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*Microbiology*

# Phenotypic Variability of *Erwinia amylovora* from Pome Fruits in Georgia

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**Since its first appearance in 2016, the fire blight has spread to several regions of the country. As a result of surveys conducted in 2020-2022, out of collected 169 samples the fire blight disease was confirmed in 35 samples of apple, pear and quince collected in 7 municipalities of four regions of eastern Georgia. A total of 55 isolates were recovered and identified by specific PCR as *Erwinia amylovora*. Morpologically typical *E. amylovora* isolates express phenotypic variability according to esculin hydrolysis and the ability to utilize certain sugars and polyols, such as galactose, maltose, xylose, sorbitol and mannitol.** *© 2023 Bull. Georg. Natl. Acad. Sci.*

fire blight, apple, pear, quince, phenotypic characterization

Fire blight, the destructive disease, caused by a bacterial pathogen *Erwinia amylovora* damages a wide spectrum of cultural and wild plants belonging to the *Rosaceae* family [1]. Apple, pear and quince [2] are among the most susceptible fruit trees which are affected by the disease. Direct damage and often eradication of the entire fruit tree orchards if the appropriate countermeasures are not taken [3] determines the economic losses triggered by fire blight. There are many reports on the destruction of the cultural as well as of the wild plants by the initial disease wave in Europe [2].

Fire blight disease symptoms can vary depending on the severity of the infection, but mostly include: burn-like damage to flowers, leaves, fruits and terminal shoots; development of necrotic lesions and cankers on woody tissues (stem, branches, trunks) [4]. Although some drugs have been shown to be effective, pruning and removal of the damaged tissue and destruction of the infected plants are effectively used to prevent the spread of the disease.

Fire blight first observed around 1780 in New York State has spread worldwide in the last two centuries. After its introduction in the United Kingdom in 1958 the disease spread progressively across Europe, the Mediterranean basin and Asia, where it now occurs in almost 60 countries (https://gd.eppo.int/taxon/ERWIAM/distribution). Among them are countries of Caucasus region and

the area surrounding the Black and the Caspian Sea, such as Turkey [5], Iran, Armenia [6] and Southern Russia [7].

Current situation of *E. amylovora* distribution in Georgia according to EPPO is: present, restricted distribution (https://gd.eppo.int.taxon/ERWIAM/distribution), categorization A1 list, 2018 (https://gd.eppo.int.taxon/ERWIAM/categorization).

First case of fire blight was observed in Georgia in 2016 (eastern Georgia, Mtskheta-Mtianeti region, Mtskheta municipality, village Ksovrisi) and was related to an imported apple seedling. Since then, it was detected on few symptomatic samples of apple, pear and quince trees collected in other regions of eastern Georgia (Shida Kartli, Kvemo Kartli) and one region of western Georgia (Imereti) [8]. In 2017-2019 the disease spread in inspected orchards and the number of affected plants by years and species were reported. Four *Erwinia amylovora* isolates were characterized phenotipically and genetically [9].

In this work we continued the study into the 2020-2022 seasons in the regions previously affected by fire blight, detecting more disease foci and collecting new isolates for phenotypic and genotypic characterization.

## Materials and Methods

Surveys were conducted during the 2020-2022 seasons in the four pome fruit-growing regions of eastern Georgia: Mtskheta-Mtianeti, Shida Kartli, Kvemo Kartli and Kakheti. Private gardens and orchards of apple (*Malus domestica*), European pear (*Pyruscommunis*) and quince (*Cydonia oblonga)* were studied in villages of different municipalities of above stated regions. Samples of flowers, leaves, stems and fruits of apple, pear and quince, showing symptoms compatible with fire blight (e.g., necrosis, burn-like damages of flowers, leaves and fruits,production of exudates, etc.) were collected.

Immunostrip assay was used for immediate detection of *E. amylovora* in collected samples. Molecular detection of *E. amylovora* in affected plant samples was conducted by PCR using the primers pairs A and B [10, 11] as described before [9].

Colonies of *E. amylovora* were isolated from positive plant samples after surface disinfection, tissue maceration and serial dilution. For morphological identification of the suspected isolates pure cultures on King's B, and NSA media were used. Observation of colony morphology, was carried out by stereoscopic microscope (Leica M50).

For phenotypic identification of the isolates Gram, oxidase, nitrate reduction, $H_2S$ formation and citrate utilization tests were performed; esculin hydrolysis, gelatin liquefaction, growth ability at $39^0$C, fluorescent pigment production in King's B (under UV) and levant formation were analyzed; ability to utilize glucose, sucrose, lactose, maltose, galactose, xylose, mannitol and sorbitol were studied [12].

A single bacterial colony of 24-hour grown culture on solid medium was picked from an agar plate with a sterile loop and suspended in 100µl TE buffer (Tris-EDTA, pH 7.4). The suspension was boiled at 100°C for10 minutes, then cooled at 4°C for 10 minutes and centrifuged for 2 min at 1000 rpm. A supernatant containing DNA was used for the PCR reaction. Conventional PCR was carried out using the primers pair A and B [10, 11] or primers pair G1-F and G2-R [13] as described earlier [9].

## Results and Discussion

A total of 44 sampling sites were examined from spring to fall in the villages of four pome fruit production regions of the country in 2020-2022. Shida Kartli – Gori, Kareli and Khashuri municipalities. Kvemo Kartli – Marneuli municipality. Mtskheta-Mtianeti – Mtskheta municipality. Kakheti – Sighnaghi, Gurjaani and Lagodekhi Municipalities.

Symptoms characteristic to fire blight were observed at some locations on pomaceous fruit hosts of *E. amylovora* – apple, pear, and quince. Symptoms were observed on leaves, blossoms, fruits and shoots. Leaves and blossoms were wilted and brown or black in colour. Infected shoots were withered with scorched appearance. Bark was cracked, underneath of which the wood had a reddish-brown color. Branches and trees look like they have been burned by a fire. Bacterial ooze – slimy liquid exude on fruits and bark were observed (Fig. 1). A total of 169 samples with symptoms suggesting fire blight infection or a mixed infection with fungal disease were collected.

Initial detection of *E. amylovora* was performed by Ea AgriStrip (Bioreba) and positive samples were further confirmed by PCR. PCR confirmed *E.amylovora* in 35 samples, with the most affected pome fruit species being apple (27/5). Number of collected plant samples and number of the samples and pome fruit species where *E. amylovora* was detected by regions and municipalities are represented in the Table. As seen the most fire blight affected region is Shida Kartli, the main pome fruit production region of the country – 19 out of 35 samples in which *E. amylovora* was detected is coming from Shida Kartli region.

Typical colonies of isolated *E. amylovora* on NSA and Kings'B are represented on Fig. 2.

Biochemical tests performed for phenotypic characterization of the *E. amylovora* isolates showed that all are oxidase test and nitrate reductase test negative with the abilities of gelatin hudrolysis and citrate utilization. They form the exopolysaccharide levan on NSA medium. None of the isolates display growth at $39^0$ C, form $H_2S$ or the fluorescent pigment on King's B medium. Studies showed that all *E. amylovora* isolates

**Fig. 1.** Fire blight diseased pome fruit trees: 1 - Apple, Kakheti, Sighnaghi municipality, village Jugaani, sample #61; 2 – Pear, Shida Kartli, Gori municipality, village Karaleti, sample # 86.

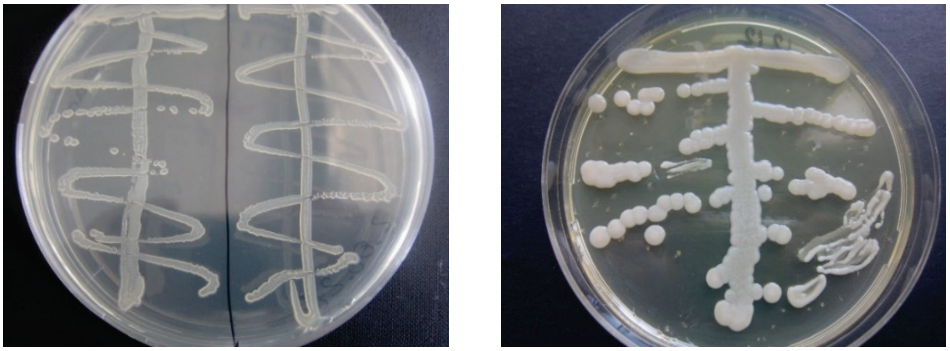

**Fig. 2.** Colonies of *E. amylovora* (#1212) on different solid growth media: A – NSA (Left); B – King's B (Right).

produce acid from glucose, fructose and sucrose. *E. amylovora*, Georgian isolates are typical representatives of the species [12, 14] and differ from each other according to utilization ability of galactose, maltose, xylose, sorbitol and mannitol, as well as the hydrolysis of a coumarin glucoside esculin.

Some differences in the ability to use certain carbohydrates as carbon sources using the API tests were demonstrated on *E. amylovora* Polish isolates [15]. Diversity in pathogen populations, expressed in their growth, carbon sources preferences and chemical susceptibility to certain antibiotics has also been demonstrated recently for *E. amylovora* isolated from fire blight outbreaks in Portugal [16].

Further characterization of *E. amylovora* isolates and study of their hyper variable regions of the genome, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) will reveal more phenotypic and genotypic diversity which are important for epidemiology and outbreak tracking [17], implementing targeted control strategies to limit the spread of the devastating fire blight disease.

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**Table. Number of collected symptomatical plants and the number of samples with confirmed fire blight by regions and years**

| Region | Municipality | Villages | 2020 | | 2021 | | 2022 | |
|---|---|---|---|---|---|---|---|---|
| | | | Number of collected plants with disease symptoms | Number of plants with con-firmed fire blight disease | Number of collected plants with disease symptoms | Number of plants with con- firmed fire blight disease | Number of collected plants with disease symptoms | Number of plants with con-firmed fire blight disease |
| Mtskheta-Mtianeti | Mtskheta | Ksovrisi, Mukhrani | 11 | 2 | 0 | 0 | 12 | 0 |
| Shida Kartli | Gori; | Karaleti, Tirdznisi, Pkhvenisi, Mereti, Mejvriskhevi, Akhrisi, Kheltubani | 15 | 2 | 29 | 5 | 24 | 0 |
| | Kareli | Kekhijvari, Tataraantubani | 1 | 1 | 4 | 0 | 12 | 2 |
| | Khashuri | Osiauri, Gomi | 10 | 1 | 11 | 8 | 0 | 0 |
| Kvemo Kartli: | Marneuli | Kachagani | 0 | 0 | 0 | 0 | 8 | 8 |
| Kakheti: | Gurjaani; Lagodekhi, Sighnaghi | Kachreti, Chabukiani, Bodbe, Magharo, Jugaani, | 9 | 1 | 11 | 5 | 13 | 0 |
| | Total | | 46 | **7** | 55 | **18** | 68 | **10** |

*მიკრობიოლოგია*

# საქართველოში თესლოვანი ხეხილიდან გამოყოფილი *Erwinia amylovora*-ს ფენოტიპური ცვალებადობა

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**ციურიხის გამოყენებითი მეცნიერებების უნივერსიტეტი (ZHAW), ბუნებრივი რესურსების მეცნიერებათა ინსტიტუტი, უოდენსვილი, შვეიცარია
§აკადემიის წევრი, საქართველოს მეცნიერებათა ეროვნული აკადემია, თბილისი, საქართველო

ხეხილის ბაქტერიული სიდამწვრე, საქართველოში 2016 წელს მისი პირველი გამოჩენის შემდეგ გავრცელდა ქვეყნის რამდენიმე რეგიონში. 2020-2022 წლებში ჩატარებული კვლევების შედეგად, მოძიებული 169 ნიმუშიდან დაავადება დადასტურდა აღმოსავლეთ საქართველოს ოთხი რეგიონის 7 მუნიციპალიტეტში შეგროვებულ ვაშლის, მსხლისა და კომშის 35 სინჯში. ბაქტერიის 55 იზოლატი გამოიყო და იდენტიფიცირებულ იქნა სპეციფიკური პჯრ-ით, როგორც *Erwinia amylovora*. მორფოლოგიურად ტიპიური *E. Amylovora* იზოლატები ხასიათდება ფენოტიპური ცვალებადობით, რაც ვლინდება ესკულინის ჰიდროლიზის და გარკვეული შაქრებისა და პოლიოლების შეთვისების უნარში, როგორიცაა გალაქტოზა, მალტოზა, ქსილოზა, სორბიტოლი და მანიტოლი.

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