georgian isolates investigated in this work. The arrays are oriented in the 3'-to-5' direction with the newest spacers next to the leader sequence on the right side of the picture. Numbering and coloring of the spacers is coherent with that proposed by Rezzonico et al. (2011). CRR2 genotypes ä and z can be derived from genotype a through the deletion of spacers 2037 and 2034–2036, respectively. The position and direction of the primers used for PCR screening is indicated above the respective arrays

Fig. 3 Distribution of *Erwinia amylovora* CRISPR genotypes in isolates retrieved from different regions and municipalities of Central and Eastern Georgia. The number of isolates, their genotype and year of isolation are coded within the shape according to the figure legend. Although fire blight is present also in Imereti, no isolate from that region is included in this study

CRR, the sporadic addition of single new spacers next to the 3'-end of the array has also been observed (Rezzonico et al. 2011; Mendes et al. 2021). Several novel genotypes have been revealed also among *E. amylovora* populations detected on wild apple and pear species in the walnut-fruit forest of Kyrgyzstan hinting to a discrete geographical distribution of the different subpopulations in the different part of the country (Doolotkeldieva et al. 2021).

In our analysis, including 56 isolates from the time frame 2016 to 2022, the (A, z, α) genotype is overall numerically predominant, accounting for 45 isolates, while the (A, a, α) and (A, ä, α) genotypes are represented by ten and one isolates, respectively (Table 1). This prevalence is particularly evident in Shida Kartli and in Kvemo Kartli, where the (A, z, α) genotype represented 87.5% and 84.2% of the analyzed isolates, respectively. On the other hand, in Kakheti ($n=8$) a small prevalence of the (A, a, α) genotype was found and in Mtskheta-Mtianeti ($n=5$) all the isolates belonged to the (A, z, α) genotype, but in both cases the sample size is possibly too small to generalize the results. Overall, these data

indicate the possible dominance of the (A, z, α) genotype in Central-Eastern Georgia, although the ancestral genotype (A, a, α) seems also to be geographically quite widespread. The single occurrence of the (A, ä, α) genotype in Kvemo Kartli is suggestive of the emergence of a new local sequence variation that was never previously reported.

The distribution of these three *E. amylovora* genotypes does not appear to adhere to discrete regional patterns. Several instances of multiple genotypes in a single garden or even a single tree underscore the complex dynamics of fire blight spread in the country. Isolates GE6162 (A, a, α) and GE6163 (A, z, α) or GE13011 (A, ä, α) and GE13021 (A, z, α) were obtained from single apple trees in Sighnaghi (Kakheti) and in Marneuli (Kvemo Kartli), respectively. In the latter municipality, another apple tree yielded a total of four isolates, three of which (GE13131, GE13132 and GE13133) belonged to the (A, z, α) genotype, while a fourth (GE13134) displayed genotype (A, a, α) (Table 1). Confirming the results of previous studies, no correlation between bacterial genotype and host plant species could be identified, indicating a broader adaptability of *E. amylovora* genotypes from the WP group on different plants of the Amygdaloideae family (Momol and Aldwinckle 2000).

This variability in genotype distribution across host plants and geographical locations emphasizes the intricate dynamics of fire blight in the country and highlights the importance of continued surveillance and deeper genetic analysis to understand its spread and impact in the region. As the sequence of the CRRs alone has revealed itself not to be sufficient to deduce the introduction routes of *E. amylovora* to Georgia, comparative genome analysis with strains from the surrounding countries and beyond will be required in the future to tackle these critical questions.

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Ethical approval Authors declare that the manuscript is entirely original and have not been, and will not be, published elsewhere if accepted for publication in JPP. The content and authorship of the submitted manuscript has been approved by all authors, and all prevailing local, national, and international regulations and conventions, and normal scientific ethical practices have been respected. Authors declare no competing interests. This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Amashukeli N, Gaganidze D, Aznarashvili M, Kharadze S, Sturua N, Rezzonico F, Sadunishvili T (2023) Phenotypic variability of *Erwinia amylovora* from pome fruits in Georgia. Bull Georgian Natl Acad Sci 17(3):69–74. http://science.org.ge/bnas/vol-17-3.html

Anonymous (2022) PM 7/20 (3) *Erwinia amylovora*. EPPO Bull 52:198–224. https://doi.org/10.1111/epp.12826

Bereswill S, Pahl A, Bellemann P, Zeller W, Geider K (1992) Sensitive and species-specific detection *of Erwinia amylovora* by polymerase chain reaction analysis. Appl Environ Microb 58:3522–3526. https://doi.org/10.1128/aem.58.11.3522-3526.1992

Doolotkeldieva T, Bobushova S, Carnal S, Rezzonico F (2021) Genetic characterization of *Erwinia amylovora* isolates detected in the wild walnut-fruit forest of South Kyrgyzstan. J Plant Pathol 103(S1):109–120. https://doi.org/10.1007/s42161-021-00752-1

DuPont ST, Munir M, Cox K, Johnson K, Peter K, Baro A (2023) Evaluation of pruning therapies in apple trees with fire blight. J Plant Pathol 105:1695–1709. https://doi.org/10.1007/s42161-023-01447-5

Dye DW (1981) A numerical taxonomic study of the genus *Erwinia*. N Z J Agr Res 24:223–229

Gaganidze DL, Aznarashvili MA, Sadunishvili TA, Abashidze EO, Gureilidze MA, Gvritishvili ES (2018) Fire blight in Georgia. Annals Agrarian Sci 16:12–16. https://doi.org/10.52340/idw.2021.488

Gaganidze D, Sadunishvili T, Aznarashvili M, Abashidze E, Gurielidze M, Carnal S et al (2021) Fire blight distribution in Georgia and characterization of selected *Erwinia amylovora* isolates. J Plant Pathol 103(S1):121–130. https://doi.org/10.1007/s42161-020-00700-5

Grissa I, Vergnaud G, Pourcel C (2007) CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res W52–57 35(Web Server issue). https://doi.org/10.1093/nar/gkm360

Gusberti M, Klemm U, Meier MS, Maurhofer M, Hunger-Glaser I (2015) Fire blight control: the struggle goes on. A comparison of different fire blight control methods in Switzerland with respect to Biosafety, Efficacy and Durability. Int J Environ Res Public Health 12(9):11422–11447. https://doi.org/10.3390/ijerph120911422PMID: 26378562; PMCID: PMC4586684

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Bergey's Manual of determinative bacteriology, 9th edn. Williams and Wilkins, Baltimore, Maryland, USA

Johnson KB (2000) Fire blight of apple and pear. Plant Health Instructor. https://doi.org/10.1094/PHI-I-2000-0726-01

Kurz M, Carnal S, Dafny-Yelin M et al (2021) Tracking the dissemination of *Erwinia amylovora* in the eurasian continent using a PCR targeted on the duplication of a single CRISPR spacer. Phytopathol Res 3:18. https://doi.org/10.1186/s42483-021-00096-9

McGhee GC, Sundin GW (2012) *Erwinia amylovora* CRISPR elements provide new tools for evaluating strain diversity and for microbial source tracking. PLoS ONE 7:e41706. https://doi.org/10.1371/journal.pone.0041706

Mendes RJ, Luz JP, Santos C, Tavares F (2021) CRISPR genotyping as complementary tool for epidemiological surveillance of *Erwinia amylovora* outbreaks. PLoS ONE 16:e0250280. https://doi.org/10.1371/journal.pone.0250280

Momol MT, Aldwinckle HS (2000) Genetic diversity and host range of *Erwinia amylovora*. In: Vanneste JL (ed) Fire blight. The disease and its causative agent, Erwinia amylovora. CABI Publishing, Wallingford, pp 55–72. https://doi.org/10.1079/9780851992945.0055

Parcey M, Gayder S, Morley-Senkler V, Bakkeren G, Úrbez-Torres JR, Ali S, Castle AJ, Svircev AM (2020) Comparative genomic analysis of *Erwinia amylovora* reveals novel insights in phylogenetic arrangement, plasmid diversity, and streptomycin resistance. Genomics 112:3762–3772. https://doi.org/10.1016/j.ygeno.2020.04.001

Paulin JP (2000) *Erwinia amylovora*: general characteristics, biochemistry and serology. In: Vanneste JL (ed) Fire blight. The disease and its causative agent, *Erwinia amylovora*. CABI Publishing, Wallingford, pp 87–115. https://doi.org/10.1079/9780851992945.0087

Pothier JF, Kałużna M, Prokić A, Obradović A, Rezzonico F (2022) Complete genome and plasmid sequence data of three strains of *Xanthomonas arboricola* Pv. *Corylina*, the bacterium responsible for bacterial blight of hazelnut. Phytopathology 112:956–960. https://doi.org/10.1094/PHYTO-08-21-0356-A

Rezzonico F, Smits THM, Duffy B (2011) Diversity and functionality of CRISPR regions in fire blight pathogen *Erwinia amylovora*. Appl Environ Microbiol 77:3819–3829. https://doi.org/10.1128/AEM.00177-11

Sambrook J, Fritsch ER, Maniatis T (2000) Molecular cloning: a Laboratory Manual, 3rd edn. Cold spring, Harbor Laboratory, New York

Smits THM, Rezzonico F, Kamber T, Blom J, Goesmann A, Frey FE, Duffy B (2010) Complete genome sequence of the fire blight pathogen *Erwinia amylovora* CFBP 1430 and comparison to other *Erwinia* species. Mol Plant Microbe Interact 23:384–393. https://doi.org/10.1094/MPMI-23-4-0384

Thomson SV (2000) Epidemiology of fire blight. CABI Books. https://doi.org/10.1079/9780851992945.00. CABI International

Van der Zwet T, Keil HL (1979) Fire blight:a bacterial disease of rosaceous plants. Agricultural Handbook 510, U.S. Department of Agriculture, Washington DC

Vanneste J (2000) Fire blight: the disease and its causative agent. Erwinia amylovora. CABI Publishing