Thesis of PhD Dissertation

BIOLOGICAL DIVERSITY OF THE HUNGARIAN *ERWINIA AMYLOVORA* ISOLATES CAUSING FIRE BLIGHT DISEASE

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1. INTRODUCTION AND AIMS

William Coxe (1817) wrote in his book „Cultivation of Fruit Trees” already in the XIX. century: “The fiery danger turns the leaves of the most healthy and developed trees within a few hours such dark brown, like they have been burned by a hot fire and a rust brown secretion is gushing out of the pores of the bark of the trees”. This statement is valid even today: the disease is causing significant troubles all over the world.

The serious disease of the apple, numerous ornamental and wild plants **(van der Zwet and Keil, 1979)**, called „fire blight” is caused by the bacteria *Erwinia amylovora* (Burrill) **Winslow et al. (1920)**. According to the recommendations of the EPPO, the European and Mediterranean Plant Protection Organization of the FAO UN, it is throughout Europe – and therefore in Hungary as well – a harmful quarantine pest.

Within the *Rosaceae* family 200 species belonging to 40 genuses can be regarded as its host plant **(Steiner and Zeller, 1996)**. The most important host plants from view of commerce are the species from the *Cotoneaster*, *Crataegus*, *Cydonia*, *Malus*, *Pyrus*, *Photinia*, *Pyracantha* and *Sorbus* genus. The hawthorn myrtle (*Stranvaesia davidiana*) and loquat (*Eriobotrya japonica*) are also host plants, though they are not as important in our country. The disease has also been detected in the USA at the thornless blackberries (*Rubus rusticanus* var. *inermis*) **(Evans, 1996)** and raspberry **(Schnabel and Jones, 2001)**, but these are not infectious at the apples and peers. Reports have been issued about the natural infection of the Japanese plums (**Mohan and Thomson, 1996**). Publication have been issued about the drying of spouts of plums and apricots in the USA, (**Mohan, 2007**), about the natural infection in of European plums in Germany **(Vanneste et al., 2002)** and apricots in Czech Republic **(Korba and Sillerova, 2010)**.

The disease endemic in the USA and known for about 200 years had been introduced to Europe (England) and the basin of the Mediterranean (Egypt) in the middle of the 1950-s, and has been spread now to almost all of the countries of the region. The illness was detected in summer 1995 close to Nyárlőrinc in a 5-6 year old 43,5 acre size apple plantation **(Hevesi, 1996)**. Since its appearance the damage caused by the bacteria can be serious in years provided the weather is favorable for the bacteria. Since then a number of isolate are deposited in the gene bank of the Corvinus University of Budapest, moreover we are collecting them continuously. In the past year more and more literature has been published about the appearance of the pest at new host plants, as well as about the characterization of the *Erwinia amylovora* on the basis of various attributes,

therefore a question is coming up, whether the pathogen has been changed during the years? Whether the isolates colony types, their biochemical attributes, their virulence, and their genetical feature are different from the isolates coming from different host plants or geographical places, or from any other Hungarian or foreign isolates, and of isolates collected from different places of the country.

The aims of this thesis work are the following:

- Collection of *Erwinia amylovora* isolates from various host plants and geographical origin,
- Identification and description of the collected isolates with classical bacteriological methods,
- The description of collected *E. amylovora* isolates and isolates from the gene bank, their comparison on different media on the basis of the colony type,
- The description of collected *E. amylovora* isolates and isolates from the gene bank, comparison on the basis of their biochemical attributes,
- The description of collected *E. amylovora* isolates and isolates from the gene bank, comparison on the basis of their bacteriophage sensibility,
- The description of collected *E. amylovora* isolates and isolates from the gene bank on the basis of their virulence in case of various pear species,
- The identification, description, comparison of collected *E. amylovora* isolates and isolates from the gene bank with molecular methods, determination of their relations.

2. MATERIALS AND METHODS

The isolates

The *Erwinia amylovora* Ea1 isolate was first isolated by Mária Hevesi from apple tree in Nyárlőrinc **(Hevesi, 1996)**. Since 1996, several Hungarian and foreign *Erwinia amylovora* isolates from different locations, years and host plants are available in the gene bank of the Corvinus University of Budapest, from which 22 have been selected for this experiment.

Plant parts with visible symptoms were collected from different locations in Hungary, Erdély and Vajdaság between 2009 and 2011. Nine isolates were made from samples, which derived from orchards, public areas and private gardens.

The morphological, biochemical, physiological and molecular properties of a total 31 *E. amylovora* isolates were studied. Pathogenicity and bacteriophage sensitivity and virulence tests were made as well.

Isolation and propagation

The infected stems were first disinfected with ethanol. Samples then were taken under sterile conditions from the border of the dead and healthy plant parts. Next the plant tissues were homogenized with distillated water and transferred to King-B agar medium **(King et al., 1954)**. The Petri-dishes were incubated on 26 ˚C for 1-2 days. The separate colonies were then inoculated on sterile medium. Clean cultures were made and incubated on 26 ˚C. The plates were then stored on 4 ˚C.

The lyophilized isolates from the gene bank were homogenized with distillated water. Then the bacteria suspensions were transferred to Petri-dishes on 26 ˚C and after 2 days they were inoculated.

Classical bacteriological tests

In case of those *Erwinia amylovora* isolates (Ea) which were collected for the purposes of this study, it was important to define the basic characteristics, which ensure selective isolation and enables us to make sure that the isolates belong to the *Enterobacteriaceae* family.

Identification of Gram-features

24 hours after inoculation, 1-2 colonies from the clean, fresh medium are placed on sterile slides. 3% potassium hydroxide were added and then homogenized. The bacteria is Gram-negative in case the potassium hydroxide dissolves the cell wall (the mixture is stingy), while the bacteria is Gram-positive if the cell wall is left intact (the mixture is watery) **(Susslow et al., 1982)**.

Hypersensitive reaction

The bacterium suspension ($5x10^7$cells/ml) was injected into tobacco leaves (*Nicotiana tabacum* L. cv. *xanthi*). After 24-48 hours the hypersensitive reaction (tissue necrosis) was monitored **(Klement, 1963)**.

Pathogenicity test

Pathogenicity tests **(Koch, 1976)** were carried out in 3-6 repetition on each isolate. Pear fruits were used for testing in case of each isolate. Besides pear, plum fruit and shoots were used as well in case of those isolates, which were isolated from plum trees. The surfaces of the plants used in the pathogenicity test were disinfected with ethanol.

The fresh, young plum shoots were 20-25 cm long when inoculated. The bacterium suspension ($5x10^7$cells/ml) was injected with a syringe into the base of the second fully matured leaf from the top of the shoot. In order to assure optimal conditions for the infection to spread, the shoots were then kept in the laboratory, in 80-90% relative humidity and on 25-27 ˚C. The untreated control was stung with a sterile syringe with distilled water. The control was kept under the same conditions as the treated ones only separated.

Contamination of the fruits (‘Eldorado’ pear and ‘d’Agen’ plum) was carried out under sterile conditions. The fruits were stung 3-6 places with bacterium suspension ($5x10^7$cells/ml). The control was again treated with distilled water. The treated fruits were incubated in 80-90% relative humidity, on 25-27 ˚C.

Evaluation of the pathogenicity test was done after 5 days on the fruits and 10-14 days on the shoots. The results were given after the analysis of the typical symptoms of *Erwinia amylovora* **(van der Zwet and Keil, 1979)**: the brownish, blackish colorization and the deformation of the shoot and the water-soaked tissue lesions on the fruits.

Comparative studies of the *Erwinia amylovora* isolates

Comparison of the colony types

Various types of media were used in this study (normal types: Nutrient agar, King-B (**King et al. 1954**), Kado-Heskett (**Kado and Heskett, 1970**), Miller-Schroth (**Miller and Schroth, 1972**), selective media: Eosine Methylene Blue agar (**Holt- Harris and Teague, 1916**), Crosse-Goodman (**Crosse and Goodman, 1973**)). The isolates were transferred into the different types of media. The Petri-dishes were incubated on 26 ˚C. The isolates were typified by colony types (**Mazzucchi, 1977**). The isolates were evaluated after 24-48 hours, under microscope. The colony types were distinguished by consistence, shape, surface, margin and color (**Puskás, 1986; Klement et al., 1990**).

Biochemical characteristics

Newly isolated *Erwinia amylovora* isolates were studied with API 20E and API 50CH (Biomérieux, Marcy l'Étoile, France) strips, while for the gene bank material, only API 50CH strips were used.

The instructions of the manufacturer (Biomérieux, Marcy l'Étoile, France) were followed during API 20E and API 50CH tests. In case of each isolate, $5x10^7$ cells/ml bacterium suspension was used for the sample places containing special media of the kits. Both tests were incubated on 36 ˚C and evaluated after 24-48 hours. The evaluation of both tests is based on color change. The evaluation of the API 20E test was done with the help of positive and negative test strips provided by the manufacturer. In case of the API 50CH test, if the bacteria utilize a given carbohydrate, the originally red solution turns into yellow, while in case of the gelatin is dissolved, the solution turns into black.

Bacteriophage sensitivity

For the characterization and comparison of bacteriophage sensitivity of the *Erwinia amylovora* isolates four different phages were used (**Schwarzinger et al., 2011**). The bacteriophages were isolated from different host plants and in different years and areas. Sensitivity of the phages was determined with double agar layer technique described by **Adams (1959)**. After a 24 hour incubation time, suspensions of the *Erwinia amylovora* isolates ($5x10^7$cells/ml) were made. Then the different phages (10 µl, 10^6 PFU/ml) were dropped on the surface of the medium. The Petri-dishes were incubated on 26 °C for 24 hours.

The evaluation was based on the plaque morphology of the phages formed on the different bacteria suspensions. The phages were divided into three groups of plaque types: A- clear plaque; B- less transparent plaque; C- no plaque **(Klement et al., 1990)**.

Virulence test – inoculation of unripe pear fruits

Seven traditional pear cultivars were used for the virulence tests of the *Erwinia amylovora* isolates. Based on the results, the susceptibility of the cultivars were determined as well. After a 24 hour incubation time, suspensions of the *Erwinia amylovora* isolates ($5x10^7$cells/ml) were made. On unripe fruits, natural infection through superficial scars was imitated *in vitro*. Fruits of 5-6 cm in diameter were used for this study (5 fruits/cultivar). Six punctures per fruit were made with a needle previously dipped into the bacterial suspensions. The same procedure was performed with needles dipped into distilled water as a control. The fruits were then incubated on 26 °C. Evaluation of the test was done 4-5 days after inoculation **(Honty et al., 2004)**. The severity of the symptoms were rated according to a scale of 0-4 index of infection **(Horsfall and Barratt, 1945)** for fruits (0-symptomless fruit; 1-low susceptibility (necrotic spots with a 1-5 mm diameter); 2-moderate susceptibility (necrotic spots with a 6-10 mm diameter); 3-susceptible (water soaked spots 11-20 mm in diameter) and 4-very susceptible (water soaked spots with a diameter of more than 21-30 mm).

<u>Molecular bacteriological tests</u>

Amongst molecular bacteriology tests, for the identification of bacteria and for taxonomy studies, determination of the sequence encoding 16S rRNA is the most common method **(Choi et al., 1996; Clarridge, 2004)**. Since detecting the presence of *Erwinia amylovora* on plum in Hungary has high significance, it was especially important to identify the isolate by determining the sequence of 16S rDNA.

In case of all the other collected isolates and of those from the gene bank, the molecular test of the plasmid was carried out to determine the number of repetitive regions. Tests based on the size of SSR (Short-Sequence-Repeat) show the differences and similarities between isolates **(Ruppitsch et al., 2004)**.

16S rDNA test

DNA was recovered 24 hours after the inoculation of bacteria on King-B media. For 16S rDNA sequence determination universal primers (63f: 5'-CAGGCCTAACACATGCAAGTC-3', 1389r: 5'-ACGGGCGGTGTGTACAAG-3') were used. After the polymerase chain reaction, the PCR product was cleaned, ligated into the pGEM-T Easy plasmid and then transferred into *Escherichia coli* (strain DH5α) **(Maniatis et al., 1989)**. The recombinant plasmid sequence was determined and then matched to homologue sequences found in the international databases.

Examination of the pEA29 plasmid

For this study, specific primers of the pEA29 plasmid (pEA29A: 5'-CGG TTTTAACGCTGG G-3', pEA29B: 5'-GGGCAAATACTCGGATT-3') of *Erwinia amylovora* were used. With the help of the primers, a 1,1 kb long fragment was amplified from the plasmid. The PCR product was cleaned and then the sequence was determined. A repetitive 8 nucleotide region (ATTACAGA), an SSR (Short-Sequence-Repeat) was found in this fragment. Then the sequence was matched to homologue sequences found in the international databases.

3. RESULTS AND DISCUSSION

Identification of the bacteria with classical methods

Symptoms caused by Erwinia amylovora

Brown or black, typically deformed, shepherd's crook-like shoots were collected from different host plants (*Malus, Pyrus, Cydonia, Crataegus* and *Prunus*). The symptoms implied the presence of *E. amylovora.*

Gram-test

Since the 3% potassium hydroxide solution dissolved the cellular wall of the bacteria, the isolates collected from the different host plants proved to be Gram-negative.

Hypersensitive reaction

On the leaves of the tobacco plants inoculated with $5x10^7$ cell/ml suspension of isolates, quick tissue necrosis formed after 24-48 hours, which is a sign of hypersensitive reaction.

Pathogenicity test

The test plants inoculated with bacterium suspensions showed different levels of infection. Each fruit (pear and plum) and the plum shoot showed intensive reaction and formed typical symptoms after 5-14 days. The *in vitro* test was successful.

Where the pear fruits were inoculated, diffuse necrotic spots were appeared in case of all isolates. On the plum fruits, saggy, brown, rotting spots formed around the inoculation. The shoots became brown or black and crook-like.

Comparison of Erwinia amylovora isolates by colony types

The different colony types of the isolates formed on common or selective media were evaluated and classified. The colonies on King-B media were uniform, milky and cream-colored. On Miller-Schroth media, each *Erwinia* species were able to form orange-colored colonies with a transparent outline. In case of the *Erwinia*-selective Kado-Heskett media, the isolates formed red-orange-colored colonies. The surface of the colonies became crater-like on the *Erwinia amylovora*-selective Crosse-Goodman media. Comparison of the colonies on Crosse-Goodman media was suitable for the separation of the different isolates. The colonies of the bacteria on the Eosin

Methylene Blue media was convex, smooth surfaced with intact outlines. This media is suitable for differentiating the lactose and saccharose utilizing (light-colored colonies with a black center) or non-utilizing (colonies without color) bacteria. King-B, Miller-Schroth and Kado-Heskett media cannot be used for separation of the different isolates, but Crosse-Goodman and Eosin Methylene Blue media are suitable for this purpose.

The biochemical properties of the *Erwinia amylovora* isolates

Result of the API 20E test

The API 20E test is used for the determination of the species belonging to the *Enterobacteriaceae* family. The collected isolates were tested with API 20E kits in order to identify them as *E. amylovora*. The isolates Eam1, Eam2, Eam4, Eam5, Eam6, Eam7, Eam8, Eam9, Eam10 and Ea-PlumBo1 showed positive reaction for β-galactosidase and citrate utilization, acetoin production and also in glucose, mannitol, sorbitol, saccharose, melibiose and arabinose reactions. The results were negative in case of arginin-dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S production, urease, tryptophan deaminase, indole production, gelatinase, inositol, rhamnose, amygdalin tests. The biochemical properties of the tested isolates matched the description of *Erwinia amylovora* given in the API 20E kit.

Result of the API 50CH test

The isolates were compared on the basis of carbohydrate utilization, for which API 50CH fast test was used. From the 49 various carbohydrates, each isolate utilized 11 types and did not utilize 27 types at all by the end of the reaction time (48 hours) **(Table 1.)**. The 11 carbohydrates were utilized by all isolates.

In addition, 11 carbohydrates (glycerol, D-xylose, mannose, inositol, amygdalin, arbutin, salicin, cellobiose, melobiose, raffinose and D-frucose) were utilized by the isolates in different ways. These carbohydrates are suitable for differentiating the isolates. In the utilization of the 49 types of carbohydrate, differences were found between the isolates from various host plants, based on which we can assume that they do not belong to the same strain, and that they are suitable for the detection of differences between isolates.

Table 1. Carbohydrates utilized or non-utilized by *Erwinia amylovora* isolates

„Non-Utilized”			
No.		**No.**	
2	Erythritol	34	Melesitose
3	D Arabinose	36	Starch
7	L Xylose	37	Glycogen
8	Adonitol	38	Xylitol
9	β-Methyl-D-Xyloside	40	D Turanose
14	Sorbose	41	D Lyxose
15	Rhamnose	42	D Tagatose
16	Dulcitol	44	L Fucose
20	α-Methyl-D-Mannoside	45	D Arabitol
21	α-Methyl-D-Glucoside	46	L Arabitol
25	Esculin	47	Gluconate
28	Maltose	48	2-Keto-Gluconate
29	Lactose	49	5-Keto-Gluconate
33	Inulin		
„Utilized”			
No.		**No.**	
4	L-Arabinose	19	Sorbitol
5	Ribose	22	N-Acetyl- Glycosamyne
10	Galactose	31	Sucrose
11	Glucose	32	Trechalose
12	Fructose	39	Gentobiose
18	Mannitol		

Comparison of the *Erwinia amylovora* isolates by bacteriophage sensitivity

In this study, the bacteriophage sensitivity of the *Erwinia amylovora* isolates was tested against 4 phages. The phage is considered to be the most effective if it forms a clear plaque on the surface of the most tested isolates, which means that it is able to lyse all bacteria on the tested area. The tests were evaluated based on the plaque types after incubation time (**Figure 1.**).

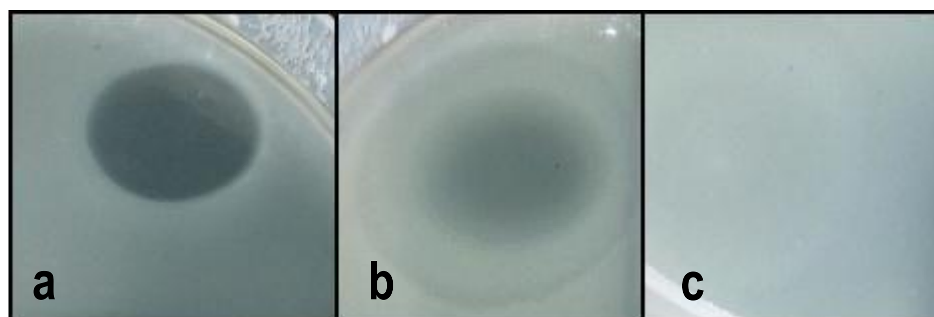

Figure 1. Plaque morphology **(Photo: Végh, 2011)**

(plaque types: a - clear plaque; b- less transparent plaque; c- no plaque)

The isolates showed different sensitivity with phages, which was evaluated based on whether the phages formed clear or less transparent types of plaque, or no plaque at all. The same group (plaque-type) of each isolates belongs to the same lysotype group.

There was only one isolate of 31 which was lysed by all four phages (Ea96). Two isolates formed clear plaques by three phages (isolate Ea15- H1A, H4B, H5A phages; isolate Ea70 - H1A, H4B, H8 phages). Six isolates also formed less transparent plaque by three phages (Eam2, Eam4, Ea26, Ea29, Ea12 and Ea47). In most cases (13 isolates: Ea10, Ea16, Ea22, Ea31, Ea50, Ea60, Ea67, Ea80, Ea88, Ea95, Eam6, Eam8, Eam10) the isolates showed sensitivity by two phages and formed clear plaque. All four bacteriophage formed less transparent plaque in case of 8 samples (Ea1, Ea6, Ea329/98, Eam1, Eam5, Eam7, Eam9 and EaPlumBo1). Only in case of the isolate Ea67 were no sensitivity detected against two phages (phage H4B and phage H8). The H5A phage was the most effective from the phages. It could lyse all of the *E. amylovora* isolates; on most of them it formed clear plaques.

Phages H1A and H5A proved to be the most effective, since they formed clear plaques with 13-15 isolates. The number of clear plaques was 7-8 in case of phages H4B and H8.

The isolates showed different reactions to phages, whether they originated from Hungary or abroad. The results proved that Hungarian phages can block *E. amylovora* (isolated from different host plants), although the sensitivity of the different *Erwinia* isolates differ.

Bacteriophages can be used for characterization of *Erwinia amylovora* isolates and for controlling the bacterial disease.

The virulence of *Erwinia amylovora* isolates on unripe fruit of pear cultivars

This study proved that the susceptibility of cultivars differs. For the evaluation of pear cultivars, the classification was carried out on the basis of the development of disease symptom (structure of the inoculated site) on the fruit. Cultivars like 'Alexander Lucas' and 'Stössel tábornok' represented the less susceptible category, while 'Eldorado', 'Serres Olivér' and 'Diel vajkörte' were moderately susceptible. The most susceptible cultivars were 'Téli esperes' and 'Drouard elnök'.

Based on the study, the same isolates did not cause the same reaction on the different cultivars. Strong reactions were detected in case of Ea 10, 19 and 29 isolates on 'Drouard elnök', while on other cultivars different isolates caused the most series symptoms. Isolate Ea50 was not virulent only on 'Eldorado'. The results show that there are a few isolates (Ea1 and Ea67) which

were virulent is case of most of the cultivars, while there were some (Eam7, Eam10, EamPlumBo1) which showed modest virulence.

The host plant did not have an impact on the virulence, since most of the isolates with high virulence were collected from *Malus* and *Cydonia*, not from *Pyrus* and those with lower virulence were collected from *Prunus* species. The isolates from *Pyrus* (Ea10, Ea26, Ea50, Ea80, Eam10) showed lower virulence in case of the cultivars ’Alexander Lucas’, ’Stössel tábornok’, ’Serres Olivér’ and ’Eldorado’ while on ’Diel vajkörte’, ’Drouard elnök’ and ’Téli esperes’ higher virulence was detected.

The tested foreign and Hungarian *E. amylovora* isolates caused different symptoms on the cultivars. The Hungarian isolates proved to be more effective compared to those from abroad. The cultivars ’Serres Olivér’, ’Alexander Lucas’, ’Eldorado’ and ’Stössel tábornok’ showed moderate susceptibility in case of the non-Hungarian isolates. The ‘Diel vajkörte’, the ‘Drouard elnök’ and the ‘Téli esperes’ were also less susceptible for isolates Ea96, Ea329/98, Eam1 and Eam10, while in case of Ea47 and Eam9 strong susceptibility were detected.

These results confirmed that *E. amylovora* strains of different origin can be characterized on the basis of the susceptibility of pear cultivars and virulence. On the basis of our results, using a mixture of *E. amylovora* isolates in experimental practice should be more effective, because the susceptibility of traditional pear cultivars gave different results

To characterize resistance, the resistance of the flower should be is the focus. The shoot and fruit tests complete the results of resistance tests and aid successful breeding programs in the future.

Molecular tests of the isolates

Molecular test of the 16S rRNA gene of the isolate collected from plum

The PCR product of the reaction was about 1300 bp long. The 16S rDNA nucleotide sequence (1323 bp) of the partially sequenced Hungarian Ea-PlumBo1 isolate were sent to the international gene bank. Here it can be found under the accession number HE610678.

The sequence of the isolate collected from plum was matched with the sequences found in the international data base. The sequence of the fragment showed 100% homology with two isolates: number fn434113 (German, host plant: *Crataegus*) and fn666575 (English, host plant: *Malus*). There were other matches with 98-99% homology, with samples from different host plants (*Malus, Pyrus* and *Rubus*). Based on the data, a phylogenetic tree was made. Because of the

homology of the sequences, *Rubus* samples form an isolated group. The isolate collected from plum shows closer relation to the isolates of *Malus, Pyrus, Cydonia* and ornamental plants.

Molecular test of the pEA29 plasmid of the isolates

The nucleotide sequence of the PCR products multiplied by means of primers specific to *E. amylovora* in the case of each isolate was determined. In the sequences, plasmid repetitive regions of the pEA29, the SSR (short sequence repeats) were found based on which the Hungarian *E. amylovora* isolates and *E. amylovora* isolates included in the international NCBI database can be well distinguished.

The analysis of the ATTACAGA sequence (repetitive copy number) of the pEA29 plasmid characteristic of *E. amylovora* showed that this section repeats 5, 7 or 12 times in the dominating populations of the Hungarian isolates. The majority of the Hungarian isolates (51%) belong to the sequence group of 7 to 8 repeats.

The Ea19, Ea22, Ea50, Ea 80, Ea47 and the Eam1 isolates have the lowest SSR number (5), while of that of the Ea95 and Ea329/98 was higher (10 and 12 SSR). The different SSR numbers of the isolates prove that they belong to separate strains.

The results of the repetitive region determination of the pEA29 plasmid were matched with all isolates found in the NCBI data base. Twenty seven isolates were found in the data base from different countries and with different SSR numbers. Most Hungarian isolates have 7-8 SSR numbers, while in case of the Austrian isolates this number is higher (10-14 SSR). The Bulgarian isolates have 8-10-11-12-13, the German and the English 4, the Egyptian 4-6-7, while the American 4-5 have SSR numbers. The most accurate match for our results is the Egyptians, which suggests that the bacteria arrived to Hungary from the southern directions. The other source could have been England, but the SSR number comparison does not support that concept **(van der Zwet, 1996)**.

4. SUMMARY OF NEW SCIENTIFIC RESULTS

- For the first time in Hungary, second time in Europe, the presence fire blight causing bacteria (*Erwinia amylovora*) on plum (*Prunus domestica* d'Agen) was confirmed with classical and molecular tests/methods.
- The *Erwinia amylovora* isolates were classified by morphological characteristics on different media for the first time in Hungary.
- The *Erwinia amylovora* isolates were characterized/typified by carbohydrate utilization for the first time in Hungary.
- Data from *in vitro* studies on the effect of 4 bacteriophages on *Erwinia amylovora* were provided.
- Data on virulence of *Erwinia amylovora* isolates and on sensitivity of the fruits in case of 7 traditional pear cultivars were provided.
- The repetitive region (SSR) of the *Pst*I fragment pEa29 plasmid was characterized/described for the first time in Hungary, and then the relations with reference isolates found in international data base were defined.

References

1. **Adams M.H.** (1959): Bacteriophages. Interscience Publishers, New York. 592 p.
2. **Choi S.T., Ahn H.K. and Chang Y.D.** (1996): Effect of crude extracts and chopped shoot application of *Allium* spp. on rice growth. *Korean Journal of Crop Science* 41: 625-633 p.
3. **Clarridge J.E.** (2004): Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Reviews* 17: 840-862.
4. **Crosse J.E. and Goodman R.N.** (1973): A selective medium for and a definitive colony characteristic of *Erwinia amylovora. Phytophatology* 63: 1425-1426 p.
5. **Evans I.R**. (1996): Fire blight of raspberries in Alberta. *Acta Horticulturae* 41:69-72 p.
6. **Hevesi M.** (1996): Az *Erwinia amylovora* (Burill) Winslow et al. hazai megjelenése almán. *Növényvédelem* 32 (5): 225-228 p.
7. **Holt-Harris J.E. and O. Teague** (1916): A new culture medium for the isolation of *Bacillus typhosa* from stools. *Journal of Infectious Diseases* 18: 596 p.
8. **Honty K., Hevesi M., Göndör M., Tóth M., Bács- Várkuti V. and Ferenczy A.** (2004): Susceptibility of soma traditional pear cultivars of Hungarian and foreign origin to the pathogenic bacterium *Erwinia amylovora. International Journal of Horticultural Science* 10: 41-45 p.
9. **Horsfall J.G. and Barratt R.W.** (1945): An improved grading system for measuring plant disease. *Phytopathology* 35: 655 p.
10. **Kado C.I. and Heskett M.G.** (1970): Selective media for isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas* and *Xanthomonas*. *Phytopathology* 60: 969-976 p.
11. **King E.O., Ward M.K. and Raney D.E.** (1954): Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* 44: 301-307 p.
12. **Klement Z.** (1963): Rapid detection of the pathogenicity of phytopathogenic pseudomonads. *Nature* 199- 300 p.
13. **Klement Z., Rudolph K. and Sands D.C.** (1990): Methods in Phytobacteriology. Budapest: Akadémiai Kiadó. 53-210 p.
14. **Koch R. (**1876): Untersuchungen Über die Aetiologie der Wundinfectionskrankheiten. Leipzig: F. C. W. Vogel. 8-62 p.
15. **Korba J. and Sillerova S.** (2010): First occurence of fire blight infection on apricot (*Prunus armeniaca*) in Chech Republic. *Abstracts of 12th International Workshop on Fire Blight*, Warsaw, Poland 16-20. August 2010. Abstracts: 107.
16. **Maniatis T,. Sambrook J. and Fritsch E.F.** (1989): Molecular cloning: A laboratory manual (3rd volume). Nem York: Cold Spring Harbor Laboratory, Cold Spring Harbor.
17. **Mazzucchi** (1977): Elementi di batteriologia fitopatologica. Vol. I. Pitagora. Editrice Bologna, 176 p.

18. **Miller T.D. and Schroth M.N.** (1972): Monitoring the Epiphytic Population of *Erwinia amylovora* on Pear with a Selective Medium. *Phytopathology* 62: 1175-1182 p.

19. **Mohan S.K.** (2007): Natural infection of shoot blight in Pluot® caused by *Erwinia amylovora. Abstracts of 11th International Workshop on Fire Blight*, Portland, Oregon 12-17. August 2007. Abstracts: 64.

20. **Mohan S.K. and Thomson S.V.** (1996): An outbreak of fire blight in plums. *Acta Horticulturae* 411: 73-76 p.

21. **Puskás A.** (1986): Ipari mikrobiológiai gyakorlatok. Kézirat, Budapesti Műszaki Egyetem, Vegyészmérnöki Kar. Tankönyvkiadó, Budapest. 48-50 p.

22. **Ruppitsch W., Stoeger R.A. and Keck M.** (2004): Stability of short sequence repeats and their application for the characterization of *Erwinia amylovora* strains. *FEMS Microbiology Letters* 234 (1): 1-8 p.

23. **Schnabel E.L. and Jones A.L.** (2001): Isolation and characterization of five *Erwinia amylovora* bacteriophages and assessment of phage resistance in strains of *Erwinia amylovora. Applied and Environmental Microbiology* 67 (1): 59-64 p.

24. **Sobiczewski P., Deckers T. and Pulawska J.** (1997): Fire blight (*Erwinia amylovora*), Some Aspects of Epidemiology and Control. *Research Institute of Pomology and Floriculture*, Poland 43-46 p.

25. **Steiner P. and Zeller W.** (1996): A tűzelhalás Magyarországon. Jelentés a Magyar Köztársaság Földművelésügyi Minisztériuma számára.

26. **Suslow T.W., Schroth M.N. and Isaka M**. (1982): Application of rapid method for Gram differentation of plant patogenic and saprophytic bacteria without staining. *Phytopathology* 72: 917-918 p.

27. **van der Zwet T.** (1996): Present worldwide distribution of fire blight. *Acta Horticulturae* 411:7-8 p.

28. **van der Zwet T. and Keil H.M. (**1979): Fire Blight – A bacterial disease of *Rosaceous* plants. Agriculture Handbook 510. US Department of Agriculture, Washington, DC, 200 p.

29. **Vanneste J.L., Lex S., Vermeulen M. and Berger F.** (2002): Isolation of *Erwinia amylovora* from blighted plums (*Prunus domestica*) and potato roses (*Rosa rugosa*). *Acta Horticulturae* 590: 89-94 p.

30. **Winslow C.E.A., Broadhurst J., Buchanan R.E., Krumwiede Jr. C., Rogers L.A. and Smith G.H**. (1920): The families and genera of the bacteria. Final report of the Committee of the Society of American Bacteriologists on characterization and classification of bacterial types. *Journal of Bacteriology* 5: 191-229 p.

Publication in the topic of the dissertation

Articles of journals

Journals with IF:

A. Végh, L. Palkovics, M. Hevesi, I. Király. and M. Tóth (2011): Susceptibility of traditional pear cultivars and virulence of several Hungarian *Erwinia amylovora* isolates. Acta Alimentaria 40, 230-239. **IF: 0.444**

A. Végh, Zs. Némethy, L. Hajagos and L. Palkovics (2012): First report of *Erwinia amylovora* causing fire blight on plum (*Prunus domestica* L.) in Hungary. Plant Disease 96(5), 759. **IF: 2.449 (2011)**

Journals without IF:

Végh A., Tóth M., Hevesi M. és Palkovics L. (2011): Régi körtefajták fogékonysága hazai *Erwinia amylovora* - izolátumokkal szemben. Növényvédelem, 47(3):83- 88.

Végh A., Némethy Zs., Hajagos L. és Palkovics L. (2012): Új gazdanövényen (*Prunus domestica* L. 'D' Agen') jelent meg az *Erwinia amylovora* Magyarországon. Növényvédelem 48(8), 378-382.

Other papers in journals:

Dr. Hevesi M., **Végh A.** és Dr. Tóth M. (2009): A tűzelhalás múltja és jelene. Agrofórum Extra 28, 90-91.

Végh A., Némethy Zs., Hajagos L. és Palkovics L. (2012): A tűzelhalás betegség kórokozója (*Erwinia amylovora*) új gazdanövényeket hódít Magyarországon. Őstermelő 2012/2. sz. 74-75.

Végh A., Hevesi M., Tóth M. és Palkovics L. (2012): Régi körtefajták fogékonyságának vizsgálata a tűzelhalás betegség kórokozójával. Biokultúra 2012/ XXIII. évfolyam, 3-4. sz. 28-30.

Conference papers

International conference (full paper)

M. Hevesi, **A. Végh**, M. Tóth, E. Benczúr and L. Palkovics (2011): Investigating the virulence of *Erwinia amylovora* isolates by using apple tissue culture and pear fruit. Proceedings of the 12 th International Workshop on Fire Blight. Acta Horticulturae 896, 223-230.

International conference (abstract)

A. Végh, L. Palkovics, I. Schwarzinger, M. Tóth and M. Hevesi (2010): Characterization of *Erwinia amylovora* isolates by colony type and bacteriophage sensitivity. 12th International Workshop on Fire Blight, August 16-20, 2010, Varsó, Lengyelország. Book of abstracts, 94.

A. Végh, L. Palkovics and M. Hevesi (2010): Differentiation of Hungarian *Erwinia amylovora* isolates by carbohydrate utilization. 2nd International Conference on Horticulture Post-Graduate Study, August 30-31, 2010, Lednice, Csehország. Book of abstracts, 10.

Hungarian conference (full paper)

Végh A., Hevesi M. és Palkovics L. (2010): Hazai és külföldi *Erwinia amylovora* izolátumok jellemzése szénhidrát hasznosítás alapján. 15. Tiszántúli Növényvédelmi Fórum, 2010. október 20-21., Debrecen. Agrártudományi Közlemények, 2010/37 különszám, 29-33.

Hungarian conference (abstract)

Végh A., Palkovics L., Tóth M. és Hevesi M. (2009): Az *Erwinia amylovora* izolálásának és szelektálásának gyors módszere. Lippai- Ormos- Vas Tudományos Ülésszak, 2009. október 28-30., Összefoglaló 248-249.

Végh A., Hevesi M., Tóth M. és Palkovics L. (2011): Hazai *Erwinia amylovora* izolátumok virulenciája régi körtefajtákkal szemben. 57. Növényvédelmi Tudományos Napok, 2011. február 22-23., Budapest, Összefoglaló, 34.

Hajagos L., **Végh A.** és Palkovics L. (2011): Dél-magyarországi *Erwinia amylovora* izolátumok jellemzése kolónia típus és szénhidrát hasznosítás alapján. Tehetségnap (2011. május 11.); "Tudós diákok az életminőség javításáért" NTP-OKA-XII-047 pályázat, BCE Élelmiszertudományi Kar, Kertészettudományi Kar.

Végh A., Némethy Zs., Hajagos L. és Palkovics L. (2012): A szilva az *Erwinia amylovora* új gazdanövénye hazánkban. 58. Növényvédelmi Tudományos Napok, 2012. február 21-22., Budapest. Összefoglaló, 52.

Others publications

Articles of journals

Journals with IF:

A. Végh, M. Hevesi, Zs. Némethy and L. Palkovics (2012): First report of bacterial leaf spot of basil caused by *Pseudomonas viridiflava* in Hungary. Plant Disease 96(1), 141. **IF: 2.449 (2011)**

M. Hevesi, A. Blazovics, E. Kállay, **A. Végh**, M. Stéger-Máté, G. Ficzek and M. Tóth (2012): Sour Cherry Activity on Bacterial Flora in Human Saliva, Food Technol. Biotechnol. 50(1), 117–122. **IF: 1.195 (2011)**

Other papers in journals:

Simon, G. és **Végh, A**. (2007): A Keep Fresh hatása különböző gyümölcsök tárolására. Kertészet és Szőlészet, 56(41):30.

Conference papers

International conferece (abstract)

M. Hevesi, J. Tornai-Lehoczki, M. Tóth, **A. Végh**, M. Petróczy and L. Palkovics (2008): Characterization of HIP 32 bacterium antagonistic to *Erwinia amylovora*. COST 864 Meeting. Izmir, Turkey, 14-16, May, 2008. 52-53.

Hungarian conference (abstract)

Hevesi M., Blázovics A., Ficzek G., Kállay T.-né, **Végh A**., Stégerné Máté M. és Tóth M. (2008): Meggyfajták antibakteriális hatásának vizsgálata. MSZKT és MTA Mikroelem Munkabizottság Munkaértekezlete, MSD Centrum, Budapest, 2008. szeptember 26., Összefoglaló 13. oldal

Hevesi M., Honty K., **Végh A**., Ficzek G. és Tóth M. (2009): Állami elismerés előtt álló alma fajtajelöltek rezisztenciája a tűzelhalással szemben. Lippai- Ormos- Vas Tudományos Ülésszak, 2009. október 28-30., Összefoglaló 170-171. oldal

Simon G., Rozsnyay Zs., **Végh A.** és Hevesi M. (2009): Cseresznyefajták pseudomonas-fogékonysága / rezisztenciája inokulációs vizsgálattal. Lippai- Ormos- Vas Tudományos Ülésszak, 2009. október 28-30., Összefoglaló 208-209. oldal

Végh A., Győrfi J., Palkovics L. és Hevesi M. (2010): *Agaricus bisporus* (J. E. LANGE) Imbach foltosságát okozó *Pseudomonas tolaasii* és *Pseudomonas reactans* gyors azonosítása. 56. Növényvédelmi Tudományos Napok, 2010. február 23-24., Budapest. Összefoglaló 31. oldal. ISSN 0231 2956, ISBN 963 8131 071