Department of Biotechnology,
Intercollegiate Faculty of Biotechnology
University of Gdansk and Medical University of Gdansk

The National Centre for Research and Development

LIDER

PAT BIO CON

Ecological interaction of lytic bacteriophages and plant pathogenic bacteria – a case study involving lytic phages and pectinolytic plant pathogens in plant-associated environment

Robert Czajkowski
Robert.czajkowski@biotech.ug.edu.pl

INTERNATIONAL SYMPOSIUM
MICROBE-ASSISTED CROP PRODUCTION-OPPORTUNITIES, CHALLENGES & NEEDS

mICROPe
Dec. 4 – 7, 2017
VIENNA, AUSTRIA

Interactions in the microworld (=world of microbes)

- quite similar to these observed in the macroworld
- the rule "*everyone with everyone/everything the whole time*"
- impossible to simulate the whole spectrum of interactions under laboratory conditions
- therefore… we need models:
  - ↑ smaller, simpler, time-wise
- but they are also:
  - ↓ limited, error-prone, finding-prone, artefact-full

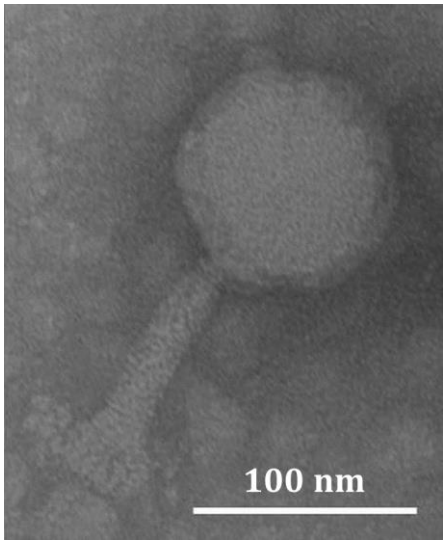
Model *def.* /ˈmɒd(ə)l/
1. a three-dimensional representation of a thing, typically on a smaller scale than the original.
2. a thing used as an example to follow or imitate.

mICROPe

The aim

- To assess the interaction of:

Lytic bacteriophage ϕD5



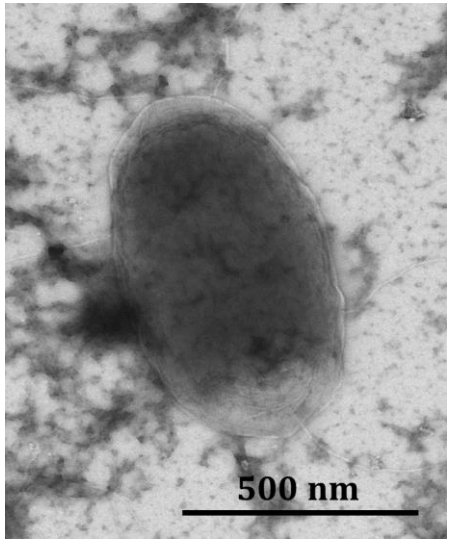
Taxonomy:
Family: ***Myoviridae***
Order: ***Caudovirales* (tailed phages)**

Host range:
broad: *Dickeya solani, D. dadantii, D. dianthicola, D. chrysanthemi D. dadantii subsp. dieffenbachiae*
Genome sequenced: KJ716335

(Czajkowski et al. 2014, 2015)

+

Dickeya solani strain IPO2222



Taxonomy:
Family: ***Enterobacteriaceae***
Order: ***Enterobacteriales***

Host range:
broad: agriculturally important crops and ornamental plants including potato, hyacinth, tulip
Genome sequenced: CP015137

(van der Wolf et al. 2014)

+

Solanum tuberosum L.

Facts
Staple crop in more than 126 countries
Not native to Europe
(introduction around 1570)

Production: more than 325 M tonnes worldwide, 80% produced in Asia and Europe
Important for food market, industry, alcohol and starch production.
Prone to bacterial diseases

Potato - as a staple crop model

- potato was introduced to Europe around 1570 (*via* Spanish explorers from South America) =non native to Europe
- is the fourth main food crop worldwide (after rice, maize and wheat)
- potato is produced in 126 countries and the area of its culitvation is increasing in developing regions
- 325 mln tonnes of potato are produced worldwide
- majority (ca. 80 %) of potato is produced in Europe and Asia
- potato is affected by different bacterial, fungal and viral pathogens

Soft Rot *Enterobacteriaceae* (SRE)

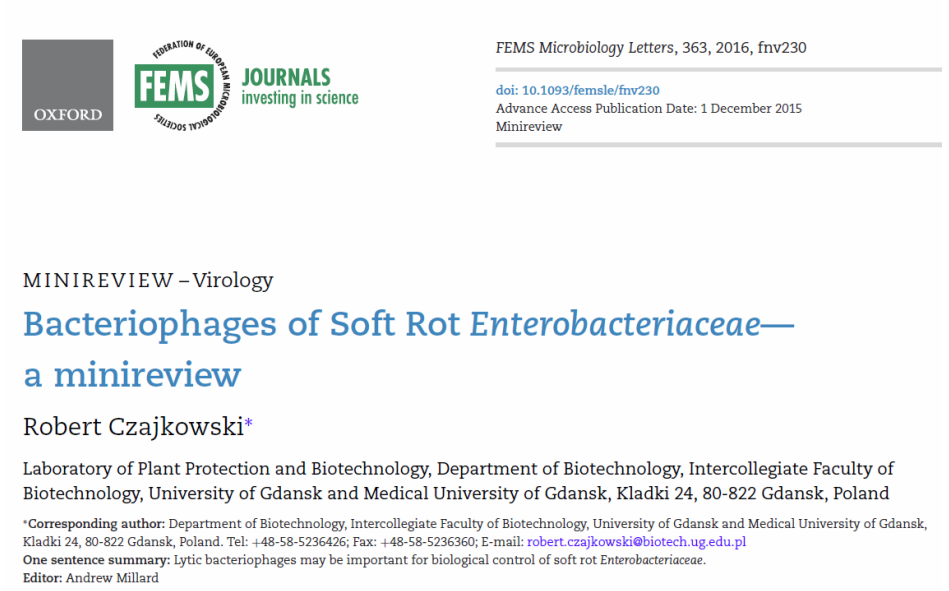
- Gram(-) *Enterobacteriaceae*
- in the past these bacteria were named pectinolytic *Erwinia* spp. (now divided into two genera *Dickeya* spp. and *Pectobacterium* spp.)
- bacteria causing soft rot symptoms on many important crops (also symptoms of blackleg in potato)
- present worldwide on different primary and secondary host plants and as saprophytes
- estimated losses – ca. 60M – 120M Euro (worldwide) annually
- difficult to control, integrative pest management is only partially successful
- biocontrol?

Bacteriophages in biological control applications

- Bacteriophages as biocontrol agents of plant pathogens
  - soft rot in Zantedeschia (*Pectobacterium carotovorum*)
  - fire blight in pear and apple (*Erwinia amylovora*)
  - bacterial spot of peach (*Xanthomonas axonopodis* pv. *pruni*)
  - bacterial blight of geranium (*Xanthomonas campestris* pv. *pelargonii*)
  - bacterial spot of tomato (*Xanthomonas campestris* pv. *vesicatoria*)
  - bacterial blotch of mushrooms (*Pseudomonas tolaasii*)
  - *Streptomyces scabies* and *Ralstonia solanacearum* in potato
- (Broad host) lytic bacteriophages against *Dickeya* spp. and *Pectobacterium* spp. (only limited data present in the literature)
- Most papers describe “proof-of-concept” experiments only

Bacteriophages of soft rot *Enterobacteriaceae*

- ca. 6000 individual phage isolates reported to date (Ackermann, 2007, 2011)
- ca. 2000 (ca. 30%) phage isolates target members of *Enterobacteriaceae*
- but only less than 20 isolates infecting specifically soft rot *Enterobacteriaceae*
- SRE bacteriophages are generally poorly-characterized (Czajkowski et al. 2016)



OXFORD FEMS JOURNALS investing in science

FEMS Microbiology Letters, 363, 2016, fnv230

doi: 10.1093/femsle/fnv230
Advance Access Publication Date: 1 December 2015
Minireview

MINIREVIEW – Virology

Bacteriophages of Soft Rot *Enterobacteriaceae*—a minireview

Robert Czajkowski*

Laboratory of Plant Protection and Biotechnology, Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Kladki 24, 80-822 Gdansk, Poland

*Corresponding author: Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Kladki 24, 80-822 Gdansk, Poland. Tel: +48-58-5236426; Fax: +48-58-5236360; E-mail: robert.czajkowski@biotech.ug.edu.pl

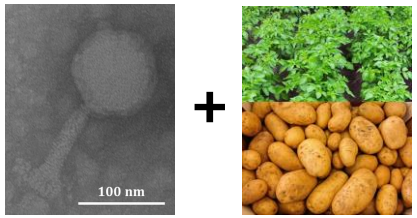
One sentence summary: Lytic bacteriophages may be important for biological control of soft rot *Enterobacteriaceae*.

Editor: Andrew Millard

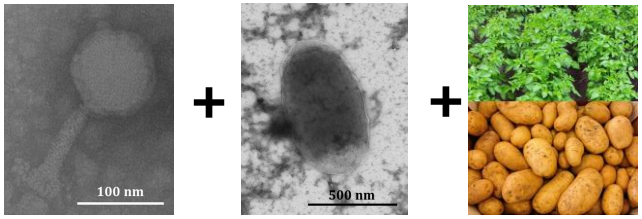
mICROPe

Experimental setup:

- stability of bacteriophage ϕD5
  - in potato tuber extract (vacuum infiltration of tubers before planting)
  - in sterilized and unsterilized soil (applied during planting)
  - in sterilized and unsterilized rain water (applied in water)
  - on surface of potato tubers at 6-8 °C (applied in storage)
  - on surface of potato (detached) leaves (applied on growing plants)
  - in solutions containing copper ions (applied together with other protective chemicals)

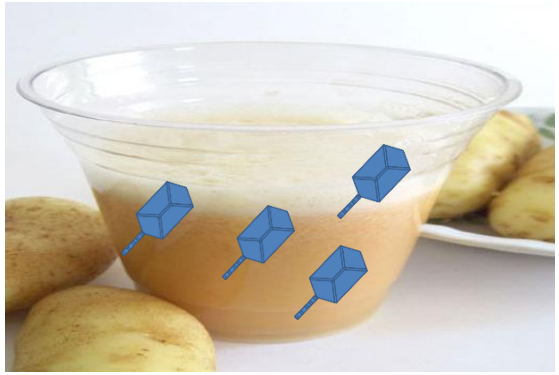


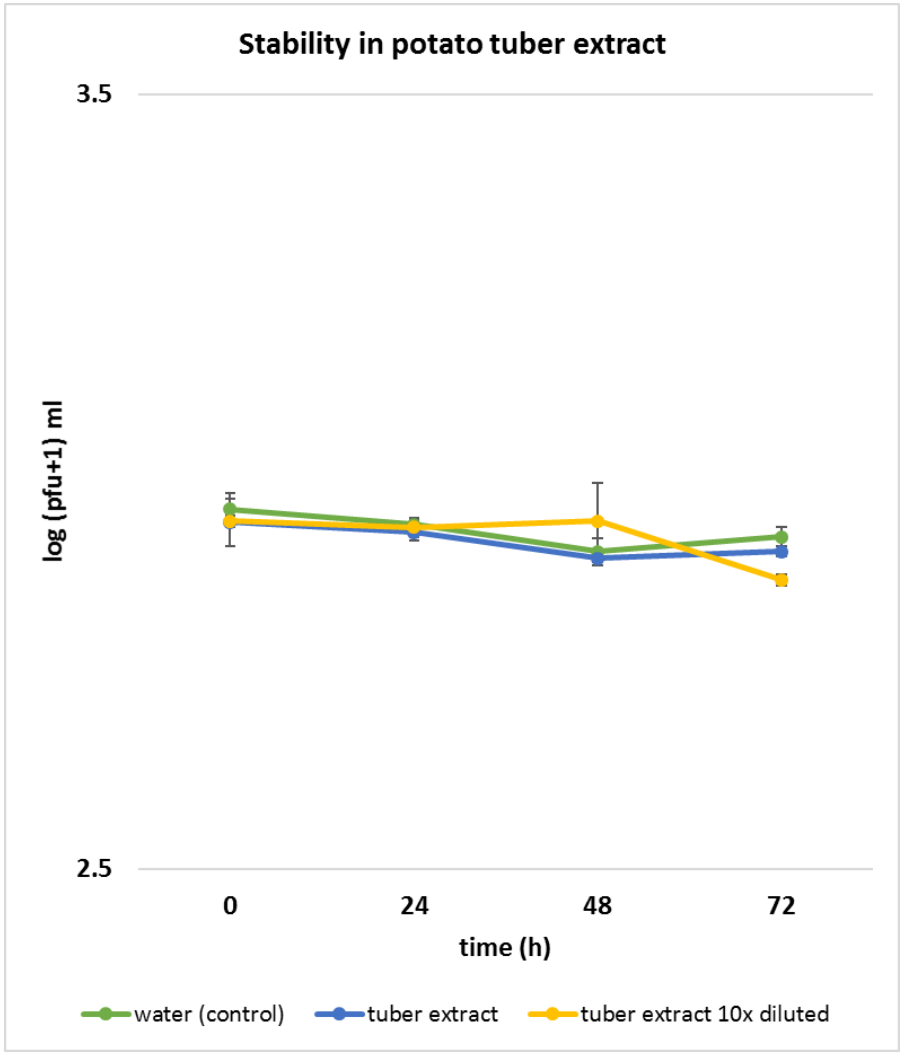
- interaction of ϕD5 with *D. solani* strain IPO2222^{T}
  - tissue culture potato plants cv. Kondor
  - potato plants grown in soil



Stability in potato tuber extract at 22 °C

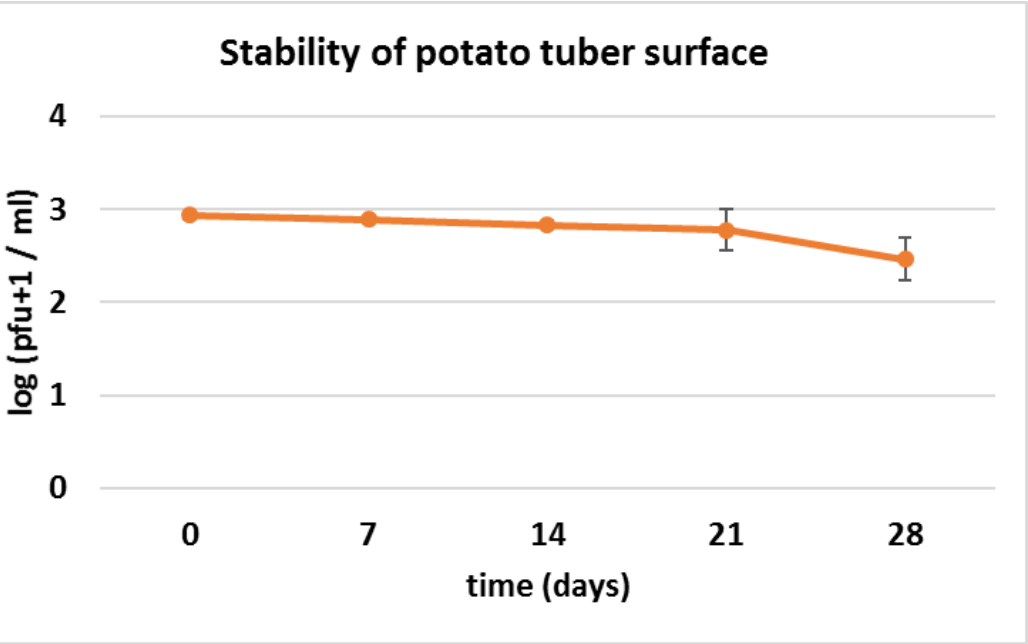
- no statistically significant differences in comparison with control
- no statistically significant reduction of phage numbers during incubation was observed
- no difference between 10x diluted and undiluted tuber extract

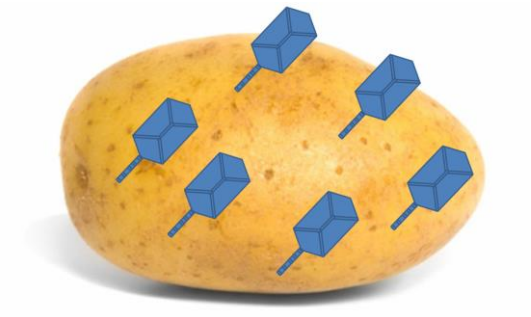




Stability on surface of potato tuber (at 4-6 °C and 80% RH)

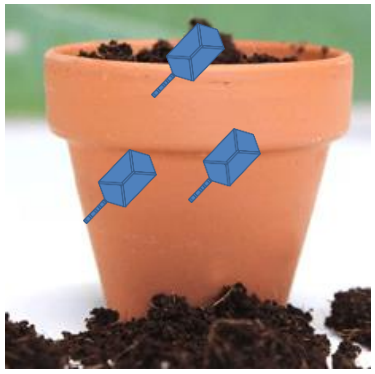
- no statistically significant reduction of phage numbers during incubation was observed
- on average only ca. 13% reduction of phage numbers observed during 28 days

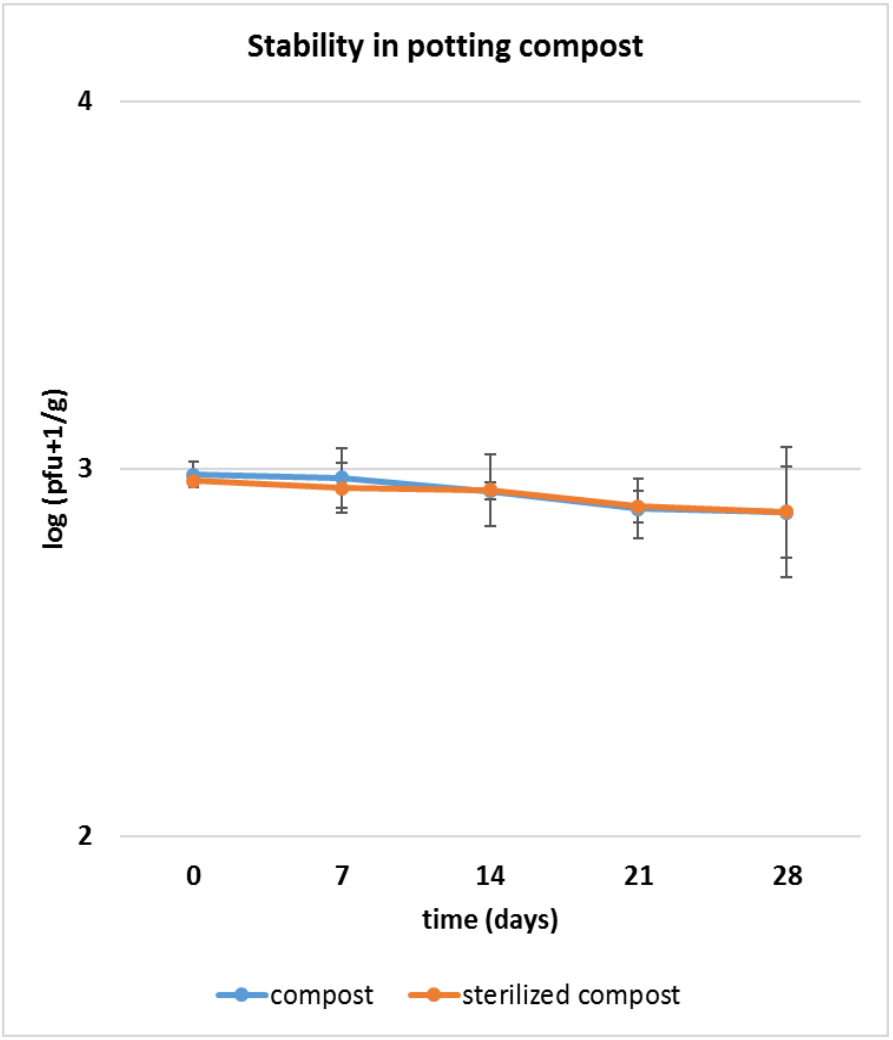




Stability in potting soil at 50% field capacity and 10 °C

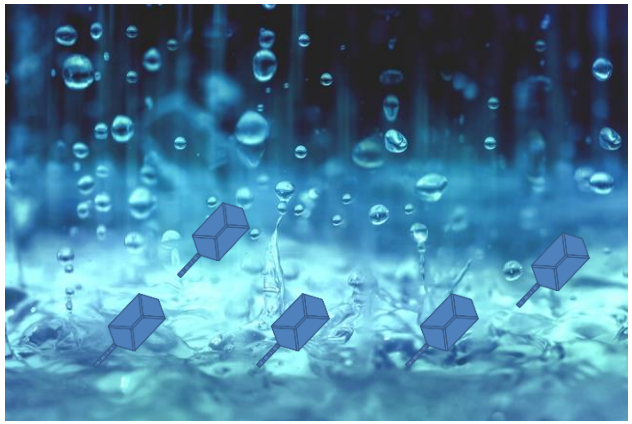
- no statistically significant differences was observed in sterilized and unsterilized potting soil
- on average only ca. 3.5 % reduction of phage numbers observed during 28 days

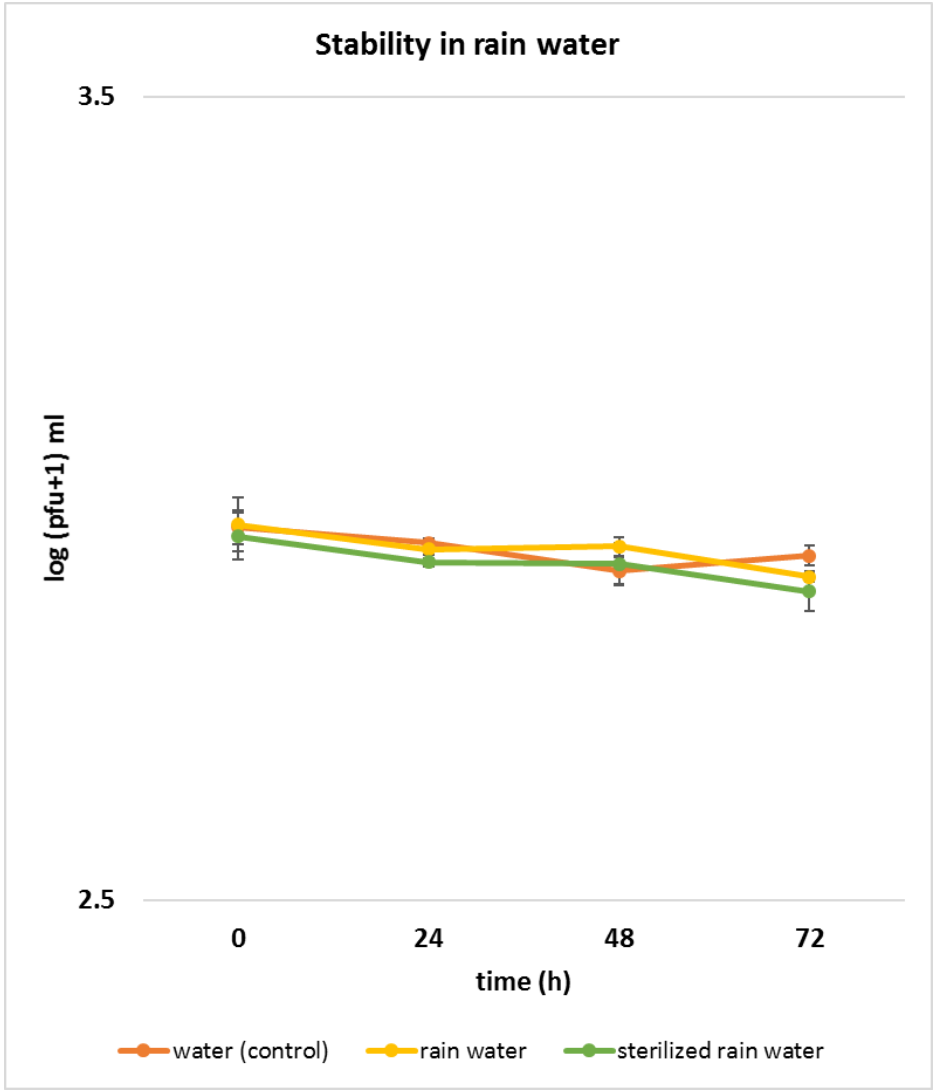




Stability in rain water

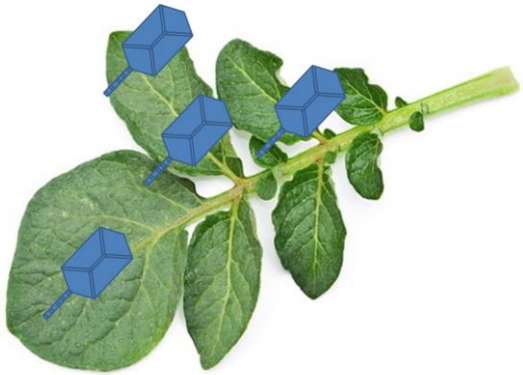
- no statistically significant differences in comparison with control
- no statistically significant reduction of phage numbers during incubation was observed

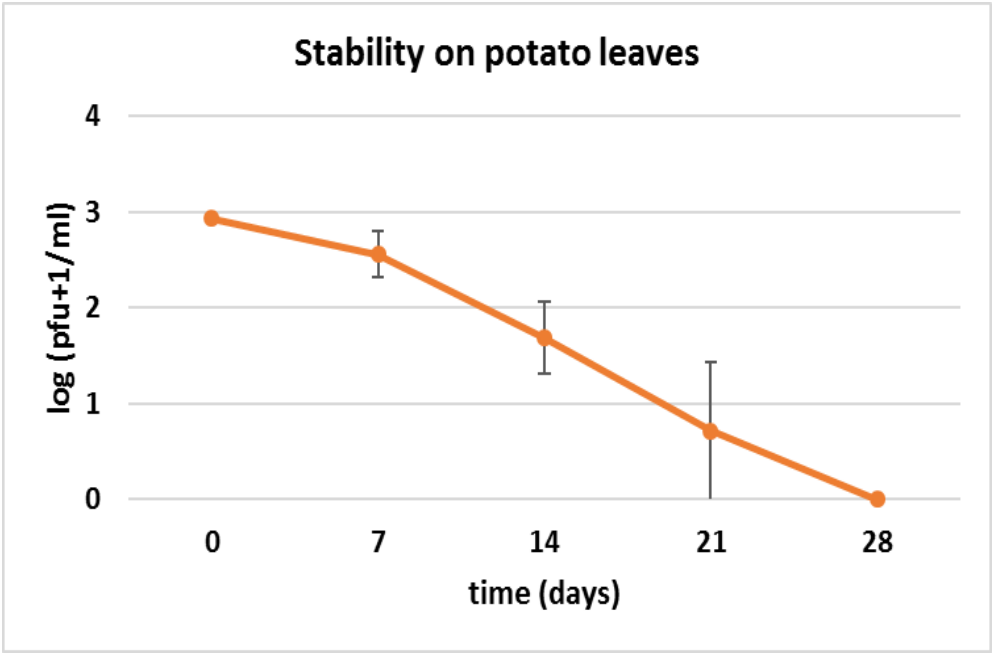




Stability on leaf surface at 26 °C (growth chamber)

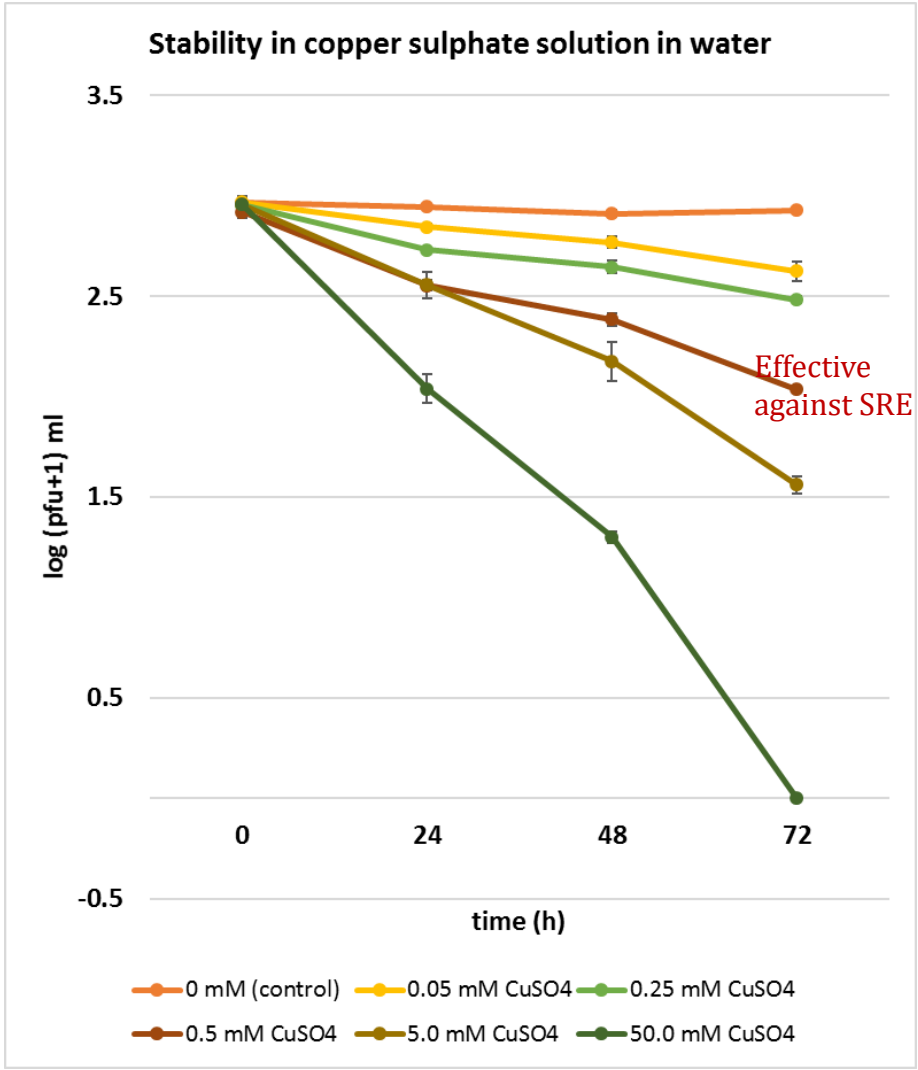
- a decline of phage numbers was observed during the entire course of experiments
- ca. 10x times reduction of phage numbers observed during first 14 days
- no phages recorded at 28 dps (days post spiking)



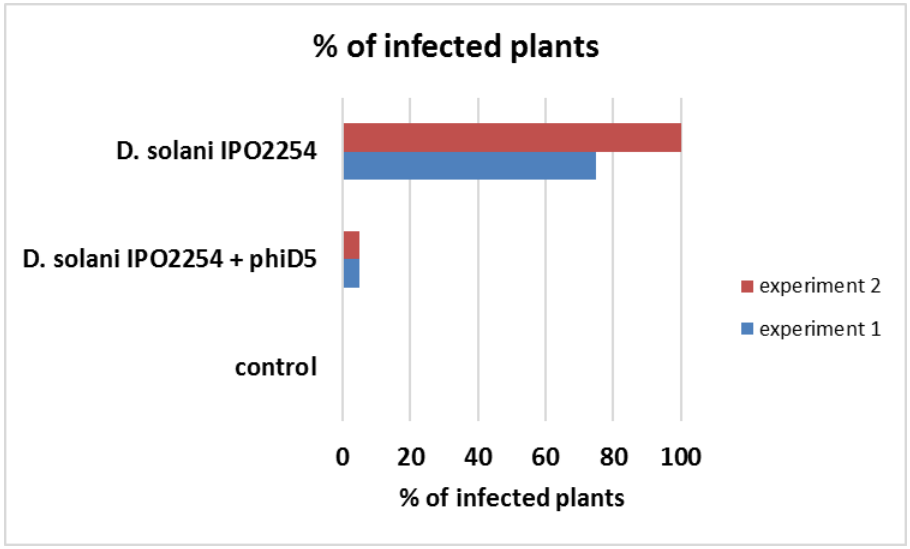


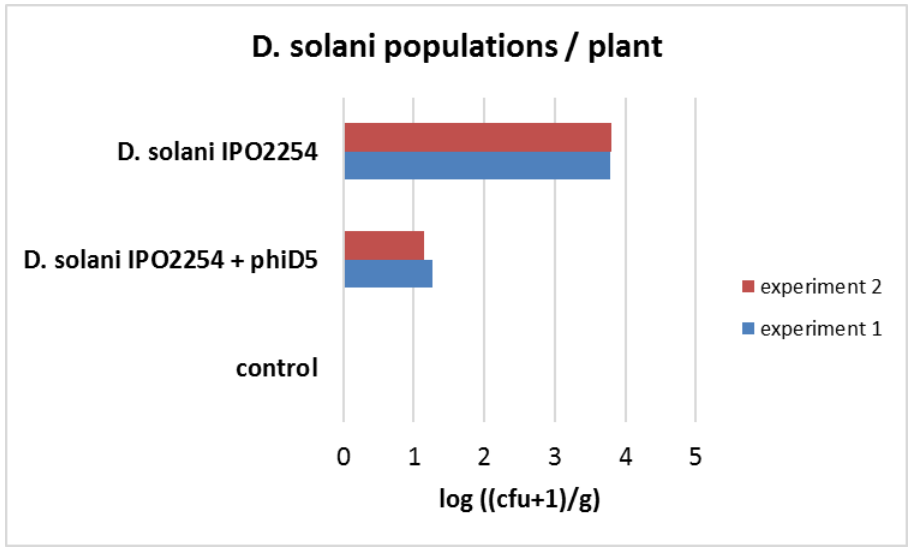
Stability in copper ions solution at 22 °C

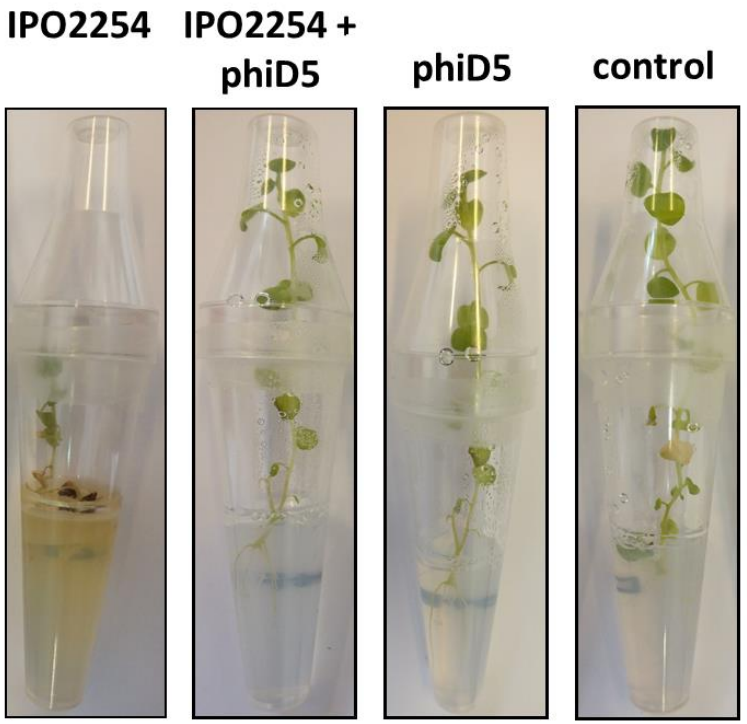
- the reduction of phage numbers was proportional to the concentration of the copper ions
- no phages survived 72 h incubation with 50 mM copper
- whereas 10 to 50% reduction of phage numbers was recorded for lower concentrations



Interaction with *D. solani* in plants grown in culture tubes



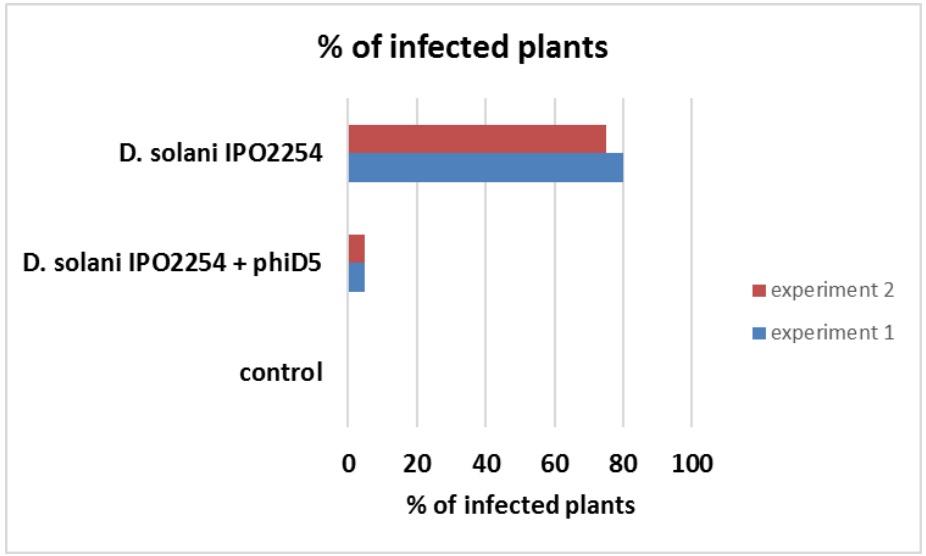




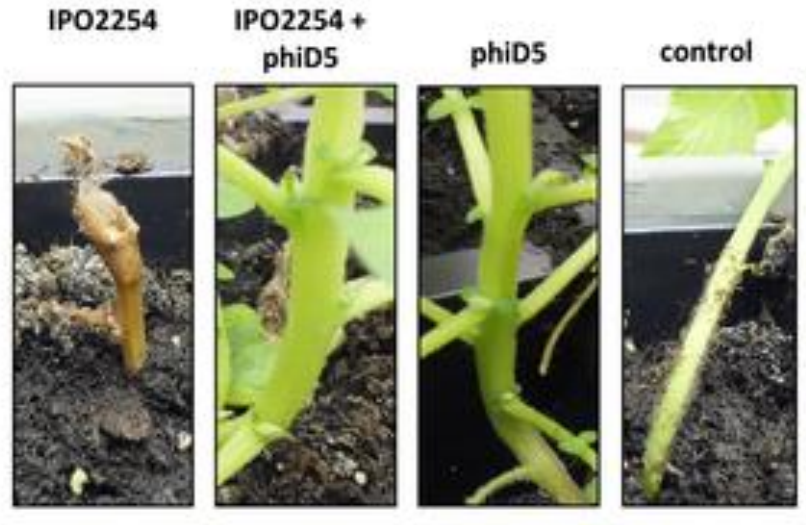
- ϕD5 applied on stem base 24 h before application of IPO2222

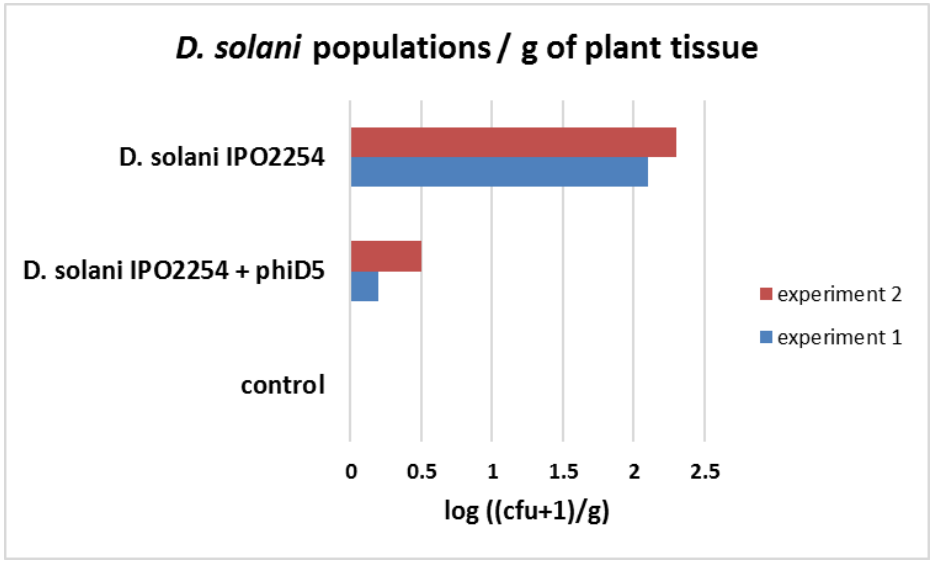
potato cv. Kondor

Interaction with *D. solani* in plants grown in soil



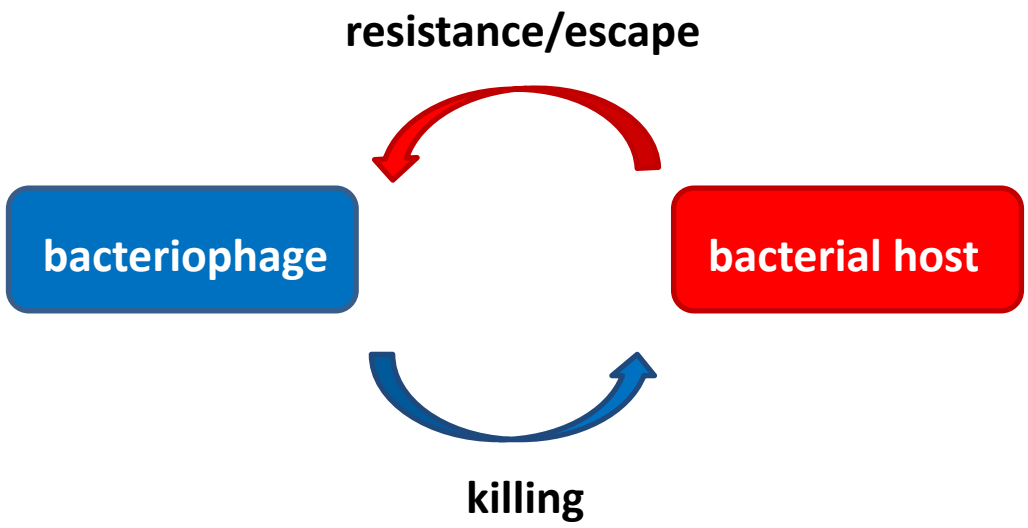
potato cv. Kondor



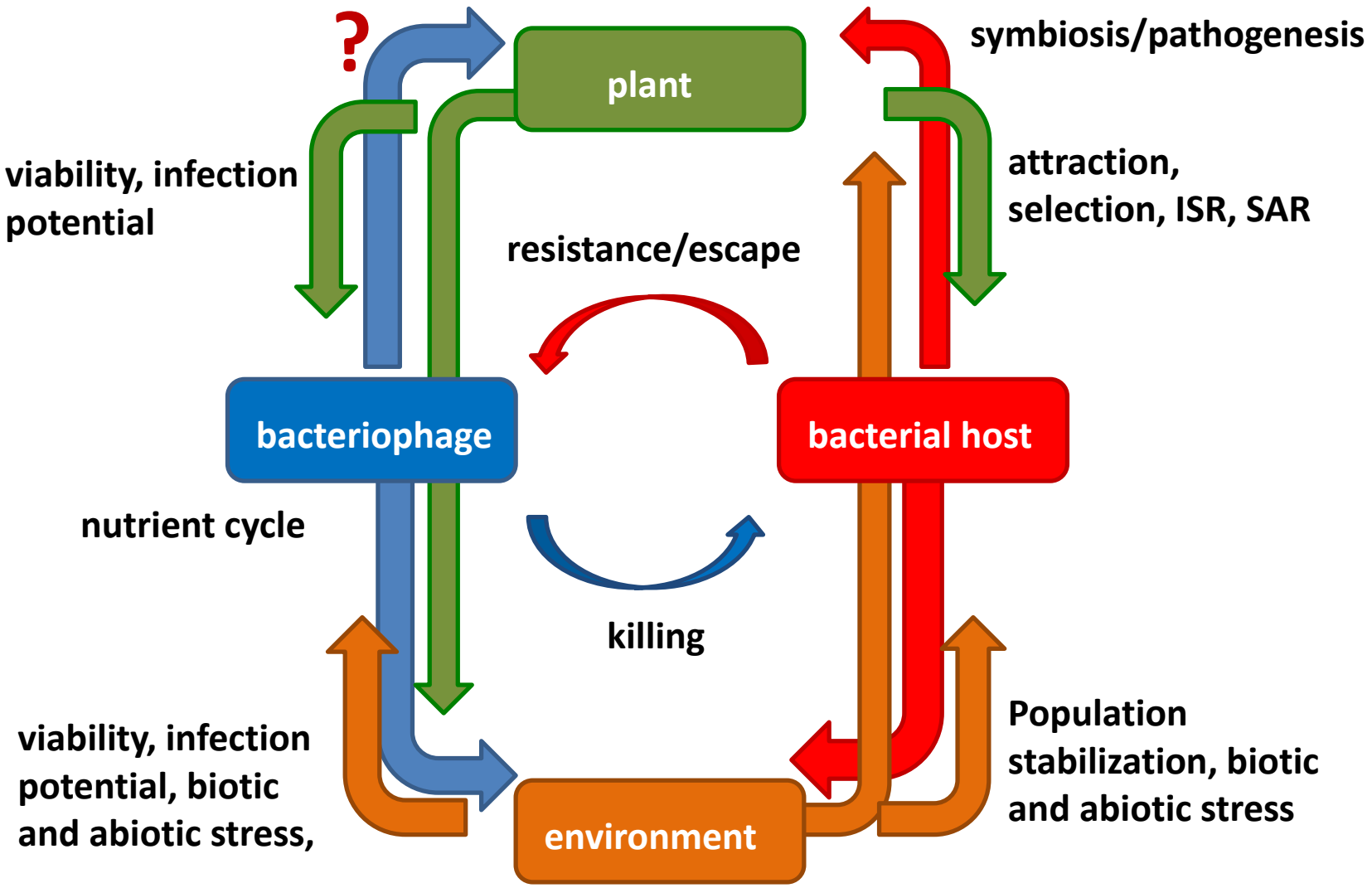


- Soil spiked with ϕD5 1 week (7 days) before application of IPO2222

Summary - The former interaction model



Summary - The new interaction model



Summary

- we assesses the viability and interaction of lytic bacteriophage ϕD5 and its host bacterium (*D. solani* strain IPO2222) on potato plants (natural environment)
- ϕD5 seems to have potential in (bio)controlling soft rot infections caused by pectinolytic bacteria *in situ*
- phages should be more considered as biological control agents in agriculture in the future (increasing importance)

Acknowledgements

The National Centre for Research and Development

LIDER

project: LIDER/450/L-6/14/NCBR/2015 (PATBIOCON) for funding

The PATBIOCON research group:

www.patbiocon.pl

RESEARCH ARTICLE

The viability of lytic bacteriophage ΦD5 in potato-associated environments and its effect on *Dickeya solani* in potato (*Solanum tuberosum* L.) plants

Robert Czajkowski, Anna Smolarska, Zofia Ozymko

Published: August 11, 2017 • https://doi.org/10.1371/journal.pone.0183200

Collaboration?? Yes, we can ☺

Thank you for your attention!!!

Advertisement:

PP-SMP-01 Dorota Krzyzanowska: PATBIOCON: Development of a biocontrol product effectively suppressing bacterial soft-rot on potato tubers in storage

PP-MI-17 Tomasz Maciąg: Antagonistic interaction between biocontrol strains, implications for biological plant control against soft rot disease