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

Isolation and characterization of novel soil-borne lytic bacteriophages infecting *Dickeya* spp. biovar 3 (*D. solani*)

Jerusalem, Israel 17-24.11.2013

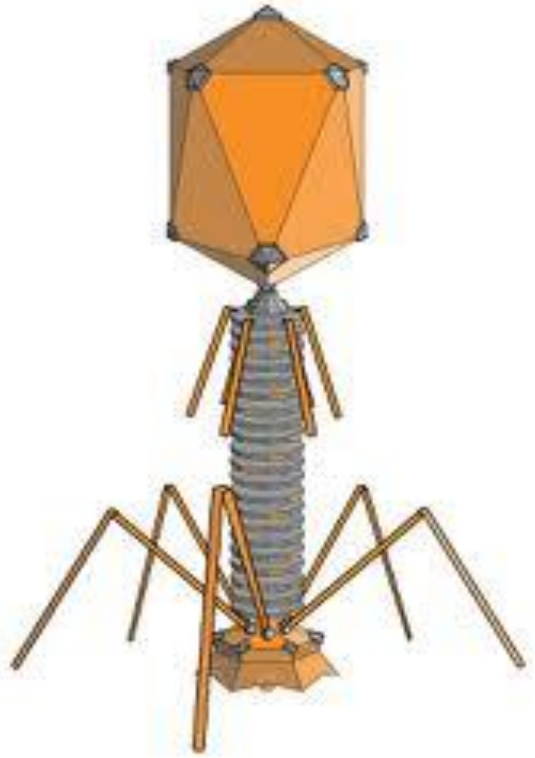
Robert Czajkowski, Zofia Ozymko, Ewa Łojkowska

Pathology Section Meeting

eapr
EUROPEAN ASSOCIATION FOR POTATO RESEARCH

Bacteriophages

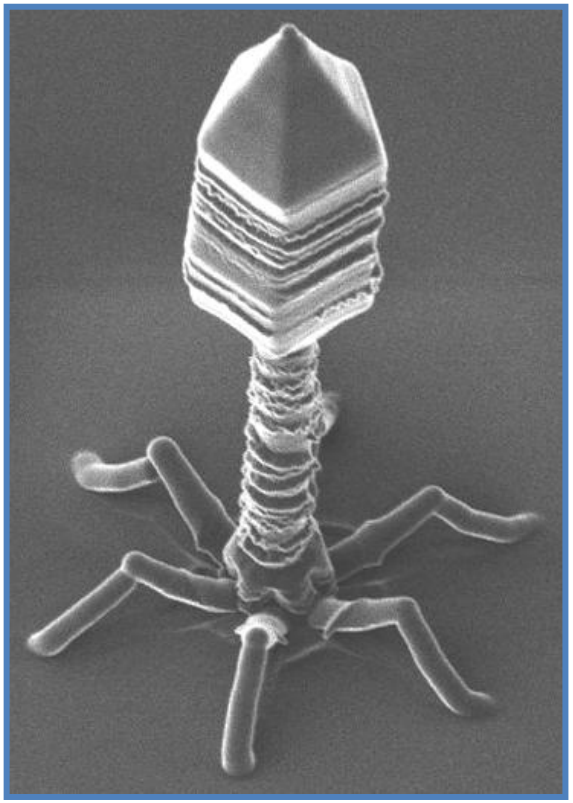
- the viruses that infect and destroy bacterial cells
- discovered by Friderick W. Twork (England, 1915) and Felix d'Herell (France, 1917)
- build up from protein coat (capsid) and nucleic acid (phage genome)
- they can multiply exclusivelly in bacterial cells (bacterial parasites)



http://en.wikipedia.org/wiki/Bacteriophage

Bacteriophages

- they are very specific to their bacterial hosts
- can be found virtually everywhere in the environment
- they can infect all bacterial species
- very diverse (at least 19 families described so far)
- divided into two groups: lytic and lysogenic phages
- widely used in molecular biology (in the past...)



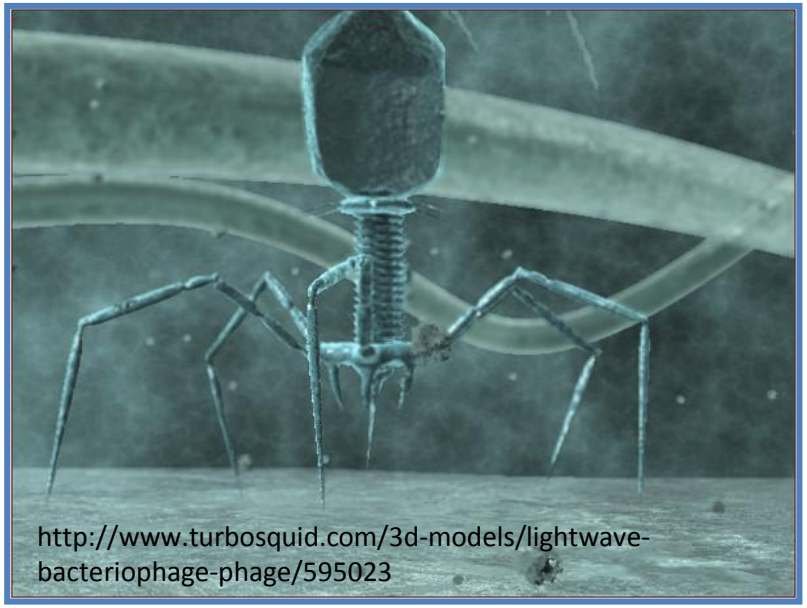
http://www.zyvexlabs.com/EIPBNuG/2005MicroGraph.html

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
- Bacteriophages against *Dickeya* spp. (Adriaenssens et al. 2011, 2012)

Managment of *Dickeya* spp. in potato

- attems to control *Dickeya* spp. in potato is ineffective as there have been no full-proven strategies developed so far:
  - no resistant potato cultivars present
  - no chemical or physical methods available
  - biocontrol of limited use
  - hygienic measures only partially successful
  - no effective detection methods available



http://www.turbosquid.com/3d-models/lightwave-bacteriophage-phage/595023

Target bacterium

- *D. solani* (van der Wolf et al. 2013)
  - isolated from potato in many European countries
  - very homogenic population
  - dominant *Dickeya* spp. in Europe
  - very virulent under European climate conditions
  - able to easily infect potato plants from soil, after stem and leaves infections
  - more virulent than *D. dianthicola* isolates
  - increase in blackleg and soft rot incidences due to the presence of this pathogen

The aim of the project

1. To isolate bacteriophages against different *Dickeya* spp. (especially against *D. solani*):

- from different environments
- with/without host bacterium present
- RFLP and TEM analysis

2. To evaluate bacteriophages for features potentially important for their stability and applications:

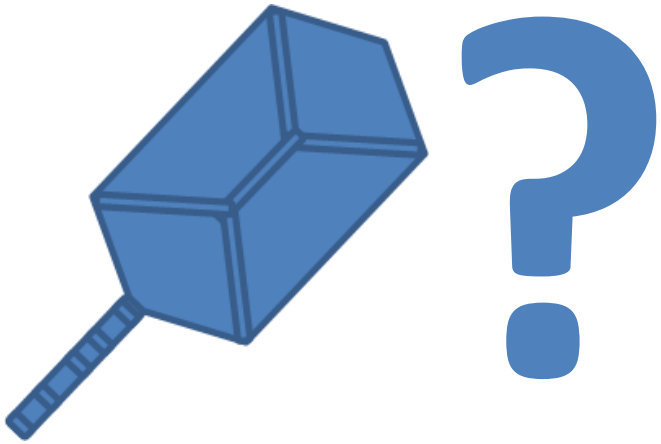
- ionic strenght, chloroform, temperature, pH, UV radiation

3. To evaluate the interaction of phages with *D. solani*

Isolation of bacteriophages

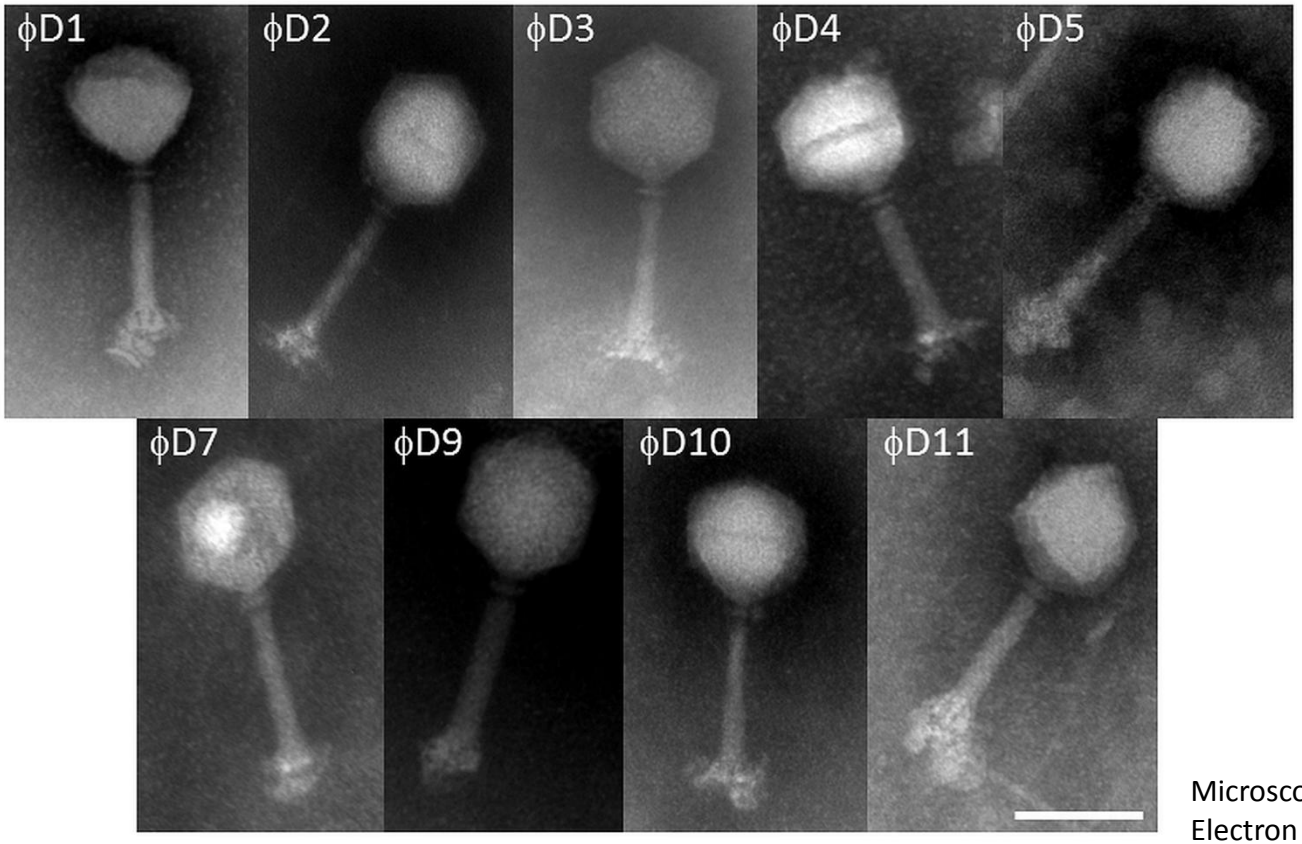
- aimed isolation from infected potato tubers, uninfested soil and potato rhizosphere
- done during the winter months (October 2012 – February 2013)
- low abundance in soil
- absent in soft rotting tubers (strange ??)
- enrichment of phages in *D. solani* cultures **always** prerequsite for isolation

Characterization of bacteriophages



Transmission electron microscopy (TEM)

- phages negatively stained with uranyl acetate



bar – 100 nm

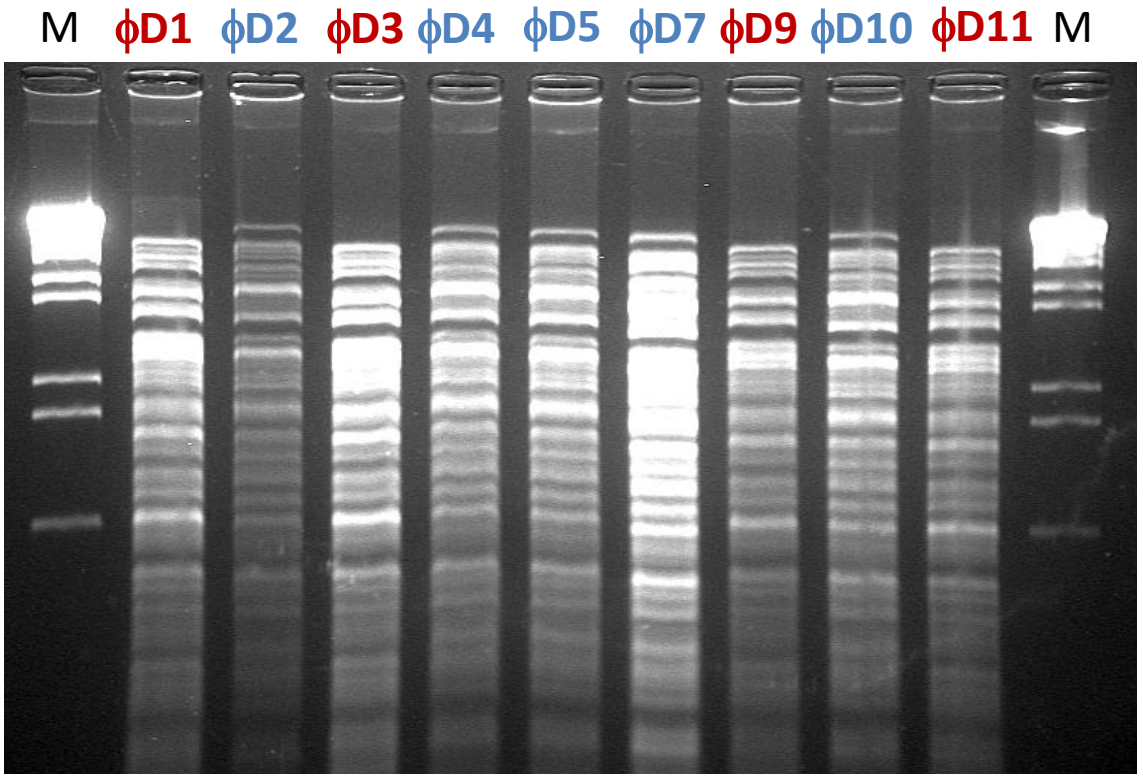
Head: 80-100 nm
Tail: 130-160 nm
contractive tail

Family: ***Myoviridae***
Order: ***Caudovirales***

Microscopic analysis performed at Laboratory of Electron Microscopy, Mossakowski Medical Center, Polish Academy of Science of Warsaw, Poland

RFLP analysis of the phages

- analysis done with 13 restriction endonucleases (only one ***Csp6I*** produces pattern)
- only two RFLP groups (**group 1** and **group 2**) found



- Difficult to assess the phylogenetic relationships of phages

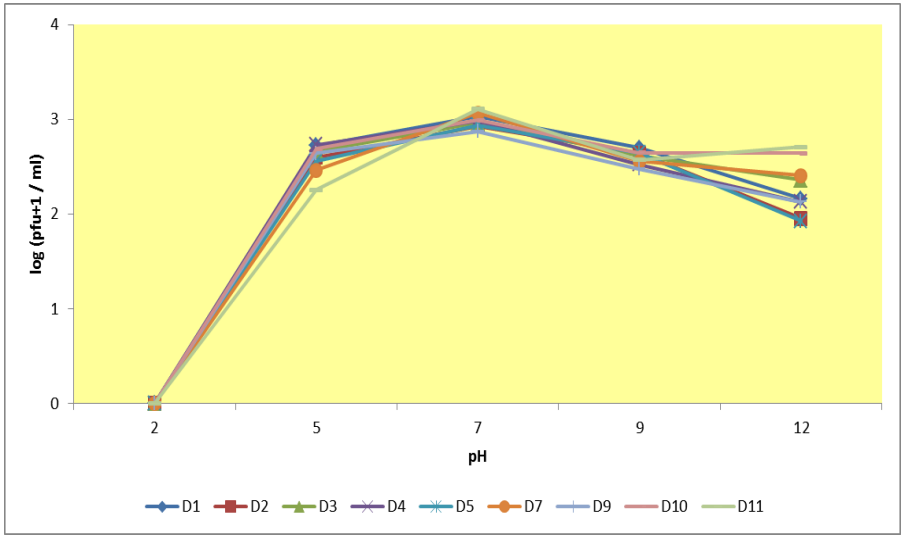
marker (M): λ DNA Hind III/ EcoRI

Effect of pH

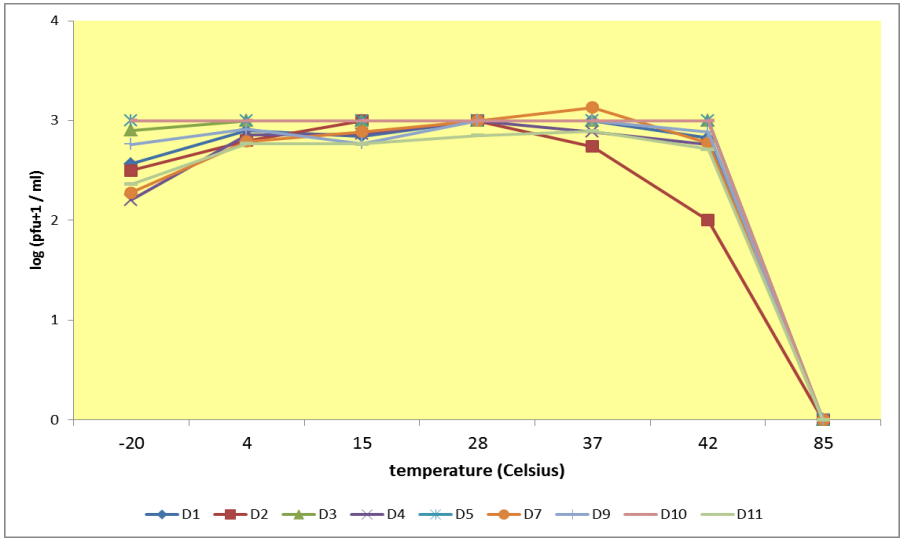
Effect of temperature

- *in vitro* experiments

- low pH more severe than high pH



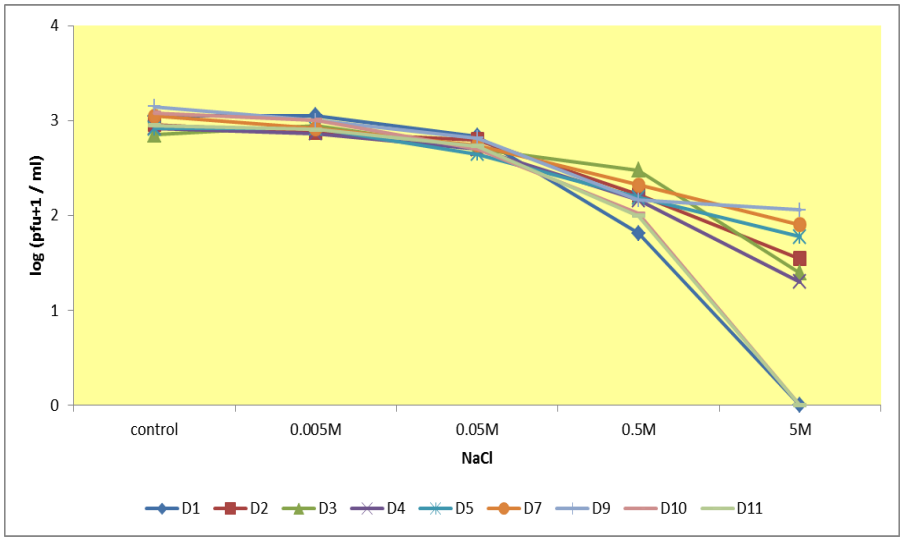
- phages unstable at 85 °C



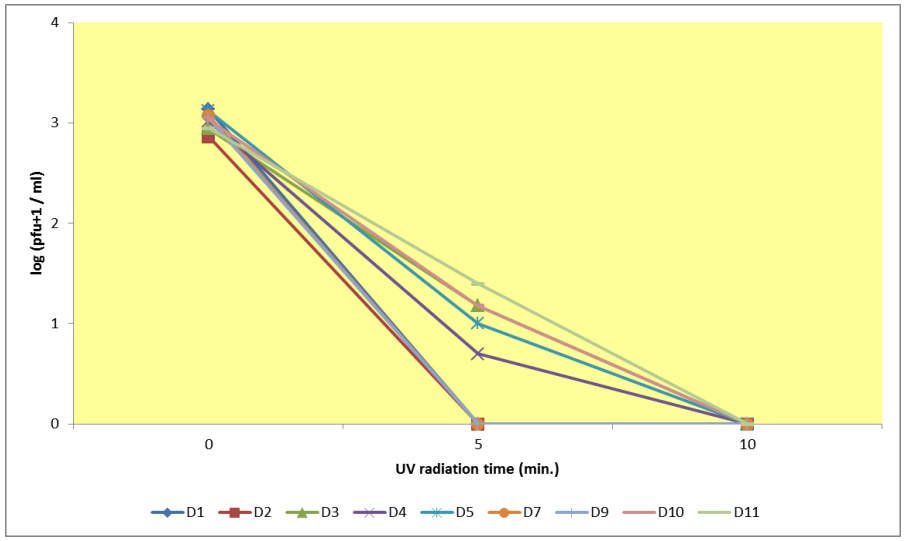
Effect of ionic concentration Effect of UV radiation

- *in vitro* experiments

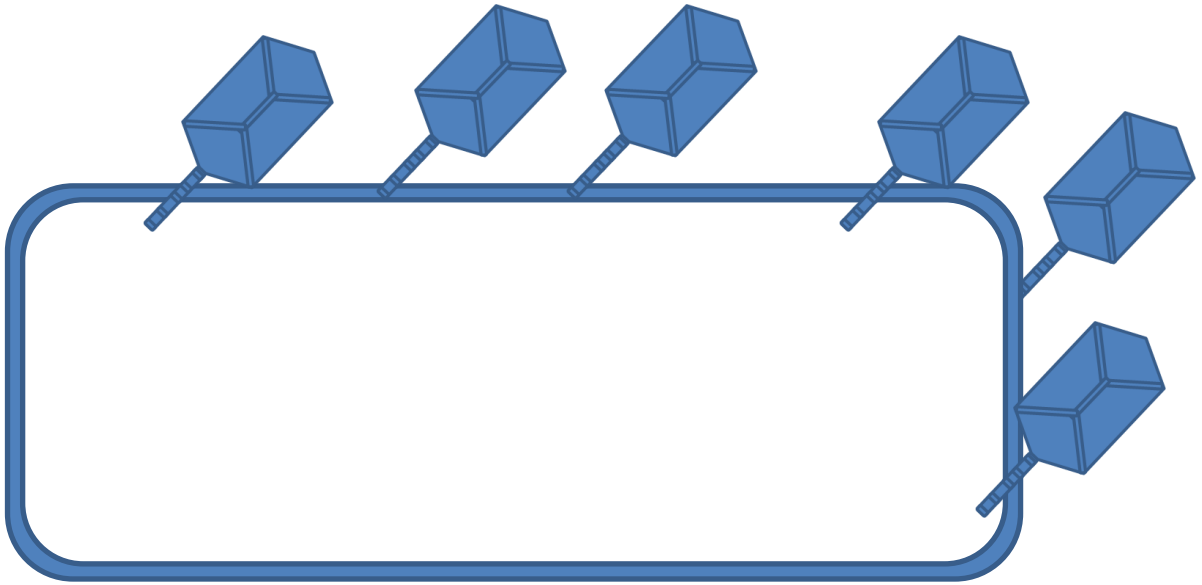
- phages unstable under high ions concentration



- phages very prone to UV light



Interaction of phages with *D. solani* IPO2222



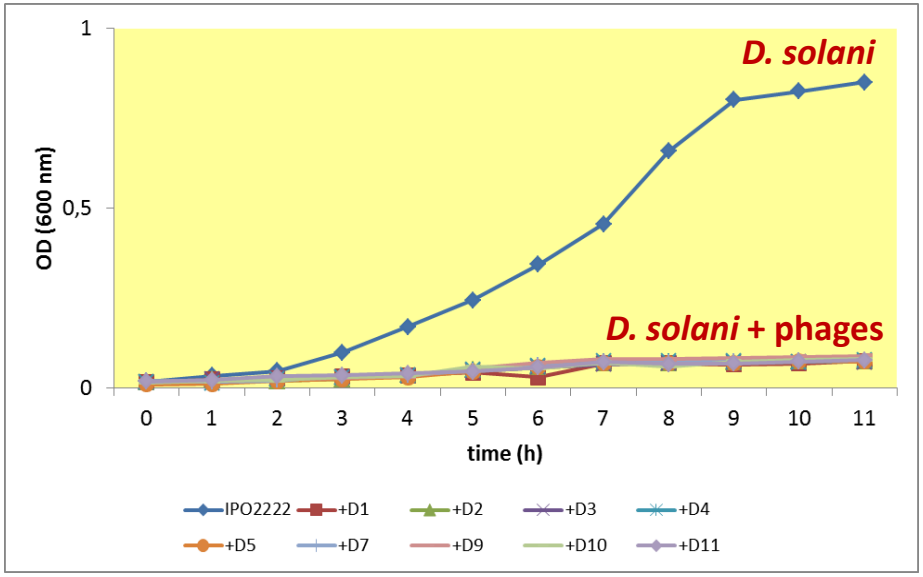
Bacteriophages host range

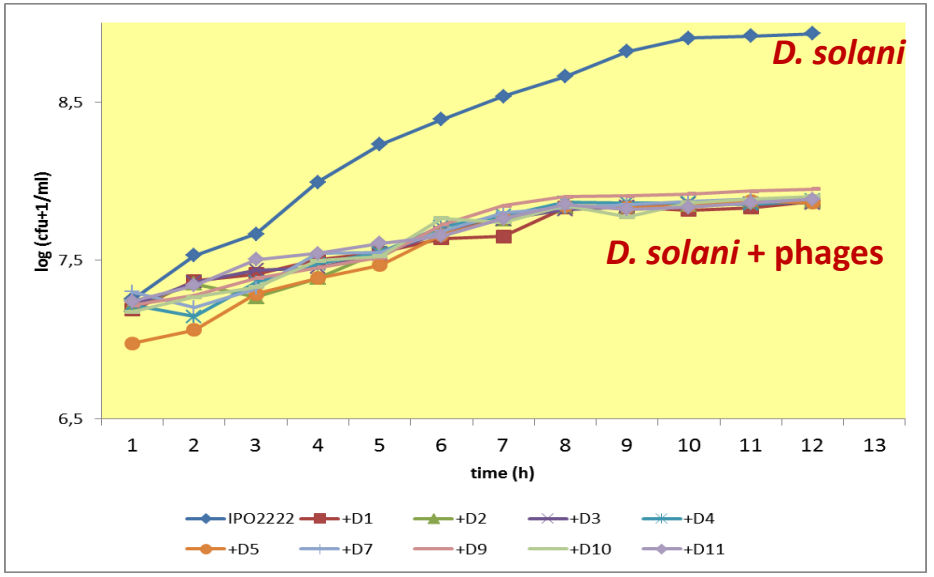
- Phages tested against:
  - **41 strains** of *Dickeya* spp. (*D. dadanti* (8), *D. dianthicola* (6), *D. zeae* (5), *D. paradisiaca* (2), *D. chrysanthemi* (4), *D. solani* (16)
  - **18 strains** of *Pectobacterium* spp. (*P. atrosepticum* (10), *P. wasabiae* (2), P. *carotovorum* subsp. *carotovorum* (5), *P. carotovorum* subsp. *brasilliensis* (1) (from **Jacquie van der Waals**, University of Pretoria, SA)

- Phages able to infect *Dickeya* spp. isolates but no *Pectobacterium* spp. isolates

Bacterial challenge in *in vitro* assay

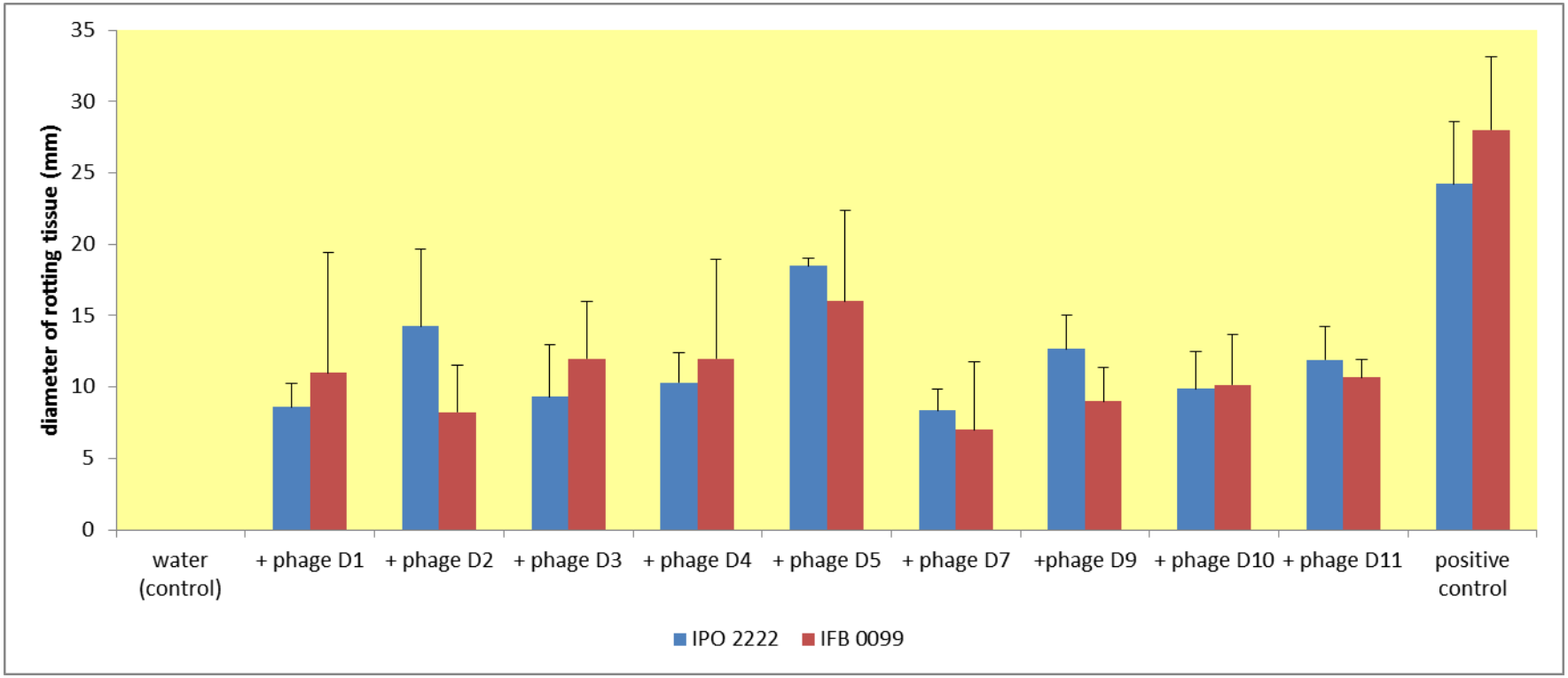
- phages were able to stop the growth of *D. solani* in co-inoculations *in vitro* :
  - OD (600 nm)
  - log (cfu+1/ml)





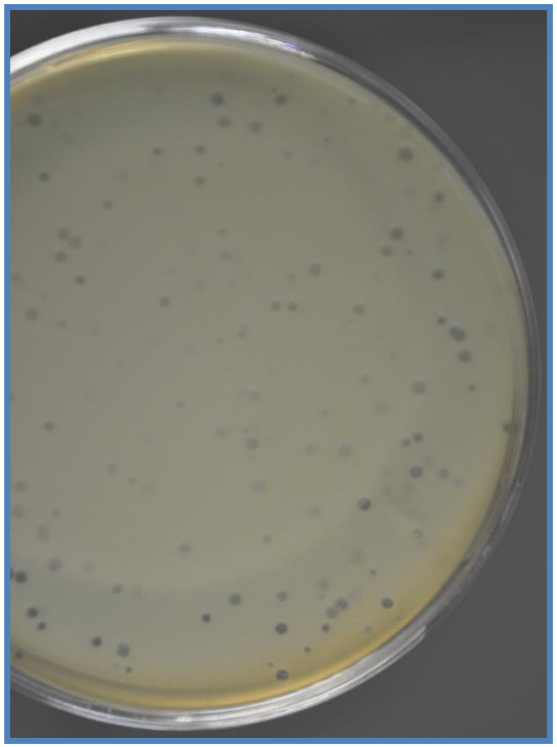
Protective effect of phages on *D. solani* infections in potato

- tested in a potato slice assay against two *D. solani* strains: **IPO2222** (Dutch strain) and **IFB 0099** (Polish strain)



Optimal multiplicity of infection (MOI)

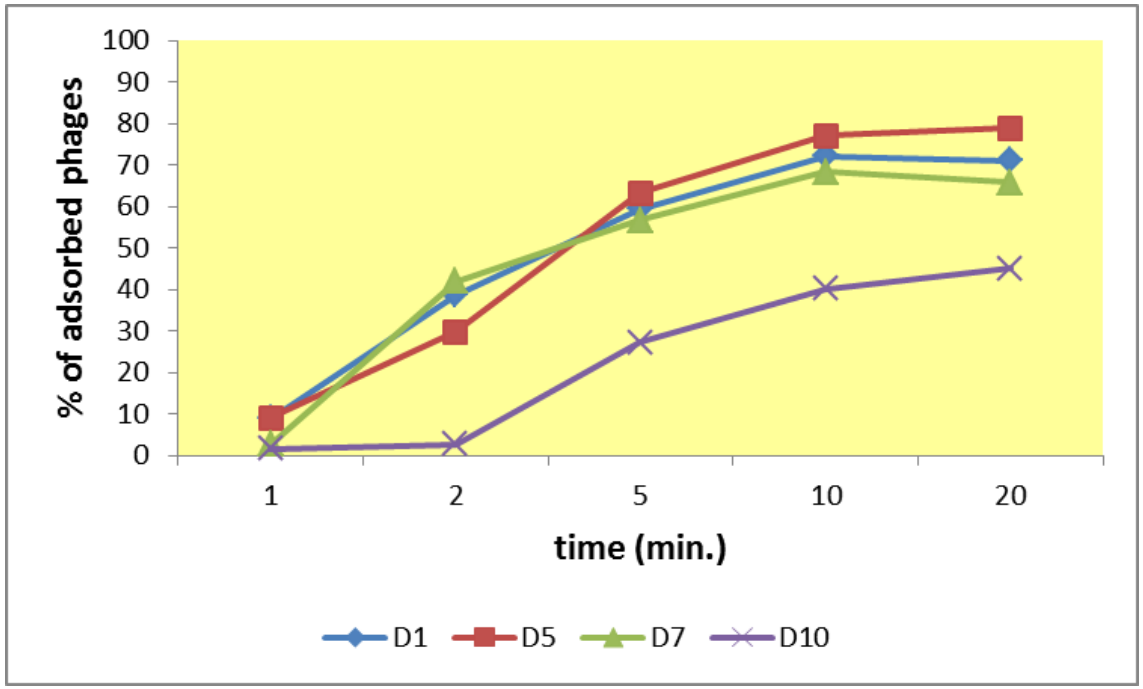
- defined as ratio of **pfu/cfu** giving the highest number of phage particles
- tested for 4 phages: ϕD1, ϕD5, ϕD7 and ϕD10
- 4 different ratios of pfu/cfu: 0.01, 0.1, 1.0 and 10.0



- Optimal MOI was 0.01 (ϕD5, ϕD10) or 0.1 (ϕD1, ϕD7)

Phage adsorption

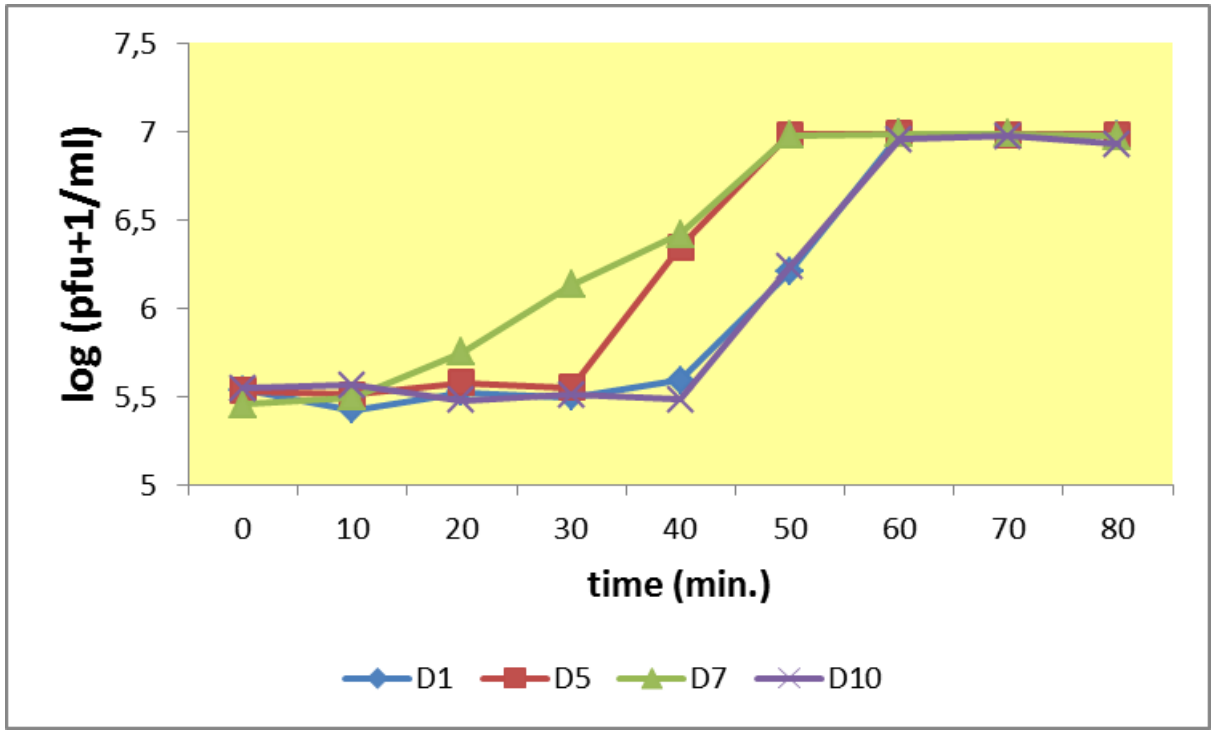
- defined as a time (min.) required by phage to attach and inject DNA inside bacterial cells (% of phages)
- tested for 4 phages: ϕD1, ϕD5, ϕD7 and ϕD10



- time required for adsorption was ca. 20 min.

Phage one step growth

- to assess the eclipse time and pfu/bacterial cell
- tested for 4 phages: ϕD1, ϕD5, ϕD7 and ϕD10



- latent period ca. 30-40 min. Burst size ca. 90 phages/cell

Conclusions

- phages against *Dickeya* spp. and specifically against *D. solani* were isolated from soil
- they were characterized in detail in this study
- the phages can be used (possibly together with other treatments) in biological control applications against *D. solani*
- are the phages similar to these described by other researchers?

Further readings:

Plant Pathology

An International Journal edited by the British Society for Plant Pathology

Original Article

Isolation and characterization of novel soil-borne lytic bacteriophages infecting Dickeya spp. biovar 3 ('D. solani').

R. Czajkowski*, Z. Ozymko, E. Lojkowska

DOI: 10.1111/ppa.12157

Issue

Plant Pathology

Accepted Article (Accepted, unedited articles published online and citable. The final edited and typeset version of record will appear in future.)

SEARCH

In this issue

Advanced > Saved Searches >

ARTICLE TOOLS

Get PDF (1127K)

Save to My Profile

E-mail Link to this Article

Export Citation for this Article

Get Citation Alerts

Request Permissions

Share

Additional Information (Show All)

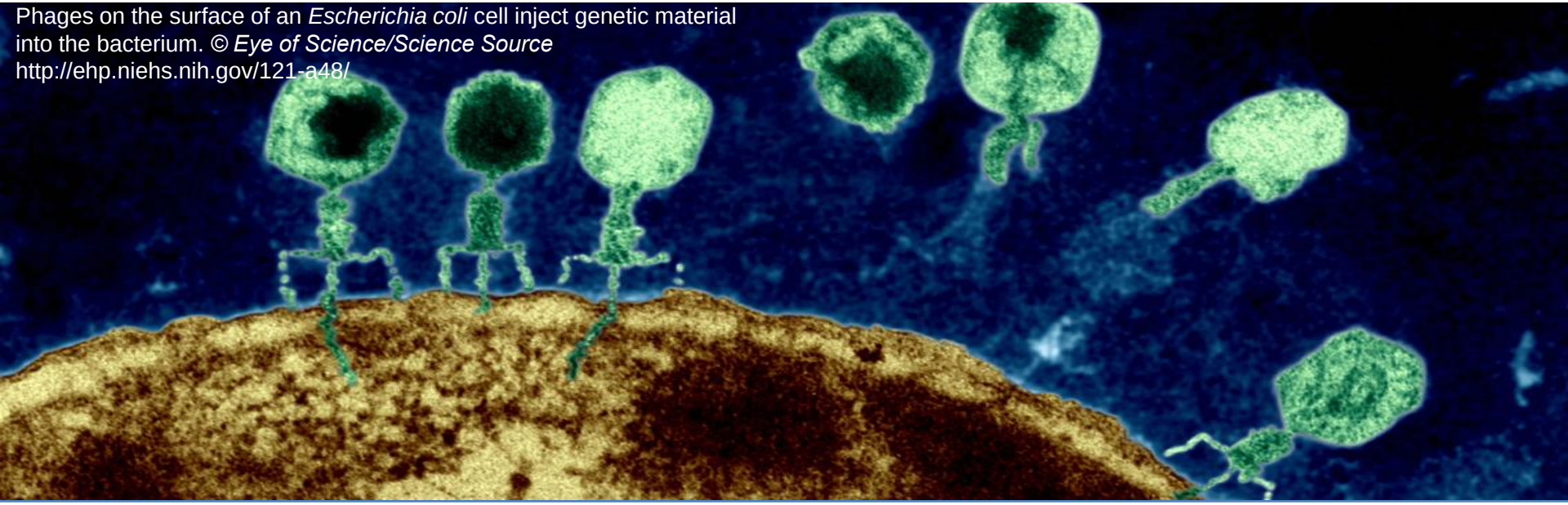
Author Information | Publication History

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ppa.12157

Acknowledgements

NATIONAL SCIENCE CENTRE
POLAND

project: **NCN 2012/04/S/NZ9/00118** for funding



Phages on the surface of an *Escherichia coli* cell inject genetic material into the bacterium. © *Eye of Science/Science Source*
http://ehp.niehs.nih.gov/121-a48/

Thank you for your attention!!!

מודה לך על תשומת לב שלך

http://www.giantmicrobes.com/us/products/T4.html