Thesis of Doctoral (PhD) Dissertation

CHARACTERIZATION OF *ERWINIA AMYLOVORA* BACTERIOPHAGES FROM HUNGARY AND THE POSSIBILITY OF THEIR APPLICATION IN BIOLOGICAL CONTROL

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1. INTRODUCTION

Fire blight of pome fruits is one of the most important bacterial diseases of plants in the rose family (*Rosaceae*). It is caused by the phytopathogenic bacterium *Erwinia amylovora* (Burrill) Winslow *et al.* (1920) which is able to induce huge economic losses in apple-, pear- and quince orchards under weather conditions optimal for its growth. The pathogen, being native to North America, first appeared in Europe in the middle 1950s. In Hungary it was first found and identified in a Nyárlőrinc apple orchard in 1995 (**Hevesi 1996**).

Currently, the control of this disease is questionable since the most effective means of protection, the timed application of the antibiotic streptomycin to open blossoms is not allowed. However, this injunction ruling in many countries including Hungary led to the research of numerous alternative control strategies one of which is based on the application of bacteriophages.

Bacteriophages, i.e. the virus pathogens of bacteria have been proved to be efficient in controlling different plant diseases of bacterial origin, among others fire blight (**Jones et al. 2007, Nagy et al. 2012**). The bacterium *E. amylovora* is regarded as a quarantine pathogen in Hungary, therefore, field experiments with this pathogen are not allowed. Our *in vitro* experiments were conducted in the accredited *Erwinia* laboratory of the Department of Biotechnology in the Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences. The purpose of these experiments was to elaborate the scientific background of bacteriophage-based biocontrol measures against *E. amylovora* in Hungary, including the isolation and characterization of Hungarian *E. amylovora* bacteriophages and investigating their impact on *E. amylovora*.

2. MAIN AIMS OF DOCTORAL RESEARCH

During our research work we have set the following aims:

- Collection and isolation of bacteriophages specific to *E. amylovora* in Hungary, in order to survey the possibility of their use in biological control against fire blight

- Characterization of the isolated Hungarian phages and their comparison with *E. amylovora*-specific phage strains originating from North America as a reference

- Selection of the most efficient phage isolates; *in vitro* investigations on the influence of phages on multiplication of *E. amylovora* in different test plants and plant samples

- Clarifying the uptake and delivery of these phages in plants, and to investigate the effect of phages introduced into the plant on fire blight symptoms.

3. MATERIALS AND METHODS

Phage isolates, phage strains and test bacteria

The phage isolates investigated were isolated from different host plants (apple, pear, quince) displaying fire blight symptoms.

For plaque morphology and comparative molecular assays we have used four reference phage strains (ΦEa1h, ΦEa100, ΦEa104, ΦEa116) originally isolated in the USA **(Ritchie and Klos 1975, Schnabel and Jones 2001).**

During our investigations we have used 27 different Hungarian *E. amylovora* isolates, nine other *E. amylovora* strains and four other *Erwinia* species (*E. billingiae* Eb661, *E. persicina*, *E. rhapontici* CFBP 3618T, *E. tasmaniensis* Et1/99), six different isolates and strains of *Pantoea agglomerans*, three *Pantoea* species and eight other bacterial species and strains (*Agrobacterium tumefaciens* C58, *Agrobacterium vitis* F2/5, *Escherichia coli* DH5α, *Xanthomonas campestris* pv. *zinniae*, *Pseudomonas cichorii*, *Pseudomonas syringae* pv. *syringae* H9, *Pectobacterium carotovorum* ssp. *carotovorum* 19111, *Pectobacterium carotovorum* subsp. *atroseptica* D2).

Phage isolation and purification

Phage isolation was done by a modified method of **Crosse and Hingorani** (**1958**), multiplication of phages was accomplished with the aid of Hungarian *E. amylovora* isolates (Ea12, Ea18, Ea26). Phage detection and purification and titer assessment of phage isolates was conducted with the modified procedures of Adams' double agar layer and drop tests (**Adams 1959**).

Characterization of phage isolates

Plaque morphology

The 22 different bacteriophage isolates obtained were distributed on LBA top agar layers supplemented with 1% sucrose and containing the test bacterium (*E. amylovora* EaCFBP1430 strain) according to the modified Adams' double agar layer method. Following incubation for one day at 28°C the phage isolates were visually characterized based on plaque size and the width of halos surrounding the plaques.

Phage particle (virion) morphology

An important basis of taxonomical identification of phages is the morphological characterization of virus particles (virions). This means recording the shape and size of phage heads and tails by electron microscopy. Sample containing the purified phage isolates (19 different isolates) were assayed by a Morgagni 268D type transmission electron microscope (TEM) following a negative staining procedure according to **Gill et al.** (**2003**).

PCR assays

During PCR (polymerase chain reaction) assays of 21 different phage isolates, primer sequences were used that are specific to the following *E. amylovora* phage genes/regions: EPS depolymerase, holing, lysozyme, terminase, peptidase, Mu-like prophage proteins, given genomic regions of the American reference phage strains used (ΦEa1h, ΦEa100 and ΦEa104), capsid genes of ΦEa1h and ΦEaH2, the Hungarian phage strain with a known genome, the *ams*F gene of the phage strain Ea7/74 and the *ams*F gene-like region of ΦEaH2. Templates for PCR reactions were obtained from the purified phage suspensions by sodium azide (NaN_3) extraction of DNA.

Sequence analysis

So far, our investigations have focused on the sequence analysis of the genomic regions encoding deploymerase in seven Hungarian phage isolates and partial nucleotide sequence determination of the genome of two other Hungarian phage isolates (H2A, H5K). In case of the phage isolate H2A, DNA fragments for sequencing were amplified during PCR with primers specific for the genes encoding peptidase, Mu-like prophage protein and terminase, while in case of the phage isolate H5K, primers specific for Mu-like prophage protein and terminase genes were used. Sequences obtained by automated DNA sequencing were analyzed and compared to homologous nucleotide sequences in international databases.

Host range analysis

16 Hungarian phage isolates obtained by us were included in host range tests. Susceptibility of the test bacteria (10^8 CFU ml^{-1}) to the phages (10^6 PFU ml^{-1}) was determined with the modified Adams' drop test. Bacterial susceptibility was characterized based on the purity of plaques in the upper agar layer containing the indicator bacteria by the following scale: ’++’ = clear plaques,’+’ = turbid (not clear) plaques, ’-’ = no plaques.

In vitro tests on the influence of phages and phage combinations on the bacterium *Erwinia amylovora*

Phage efficiency tests in liquid culture

1 ml phage suspensions (10^{10} PFU ml^{-1}) or a 1:1 combination of given phage suspensions and 1 ml test bacteria (streptomycin resistant *E. amylovora* strain Ea1/79Sm, 10^5 CFU ml^{-1}) were added to liquid LB medium in glass vials. The cultures were incubated for 22 hours at 28°C. Optical density (OD) of liquid cultures was checked at 600 nm. These OD_{600} values were used for evaluation of the experiment.

Phage efficiency tests on apple and quince flowers

Phage isolates proved to be the most effective in the liquid culture tests (H2A, H5K and H7B) and their combination (H2A+H5K+H7B) were used for testing their capability to reduce bacterial numbers in apple flowers displaying different levels of susceptibility to fire blight ('Idared', 'Golden delicious Reinders', 'Gala Schniga', 'Pinova') and in flowers of a susceptible quince cultivar ('Berecki'). Flowers were collected in the balloon phenophase and placed individually into small glass vials filled with 1% sucrose. Within 12 hours, flowers opened and 20 µl of a 1:1 mixture of phage lysate (10^{12} PFU ml^{-1}) and bacterial suspension (*E. amylovora* strain Ea1/79Sm, 10^5 CFU ml^{-1}) were pipetted onto pistils. Flowers were incubated in a growth chamber for 4 days at a relative humidity of 80%. Number of samples per treatment was 15 (in case of positive controls 20).

Following incubation of flowers, petals were removed and flower stalks with pistils placed in sterile Eppendorf tubes with 1 ml sterile double distilled water. After an incubation of 10 minutes at 22-23°C samples were briefly centrifuged and the suspension obtained with this bacterium re-isolation procedure was mixed and diluted. 50 µl bacterial suspension diluted 10 000 x with water was pipetted and plated onto a solid LBA medium plate containing 500 ppm streptomycin (LBA_{500Sm}). Results were evaluated following an incubation for 2 days in the dark at 28°C based on colony numbers.

In samples of cv. 'Idared' collected in 2012 the titer of *E. amylovora* was also checked by real time qPCR. Control dilutions of *E. amylovora* (serving as standards) and samples from treated flowers (see above) were extracted with sodium azide to obtain DNA templates. Primers used for PCR reactions are specific for the pEA29 plasmid of *E. amylovora*: P29TF (5' CACTGATGGTGCCGTTG 3') and P29TR (5'CGCCAGGATAGTCGCATA 3') (**Salm**

and Geider 2004). Results obtained were compared to those of bacterial colony counts (see above).

Phage efficiency tests on unripe pear slices

The anti-bacterial effect of phage isolates and their combinations were also tested on unripe (green) fruit slices of three pear cultivars (’Conference’, ’Dr. Guyot Gyula’, ’Erdei vajkörte’). The 0.5 cm thick pear slices (6 slices/treatment) have been placed into glass Petri dishes and soaked in either of the following solutions: 10 ml phage suspension (10^{12} PFU ml^{-1}), water or 100 ppm streptomycin. Both sides of the slices were soaked for 5 minutes each. Afterwards, pear slices were artificially inoculated with 10 µl (5 x 10^5 CFU ml^{-1}) of the pathogenic bacterium (*E. amylovora* wild type strain Ea1/79) by pipetting into the middle of the slices. Inoculated pear slices were incubated in close Petri dishes in growth chambers for 4 days.

Following incubation, the severity of symptoms due to bacterial infection was evaluated with the aid of a bonitation scale as following: (0) symptomless; (1) browning of the middle part of slices, around the inoculation site, with mucus; enhanced mucus production accompanied by browning of (2) 1/8-th of the slice; (3) 1/4-th of the slice; (4) 1/2 of the slice; (5) 3/4-th of the slice; (6) the whole slice.

Phage efficiency tests in ornamental plants

Phage isolates H2A, H4A and H5A and their combination (H2A+H4A+H5A) were used for testing their capability to reduce bacterial numbers in cuttings removed in full bloom. The following *E. amylovora*-susceptible hosts were checked: firethorn, garden cotoneaster, sorb and common hawthorn. Removed cuttings were placed in water and sprayed first with a suspension of given phages or phage combinations (10^4 PFU ml^{-1}), 20 minutes later followed by spraying with a bacterial suspension (*E. amylovora* strain Ea1/79Sm, 10^9 CFU ml^{-1}). Cuttings were incubated in a growth chamber for 4 days.

The effect of phage treatment on cuttings was evaluated by screening for lair (receptacle) browning (% bacterial infection) within 100 randomly chosen flowers.

Potential enhancement of phage treatments: penetration and translocation of bacteriophages *in planta* and their influence on symptoms of *E. amylovora* infection

Preliminary experiment to test the uptake and translocation of phages in apple seedlings

In this experiment the root uptake of phage strain H5K was tested in 5 month-old apple seedlings in sterile hydroponic culture and in perlite medium. Phages at a concentration of 10^{13} PFU ml^{-1} (suspension in water) were applied to the root zone of five plants per treatment: Subsequently, samples were taken at two time points by extracting the upper stem parts and leaves together. Considering that the titer of phage suspensions applied will possibly drop below levels detectable with direct re-isolation, 200µl of samples taken after a two-day incubation were shaken for a further four days in liquid medium (LB_{500Sm}) with bacterial culture (*E. amylovora* Ea1/79Sm).

For phage detection the modified Adams' drop test was used.

Translocation of phages in apple seedlings

Phage suspensions (ΦEa104 and H5K, 10^{13} PFU ml^{-1}) were applied to 17-18-week-old apple seedlings by two different methods, either drenching the perlite surface with 10 ml/seedling or spraying of aerial plant parts with 500 µl/plant of phage suspension. To avoid contamination of untreated plant parts with phages, drenching of the perlite medium was carried out by careful pipetting. Spraying of phage suspensions was performed after the opening of the vessel around the stem had been properly covered with aluminium foil and parafilm. Control plants were treated with water. Treatments included fifteen seedlings, i.e. three seedlings per each time point (1, 2, 3, 5 and 7 days after treatment). Following phage application to the root zone, plant parts with one centimetre above ground level were cut in two and weights of the lower stem parts (including cotyledons) and those of the leafy upper stem segments were separately determined and assayed for phage titres. In case of spraying phages onto the stem and leaf surface, stems were cut at one centimetre above ground level, root weights were measured and roots were examined for the presence of phages. Samples were extracted in SM buffer (100 mM NaCl, 8 mM MgSO4 · 7H2O, 50 mM Tris-Cl (1 M, pH 7.5), 0,002% (w/v) gelatine) and homogenized in sterile mortars.

Phage detection was carried out the modified Adams' drop test either directly after sampling or following further multiplication of phages for two days in liquid medium with bacterial culture (multiplication sampling). Samples positive for plaques were checked for the

presence of phages also by PCR. In case of phage isolate H5K a primer pair specific for the terminase gene, while in case of phage strain ΦEa104 a primer pair designed by us was used.

Effect of phage applications on* E. amylovora *symptoms - phages applied to apple seedling roots or plant parts above ground level

Phage suspensions were applied similarly as in the translocation experiment (two different modes of application) to 20-week-old apple plants grown in perlite. On the next day seedlings were artificially infected with the *E. amylovora* strain Ea1/79Sm (except for negative controls): 10 µl of bacterial suspension (5 x 10^5 CFU ml^{-1}) was pipetted to the node at the second or third young leaf, then the tissue was pricked through the drop using a sterile needle. Three plants per treatment were used for both phage isolates (ΦEa104 and H5K) at two concentrations (10^3 and 10^{13} PFU ml^{-1}, respectively). Disease symptoms were observed and recorded on the 5th and 13th days after treatments according to a bonitation scale from 0 to 5 as follows: (0) no symptoms; (1) infection site is brownish black; (1.5) 1/3 portion of the petiole around the site of infection is brownish black; (2) 1/2 portion of the cotyledon is brownish black and wilting; (2.5) the whole petiole is brownish black and wilting; (3) also the stem around the site of infection is brownish black; (3.5) necrosis to the extent of 1/4 portion of the plant; (4) necrosis to the extent of 1/3 portion of the plant; (4.5) necrosis to the extent of 1/2 portion of the plant; (5) large-scale necrosis.

Effect of phage applications on* E. amylovora *symptoms - injecting phage suspensions into the cotyledon of apple seedlings

Phage suspensions at a concentration of 10^{13} PFU ml^{-1} were injected into one of the cotyledons of 8-week-old apple seedlings. This was followed by immediate bacterial inoculation with Ea1/79Sm by pipetting 10 µl of bacterial suspension (10^6 CFU ml^{-1}) to completely soaked cotyledons and a second pricking through the droplet. Following five days of incubation fire blight symptoms developed on the cotyledon were recorded according to a bonitation scale grading from 0 to 5 as follows: (0) intact green cotyledon; (0.5) small necrotic spots at the site of infection; (1) 1/6 portion of the treated cotyledon is brown; (1.5) 1/5 portion of the cotyledon is brown (2) 1/4 portion of the cotyledon is brown; (2.5) 1/3 portion of the cotyledon is brown (3) 1/2 portion of the cotyledon is brown; (3.5) 3/4 portion of the cotyledon is brown; (4) the whole cotyledon is brown; (4.5) the untreated cotyledon is also brown; (5) both cotyledons are brown.

4. RESULTS AND DISCUSSION

Phage isolation

We have altogether 22 phage isolates, out of these 16 have been characterized in detail.

Characterization of phage isolates

Plaque morphology

The morphology of plaques produced on a test bacterium is an important characteristic of phages investigated in detail by, among others, **Gill et al.** (**2003**). Our phage isolates formed plaques of different sizes, with a diameter of 0.5 – 7.1 mm on the soft agar layer containing the test bacterium. The halo around plaques – when present – had a diameter between 0.1 – 5.0 mm in the different isolates. The smallest plaques are formed by H10A (0.5 – 0.6 mm), while plaques of H5K are the smallest (including halos), just a little bit smaller than plaques of the American phage strain ΦEa116. One of the largest sizes of plaques belongs to H7B; these plaques have a much broader halo then those of ΦEa100, an American strain giving the largest plaques in our assays. This indicates a higher lytic enzymatic activity of H7B.

Phage particle (virion) morphology

*E. amylovora p*hages that we have isolated in Hungary were assigned by us to the order *Caudiovirales* and, according to morphotyepes C1 and A1, in the *Podoviridae* and *Myoviridae* families (**Ackermann 2007**). Among these Hungarian phyages, the smallest is H11, which is smaller than phages from Germany and the USA described in the literature. The largest is H4A which has a larger size than the reference (German and American) phages mentioned previously. Head diameter of Hungarian phages is in line with literature data (**Richie and Klos 1979, Müller et al. 2011a**). Isolates assigned to the *Podoviridae* family have a head diameter of 60 nm, while those belonging to *Myoviridae* have a head diameter of ca. 70 nm. Our Hungarian phage isolates markedly differ from the two Hungarian isolates previously described in the literature and belonging to the *Siphoviridae* family [ΦEaH1 (**Meczker et al. 2014**), ΦEaH2 (**Dömötör et al. 2012**)].

PCR assays

Based on results of PCR assays we have assigned the characterized Hungarian phage isolates into four major groups and concluded that none of them are identical to the four American reference *E. amylovora* phage strains. In case of our own (Hungarian) isolates PCR

products were not obtained with primer pairs designed based on the *ams*F-like gene or capsid protein gene of ΦEaH2, the Hungarian phage strain with a well characterized genome.

Sequence analysis

Nucleotide sequence of the depolymerase gene of the investigated phage isolates (H4A, H4B, H5A, H5B, H6A, H7A, H9B) is 98-99% identical to that of ΦEa1h (**Müller et al. 2011b**). The partial sequence analysis revealed that in the genomes of H2A and H5K genes encoding the large terminase subunit and Mu-like prophage protein are indeed present. These genes show a high similarity to those of the vB_EamM-M7 *E. amylovora* phage (**Born et al. 2011**). Since the peptidase encoding genomic region of isolate H2A is also 99% identical to this phage strain, we plan to include the phage vB_EamM-M7 in our future experiments.

Host range analysis

Our phage isolates tested have a broader host range than the four American reference phage strains used in our investigations. Out of these isolates H1B, H2A, H2B, H4A, H7A and H7B have lysed most of the tested bacterial isolates and strains, while ΦEa116 was the most effective among the American phages. On the other hand all of the Hungarian *E. amylovora* isolates were susceptible to all of the phage isolates tested, irrespective of their origin. Among the non-plant pathogenic bacteria included in our assays *Pantoea agglomerans* MB96 was the most susceptible to the tested phages, except for H4B, all other phages gave clear plaques on this bacterium strain.

In vitro tests on the influence of phages and phage combinations on the bacterium *Erwinia amylovora*

Phage efficiency tests in liquid culture

We have detected decreased optical density values at the end of the incubation period in liquid medium that contained the given phage and the bacterial plant pathogen *E. amylovora*, similarly as described earlier by **Schnabel and Jones** (**2001**). The tested Hungarian phage isolates significantly decreased bacterial concentration as compared to positive controls without phages, with the exception of H1A, H4A and H6B. In case of the American phage strains only ΦEa100 displayed an effect significantly different from positive controls. Being aware of the fact that phages may have an increased efficiency in combination than alone (**Schnabel et al. 1999**) we have tested the three most efficient phages in different

combinations. The phage cocktail H2A+H5K+H7B gave the lowest OD_{600} values as compared to untreated (positive) controls. However, differences among treatments were significant in only two cases.

Phage efficiency tests on apple and quince flowers

Phage isolates and their triplicate combinations (3 phages / combination) most effective in inhibition of *E. amylovora* multiplication in liquid culture were also tested on apple and quince flowers *in vitro*. In case of all apple cultivars and quince, phage treatments suppressed multiplication of *E. amylovora*. The most optimal treatment was conferred by a phage combination that turned to be significantly more efficient on the partially resistant cultivar 'Pinova', as compared to cv. 'Idared' which is regarded as susceptible. Our results gained by colony counts (cv. 'Idared', 2012) were also confirmed by quantitative PCR.

Phage efficiency tests on unripe pear slices

The antibacterial effect of phage isolates and their triplicate combinations previously checked on flowers were also tested on unripe fruit slices of pear cultivars. We found that phage treatments reduced the symptom severity of fire blight as compared to positive controls. On the other hand, the antibacterial effect of phages was markedly lower than that conferred by streptomycin sulfate in all experiments. Based on these results phage treatments proved to be more efficient on cultivars 'Erdei vajkörte' and 'Dr. Guyot Gyula', as compared to cultivar 'Conference'. It is noteworthy to mention, however, that phage treatments of pear slices were much less efficient than that of flowers, similarly as found by **Müller et al.** (**2011a**).

Phage efficiency tests in ornamental plants

Efficiency of phages H2A, H4A and H5A and their combination was tested on flowers of four different ornamental plant species susceptible to fire blight. The most optimal effect was conferred by the phage cocktail (combination). In fact, symptoms resulting from infection by *E. amylovora* were most effectively reduced by this phage combination on flowers of firethorn. However, the phage cocktail was not able to significantly reduce the extent of flower necrosis on sorb and hawthorn.

Potential enhancement of phage treatments *in vitro*: penetration and translocation of bacteriophages *in planta* and their influence on symptoms of *E. amylovora* infection

Preliminary experiment to test the uptake and translocation of phages in apple seedlings

Two days after a single treatment of the root zone by a water-based phage suspension (H5K) we have detected phages in the upper stem parts and leaves of plants. Phages were identified by direct sampling in one plant sample grown in perlite. However, employing the so-called multiplication method, lead to the detection of phages in all plants grown in perlite and in one plant grown in hydroponic medium. In case of perlite-grown plant samples, the plaques obtained were translucent and coalescing which indicates a high concentration of phages. After incubation for six days we could detect phages in all plants grown in hydroponic medium and in one perlite-grown plant sample. All water-treated controls were free of phages.

Translocation of phages in apple seedlings

Following a single treatment of apple seedlings with phage suspensions (H5K andΦEa104) we could detect phages both in the upper plant parts (following treatment of the root zone) and in the roots (following spraying of lower and upper stem parts). The fact that both *E. amylovora*-specific phages tested by us were capable of penetration and translocation in experimental plants under the given circumstances is in accordance with earlier results obtained with other bacteriophage-plant interactions (**Rao and Srivastava 1973, Ward and Mahler 1982, Iriarte et al. 2012**). In case of both phage isolates the re-isolation of phages was possible from almost all plant samples on the first day after treatments. On the other hand, in samples containing viable phages and extracted by direct sampling phage density has significantly declined. In positive, plaque-producing samples extracted by the multiplication method the *in planta* presence of phages was also confirmed by PCR: in case of H5K in 7 out of 11 samples, in case of ΦEa104 in 10 out of 13 samples.

Effect of phage applications on* E. amylovora *symptoms - phages applied to apple seedling roots or plant parts above ground level

In case of both types of application phage treatments resulted in a significant decline of fire blight symptoms following inoculations with suspensions of the bacterium *E. amylovora* as compared to untreated controls. Comparing effects of the two different phage concentrations applied it is obvious that in the second experiment ΦEa104 applied in a concentration of 10^{13} PFU ml^{-1} could significantly reduce bacterial symptoms following application either to the

roots or upper plant parts (canopy). In the second experiment treatment of upper plant parts (stem and leaves) with H5K at 10^{13} PFU ml^{-1} proved to be significantly more effective in reducing fire blight symptoms than treatment at the lower concentration. On the other hand, in the second experiment none of the phage strains and concentrations applied gave a significantly different effect when comparing the types of application (roots vs. upper plant parts), although H5K at 10^{13} PFU ml^{-1} was slightly more effective in case of canopy application than following root zone treatment. Repetitions (i.e. the first and second experiment) did not differ significantly. Thirteen days after treatments the evaluation of phage effects was not possible any more due to severe necrosis of most of the seedlings.

Effect of phage applications on* E. amylovora *symptoms - injecting phage suspensions into the cotyledon of apple seedlings

Following phage treatments and artificial inoculation by *E. amylovora* the severity of fire blight symptoms significantly declined in comparison to untreated positive controls, as evaluated according to a bonitation scale. Fire blight symptom severity during the five day incubation period was reduced (in average of three repetitions) by 45% in case of H5K and 67% in case of ΦEa104 treatments. There was no significant difference among the effects of these two phages.

5. NEW SCIENTIFIC RESULTS OF DOCTORAL RESEARCH

- We were the first to isolate and characterize *E. amyovora*-specific bacteriophages that belong to the *Myoviridae* and *Podoviridae* families in Hungary.
- We present the first determination of the nucleotide sequence of the *dpo* gene of seven Hungarian *E. amyovora* phage isolates that belong to the *Myoviridae* and *Podoviridae* families and sequences of genes encoding a peptidase, two Mu-like prophage proteins and a terminase of an additional phage isolate. According to GenBank data these DNA regions show a high degree of homology to the same DNA regions of other *E. amyovora* phages.
- We present novel data on inhibition of multiplication of the bacterium *E. amyovora* by bacteriophages and their combinations isolated and selected by us under in vitro conditions in apple and quince flowers, unripe pear fruit slices and ornamental plants.
- We have elaborated novel methods (application of perlite medium, injection of bacteriophages into cotyledons) for investigating the ability of host plants to uptake phages through the roots or leaves and the *in planta* influence of phages on symptoms caused by *E. amyovora*.
- We were the first to show that *E. amyovora* bacteriophages are capable to enter and translocate through the roots of apple seedlings.

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