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# Stability and interaction of bacteriophage $\phi$ D5 with *Dickeya solani* IPO2222 on potato

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## Soft rot *Enterobacteriaceae*

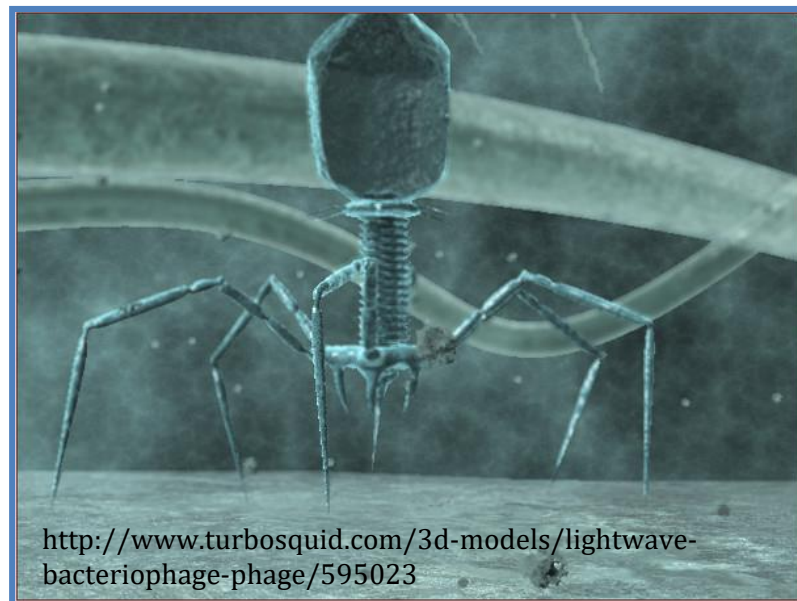
- plant pathogenic bacteria infecting a number of plant species worldwide
- causing major problems in potato production
- difficult to control (no effective control measures developed so far)
- ubiquitous in the environment
- new (virulent) species spreading in Europe cause even greater problems (e.g. *Pectobacterium brasiliense*)

# Target bacterium (a model system)

- *Dickeya solani* IPO2222 (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 in Europe
  - **together with *P. carotovorum* subsp. *carotovorum* and *P. wasabiae* – dominant potato pathogens**

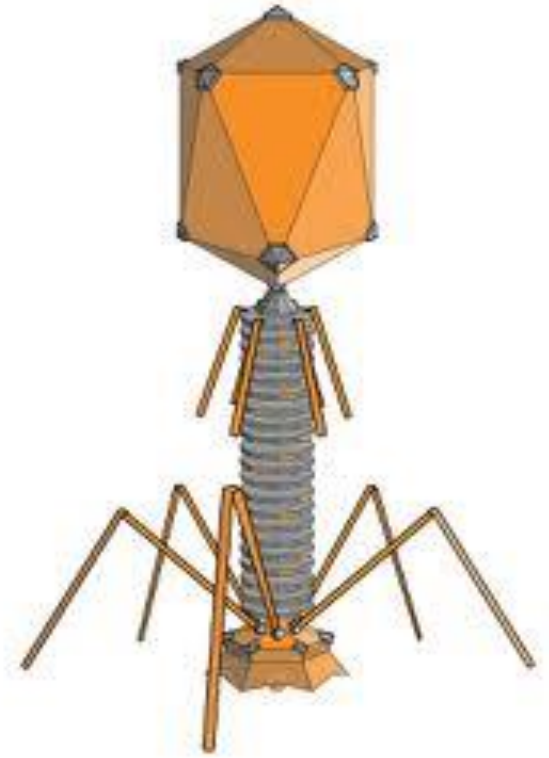
# Management of *Dickeya* spp. in potato

- attempts 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



# Bacteriophages

- the viruses that infect and destroy bacterial cells
- discovered by Friderick W. Twork (England, 1915) and Felix d'Herell (France, 1917)
- they can multiply exclusively in bacterial cells (bacterial parasites)
- **they can be considered as biological control agents against bacterial infections**



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

# 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)



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Minireview

MINIREVIEW – Virology

## Bacteriophages of Soft Rot *Enterobacteriaceae*— a minireview

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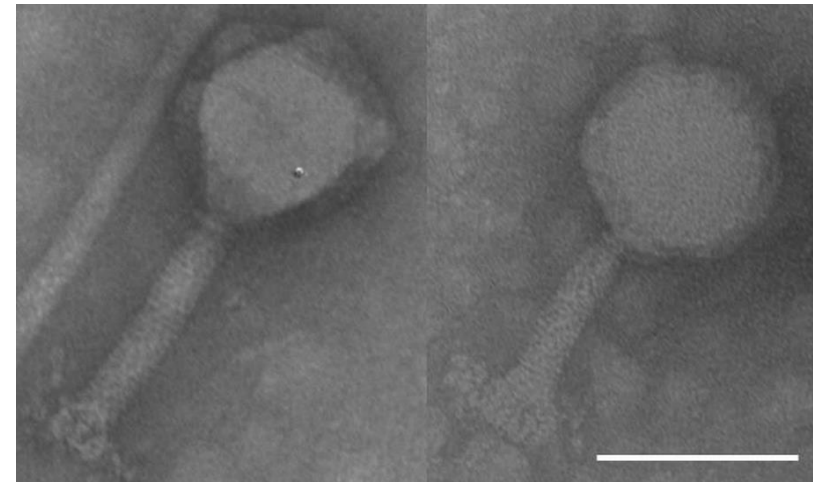
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One sentence summary: Lytic bacteriophages may be important for biological control of soft rot *Enterobacteriaceae*.

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# $\phi$ D5 bacteriophage (Czajkowski et al. 2013, 2014)

- lytic bacteriophage (no lysogeny)
- isolated primarily on *D. solani* host from arable soil in Poland
- broad host range (able to infect *D. solani*, *D. dadantii*, *D. dianthicola*, *D. zea*, *D. chrysanthemi*)
- sequenced and annotated genome
- info on its stability under different pHs, temp., osmotic pressure, UV, chloroform
- info on some infection details (adsorption time, burst size, latent period in *D. solani*)
- effective against *D. solani* on potato slices



bar - 100 nm

Head: 100 nm

Tail: 140 nm

contractive tail

Family: *Myoviridae*

Order: *Caudovirales*

Microscopic analysis performed at Laboratory of Electron Microscopy, Faculty of Biology, University of Gdansk, Poland

# The aim of the study

- to acquire knowledge on stability of  $\phi$ D5 in different potato-related environments (e.g. in soil, on leaves, on tuber surface) – with the idea to use this knowledge to develop a working biological control agent
- to better understand interaction of  $\phi$ D5 with its host bacterium and infection process on plants (*in vitro*, in soil) – to maximize effectiveness of the biological control agent



# Experimental setup:

## ■ stability of $\phi$ D5

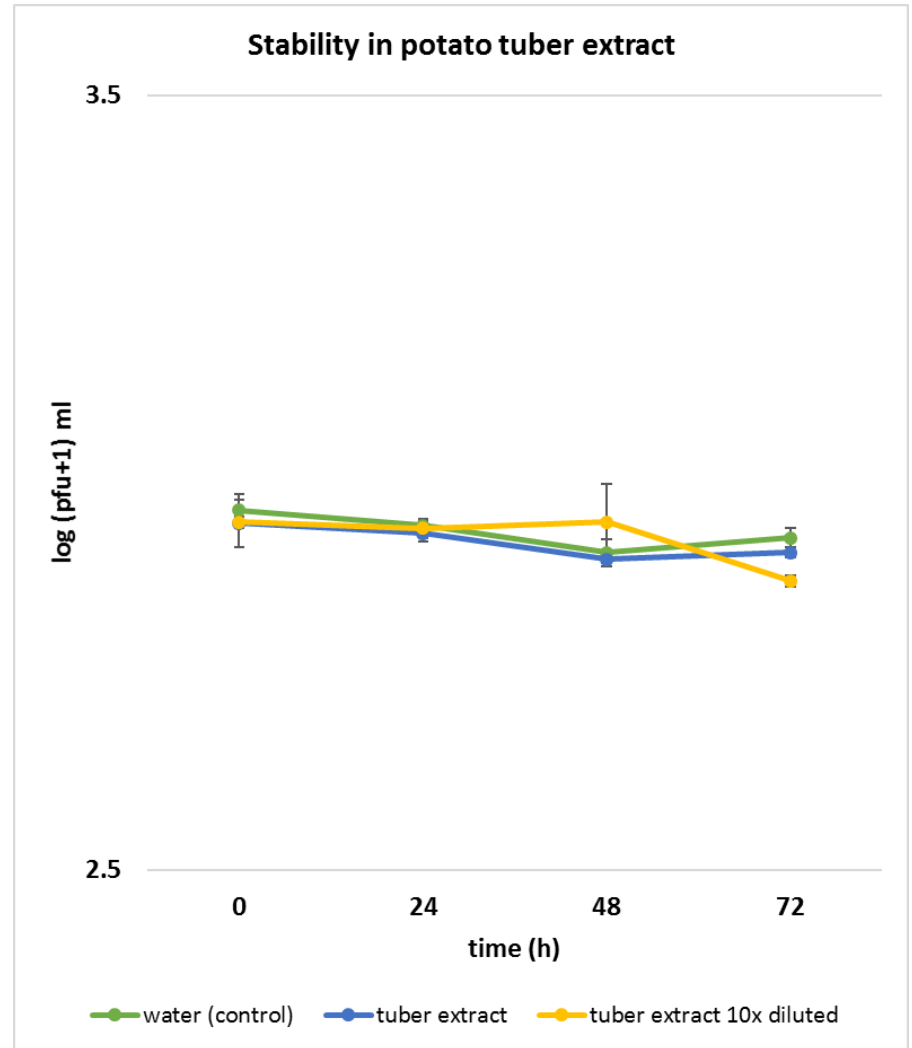
- in potato tuber extract (vacuum infiltration of tubers before planting)
- in sterilized and unsterilized soil (applied during planting)
- in sterilized and unsterilized rain water (applied in water)
- on surface of potato tubers at 6-8 °C (applied in storage)
- on surface of potato (detached) leaves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

## ■ interaction of $\phi$ D5 with *D. solani* IPO2222

- tissue culture potato plants cv. Kondor
- potato plants grown in soil

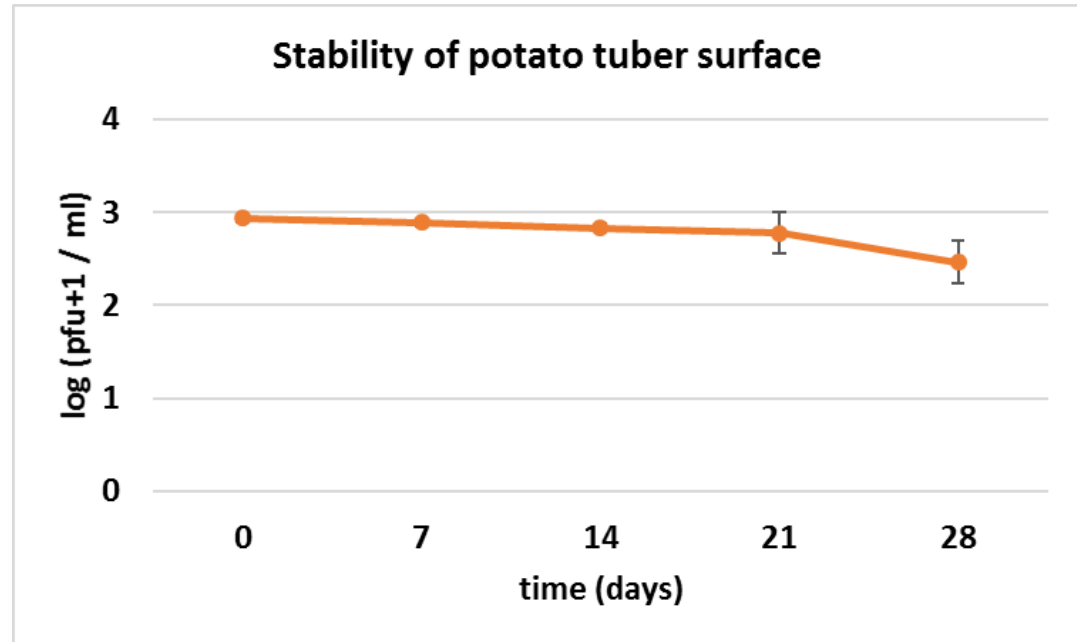
# Stability in potato tuber extract at 22 °C

- 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



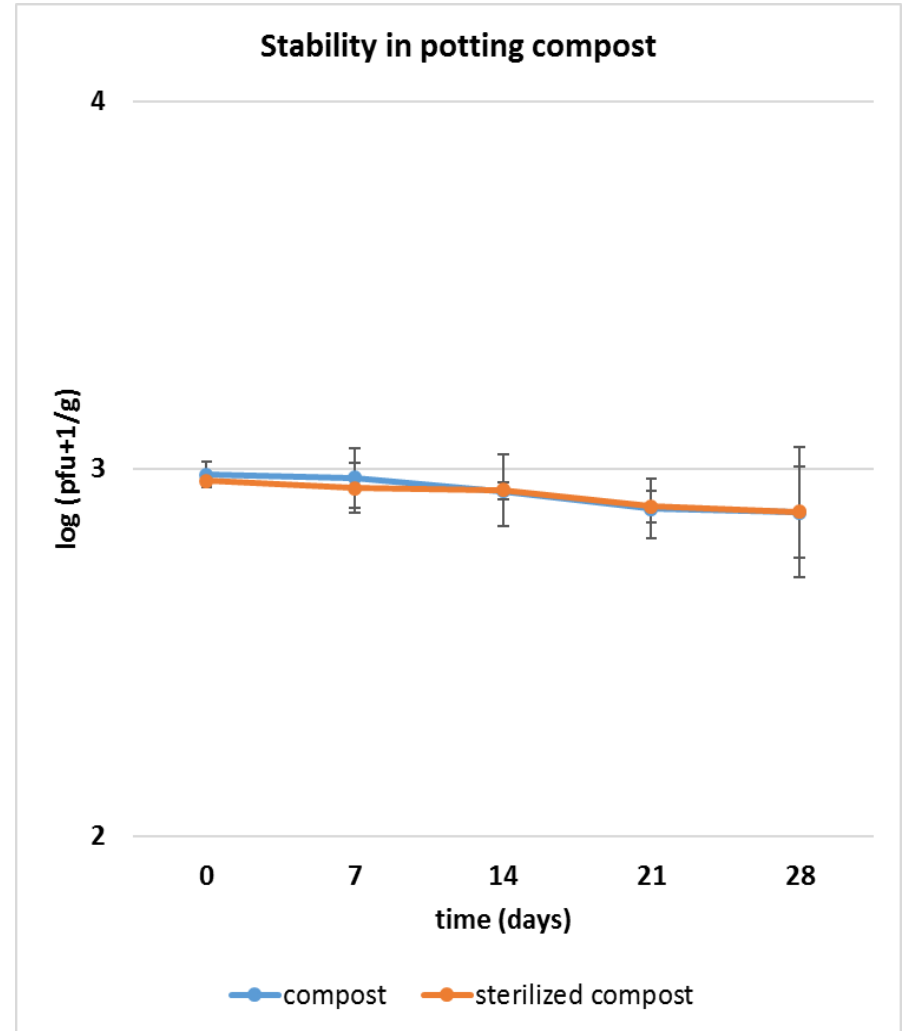
# Stability on surface of potato tuber (at 4-6 °C and 80% relative humidity)

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



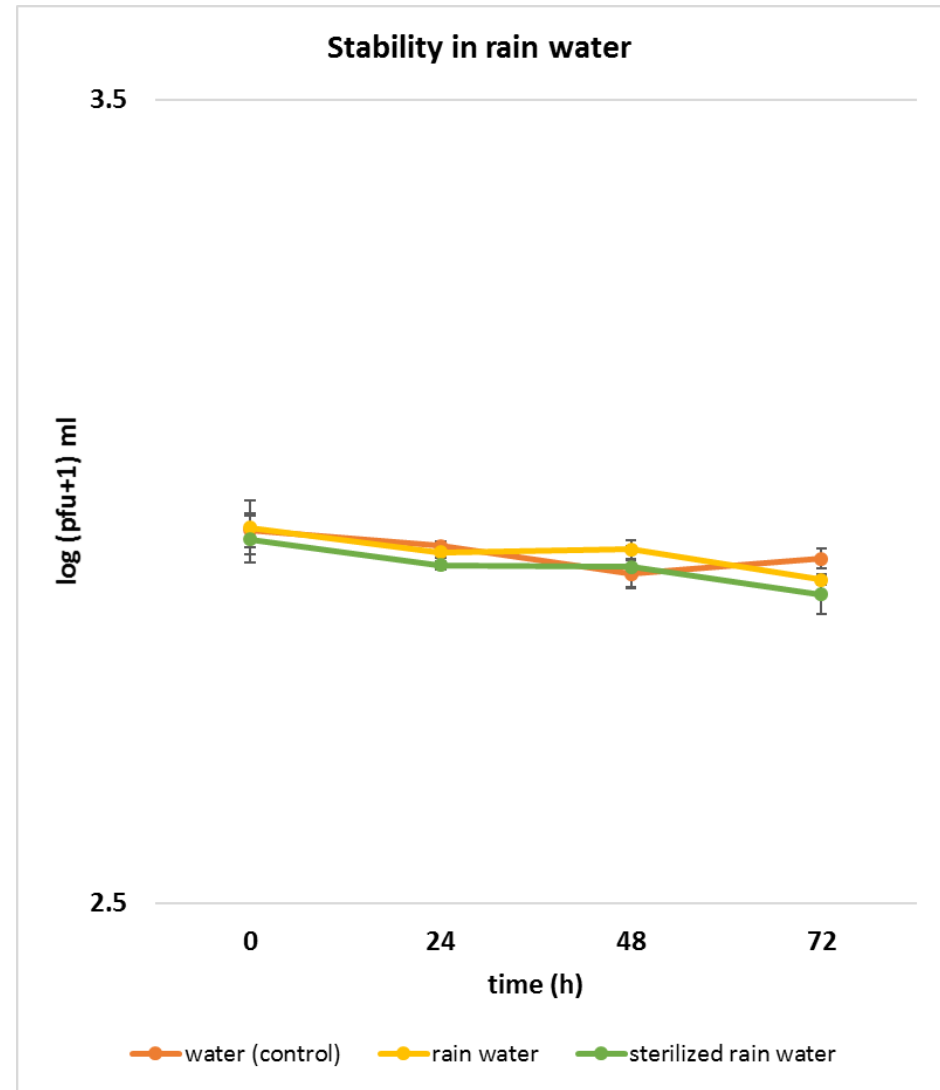
# Stability in potting soil at 50% field capacity and 10 °C

- 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



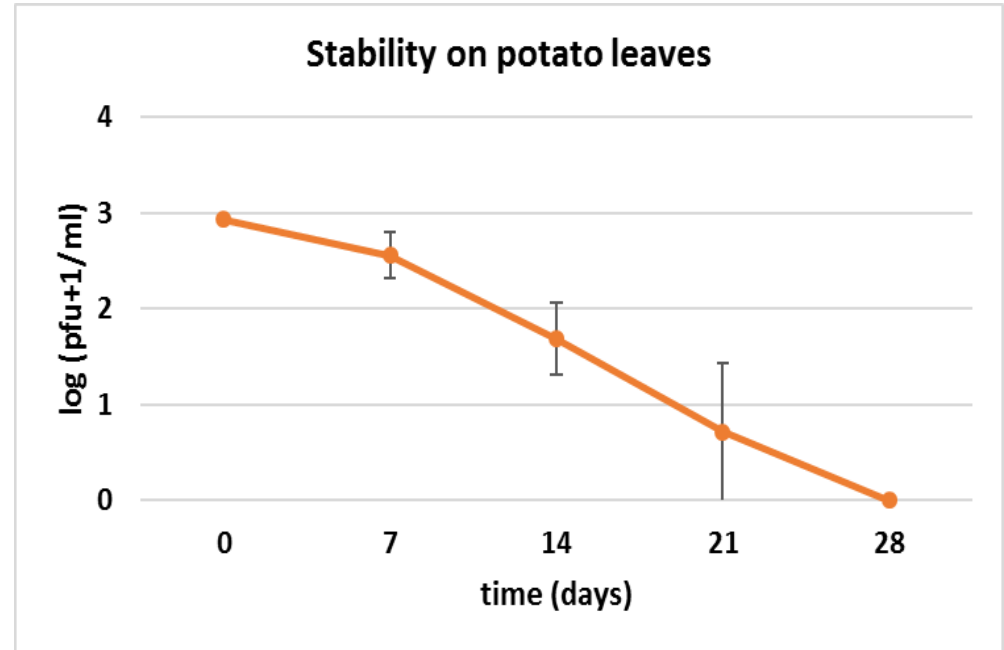
# Stability in rain water

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



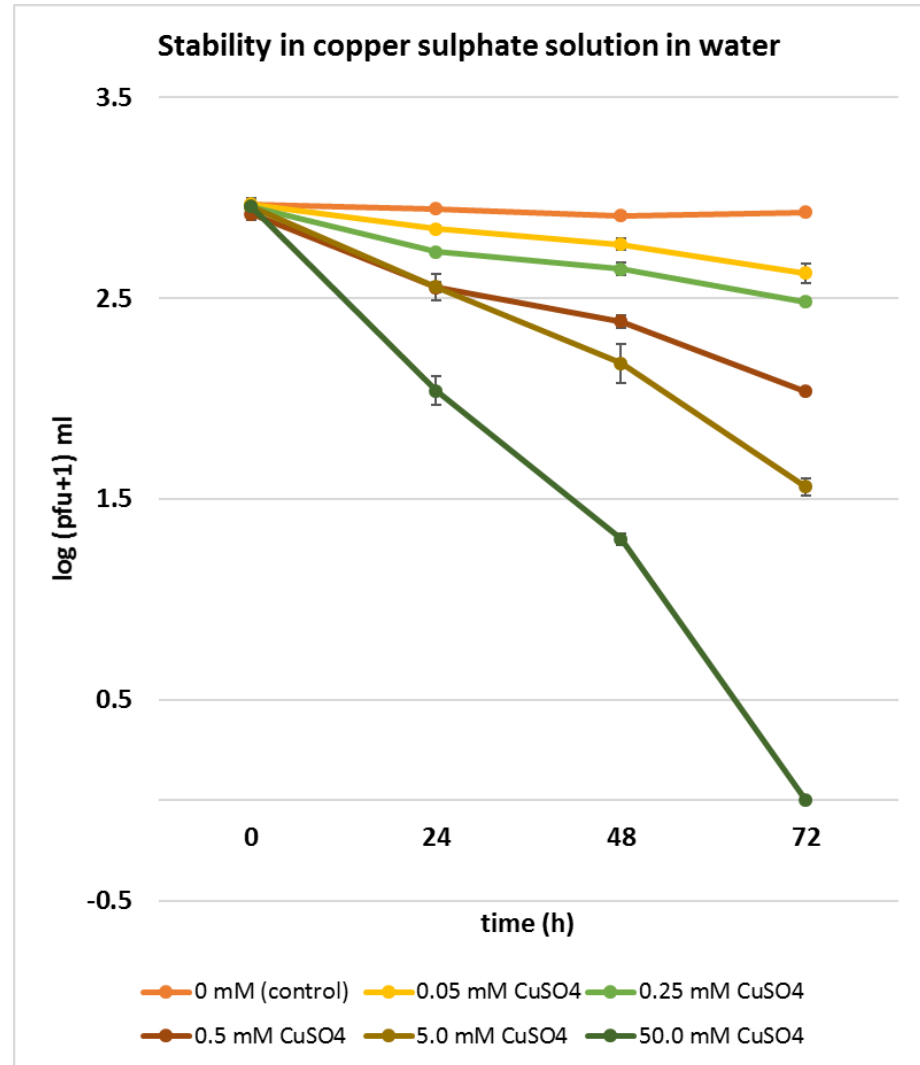
# Stability on leaf surface at 26 °C (growth chamber)

- 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)

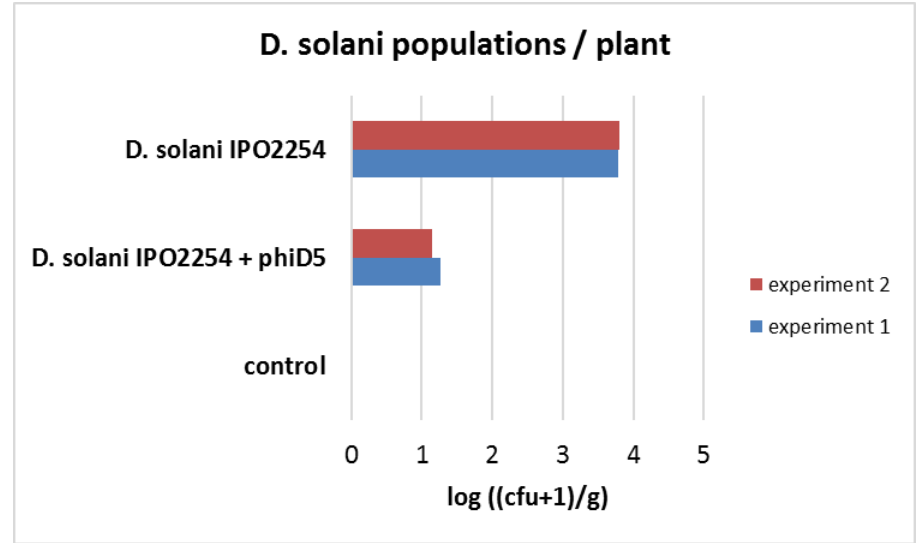
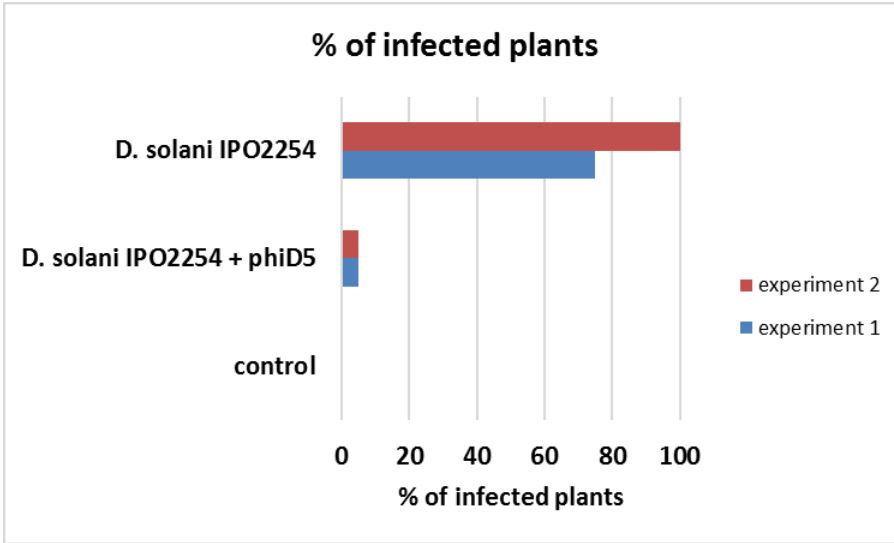


# Stability in copper ions solution at 22 °C

- 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



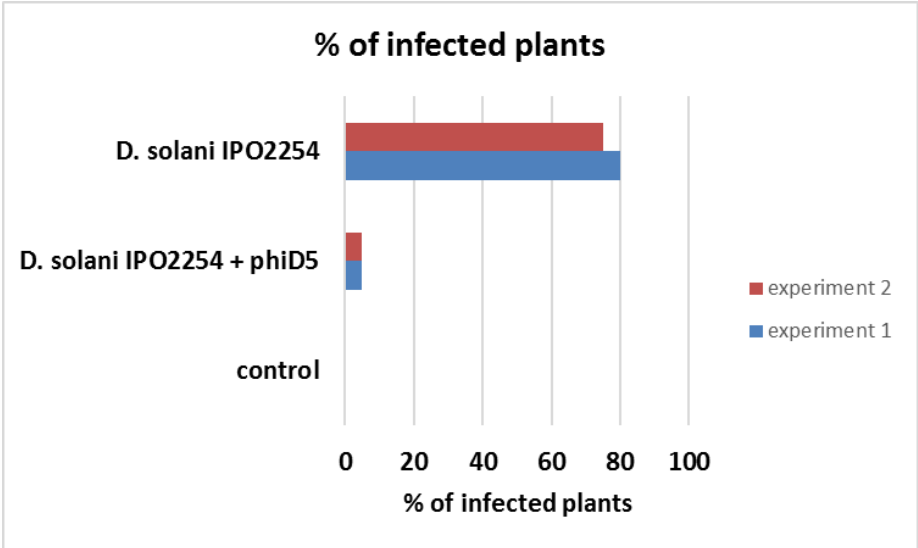
Infection symptoms on potato plants cv. Kondor inoculated with *D. solani* 14 days post inoculation



control plants

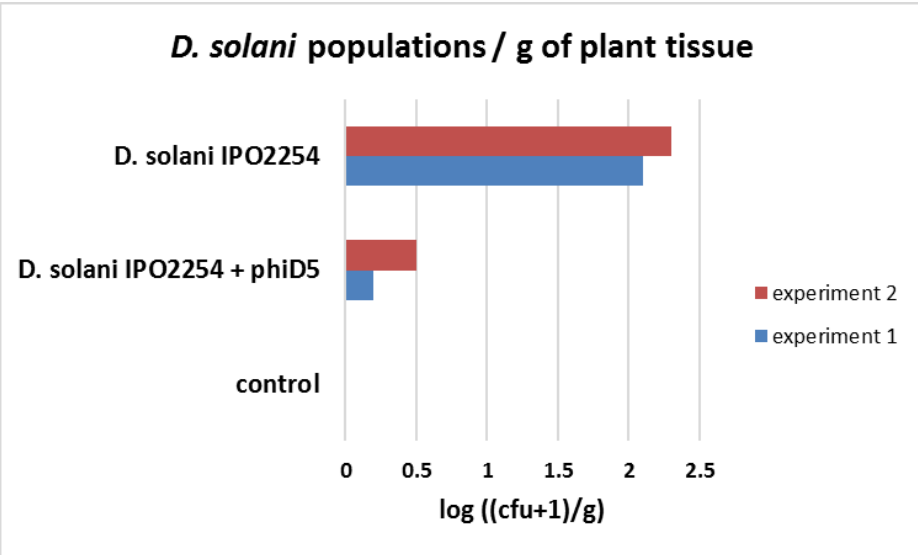


# Interaction with *D. solani* in plants grown in soil



Infection symptoms on potato plants cv. Kondor inoculated with *D. solani* 21 days post inoculation

Plants treated with *D. solani* and phiD5



Potato slices inoculated with *D. solani*

Potato slices inoculated with *D. solani* and phiD5



# Summary and conclusions

- the  $\phi$ D5 phage can survive in potato-associated environment except the phyllosphere for relatively long time (formulations are not required?)
- application of  $\phi$ D5 can reduce development of infection symptoms caused by *D. solani* in growing potato plants under *in vitro* and in soil

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attention!!!

