BACTERIOPHAGES AS AN ALTERNATIVE TO ANTIBIOTICS

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Agenda

- History of bacteriophage therapy
- Biology of bacteriophages
- The rise of antibiotic resistance
- Microbiome research
- The comeback of bacteriophages
- Bacteriophages for animal health applications
- Bacteriophages for poultry
- Bacteriophages for aquaculture
- Regulatory and technological challenges

History of bacteriophage therapy

History of bacteriophage therapy

The begining

Felix d'Herelle

Bacteriophages have been known for over a century and were used before the era of antibiotics

In the beginning of the 20th century two scientists Frederic Twort (1915) and Felix d'Herelle (1917) independently discovered bacteriophages, agents that can infect and kill specific bacteria by lysis. Felix d'Herelle was immediately interested in the therapeutics application of bacteriophages in the treatment of bacterial infections. Initial attempts were very promising. Bacteriophages were successfully applied in the treatment of dysentery and fowl typhus

History of bacteriophage therapy

The defeat

Attempts to produce therapeutic bacteriophages on an industrial scale failed due to a number of technological problems.

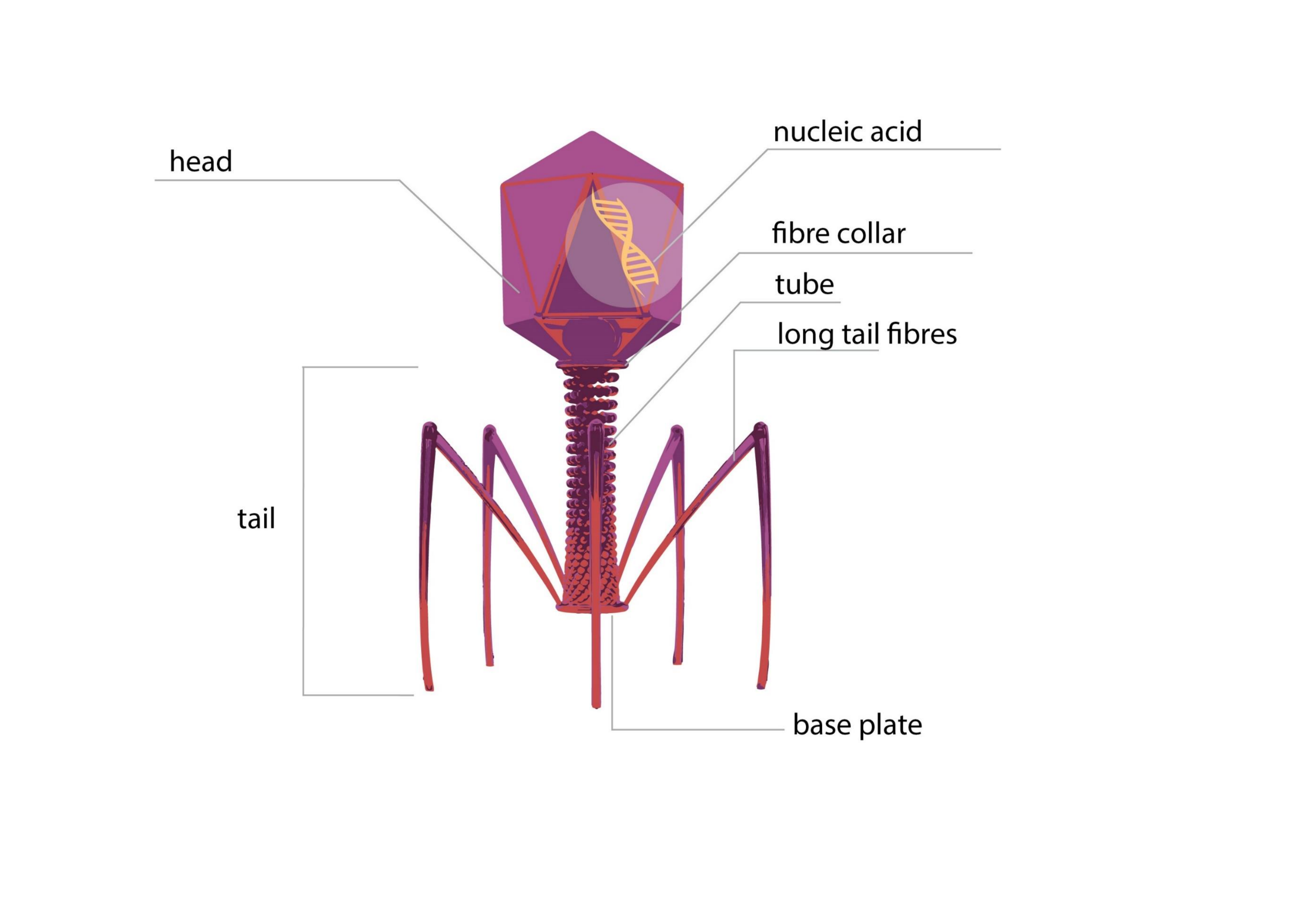
In 1940, the era of antibiotics began. Antibiotics, the new magic cure, were easier to produce in a stable reproducible fashion.

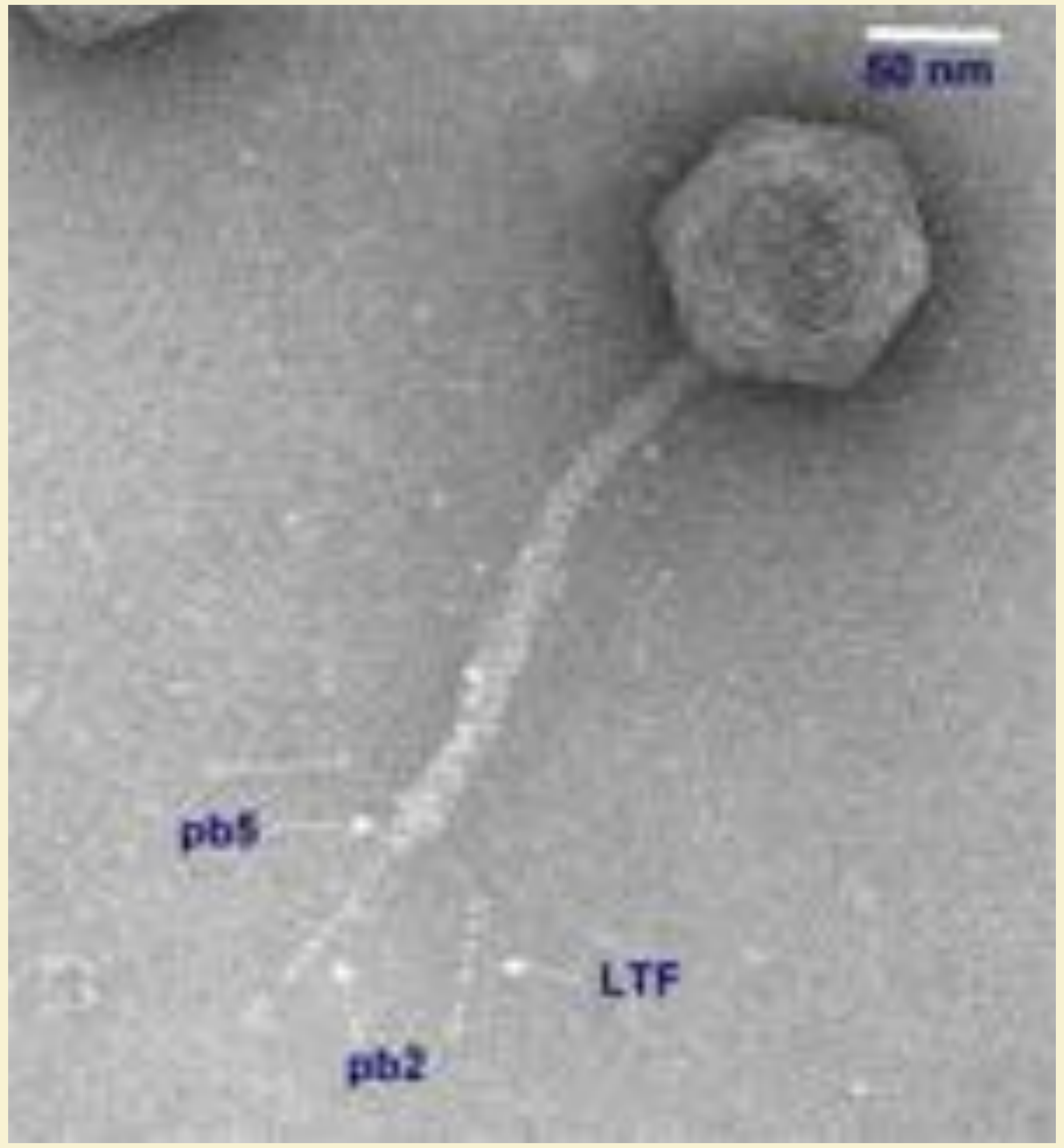
In the new era, research on therapeutic applications of bacteriophages was mostly conducted in the former Soviet Union and few other countries including Poland.

Tibilisi i Wrocław
Two research centers with a long undisrupted tradition of clinical application of bacteriophages

Biology of bacteriophages

Morphology

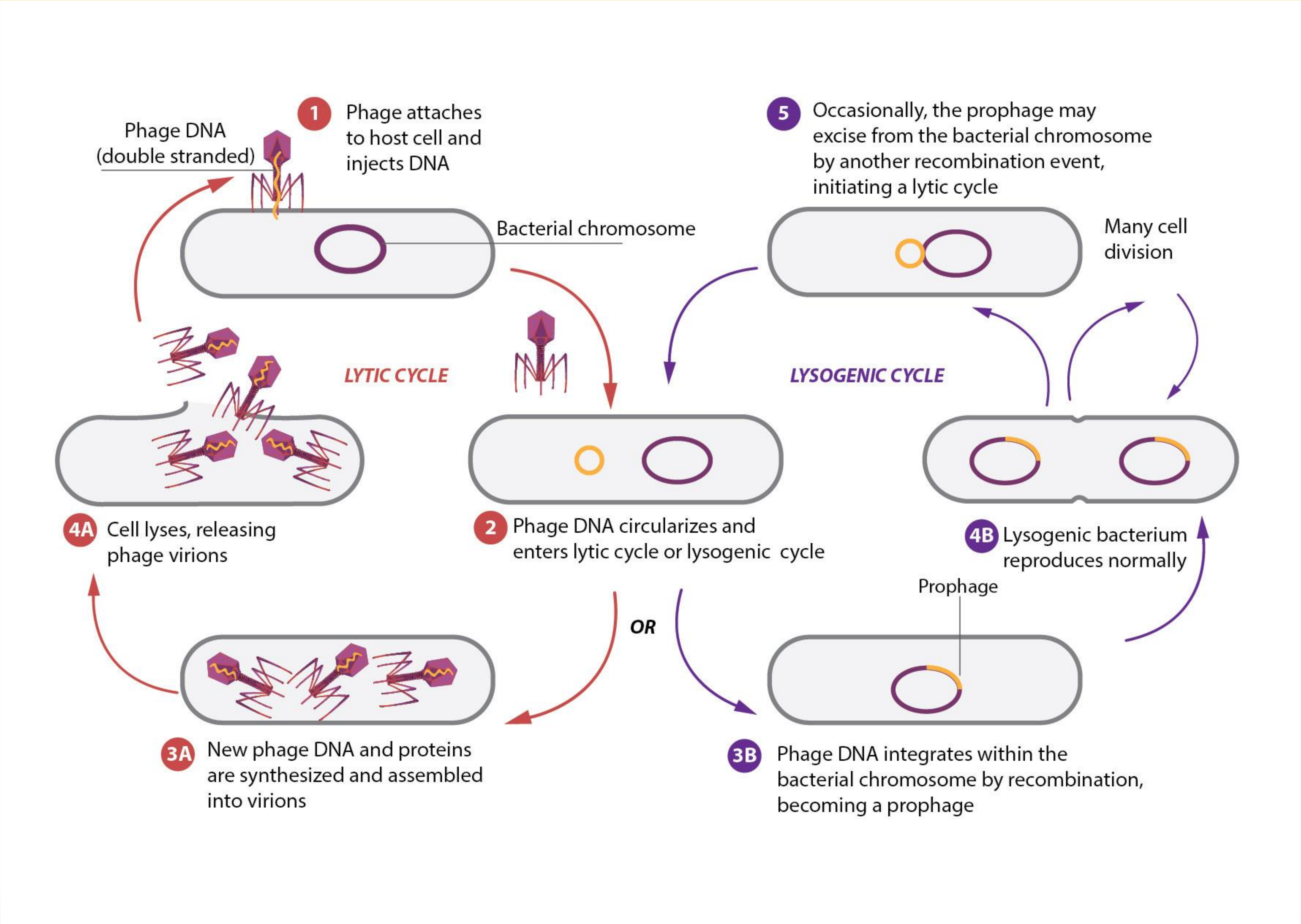




EM picture of T5 family bacteriophage

Biology of bacteriophages

Two different cycles of bacteriophage infection



Biology of bacteriophages

The mechanism of bacterial wall lysis by T5 family of bacteriophages

(1) The adsorption of viral particles on the receptors

(2) Introduction of the pre-early region into the host cell and its transcription

(3) Expression of the remaining proteins and the DNA replication

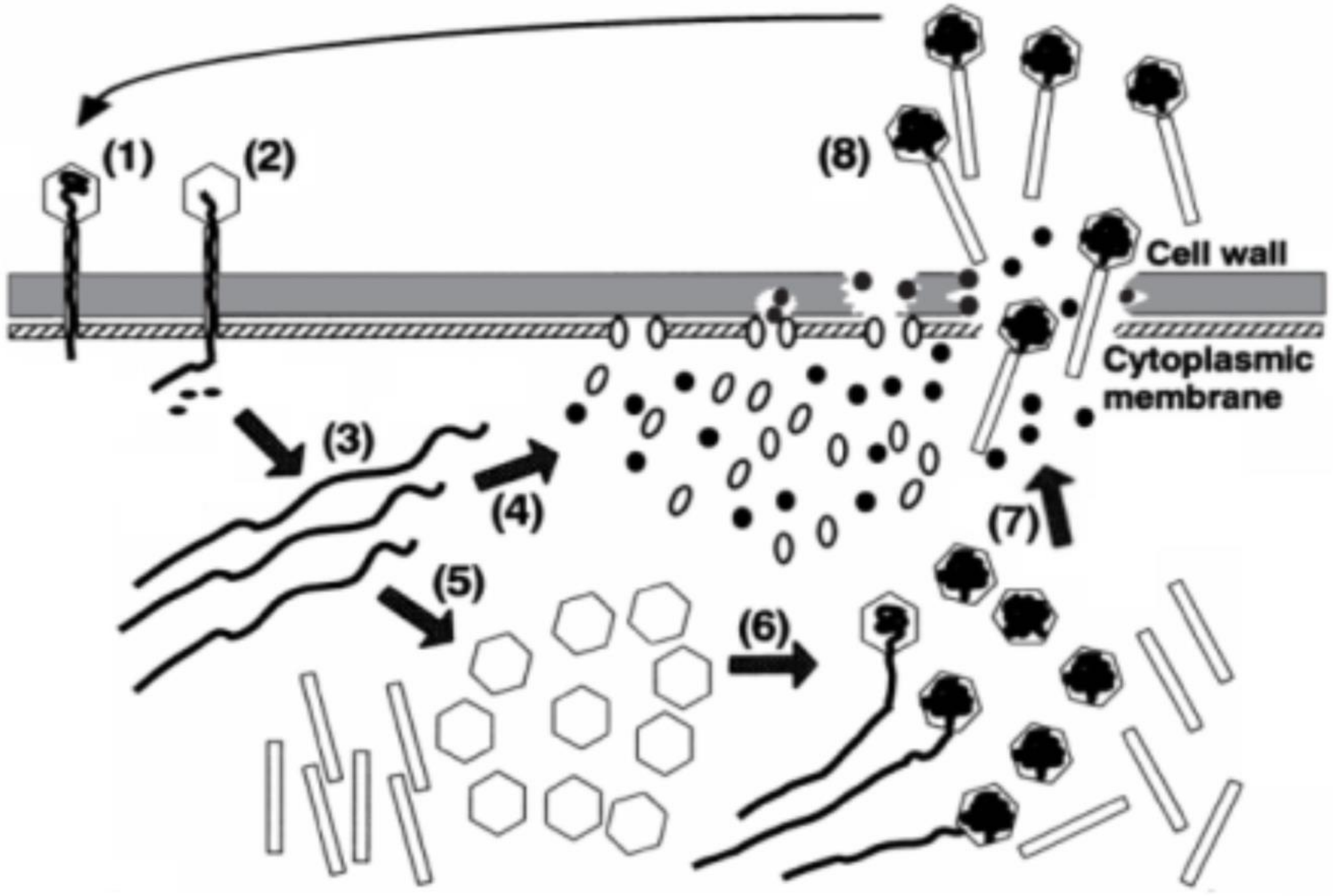
(4) Holin and lysozyme synthesis

(5) Head and tail production

(6) DNA packaging

(7) Complementation of viral particles and accumulation of appropriate amount of holin and lysozyme

(8) Cell wall degradation using holin and lysozyme and releasing of viral particles



Microbiome research

Metagenomic provide a new perspective

Source: Credit: Joana Ricou / Steven H. Lee / Studio Graphiko

- The latest scientific analysis set a number of all bacterial species on the planet as close to one trillion (10^{12})
- We are able to culture only around 10 000 bacterial species
- We have full genomic information for 100 000 bacterial species

Kenneth J. Locey et al. 2016

The rise of antibiotic resistance

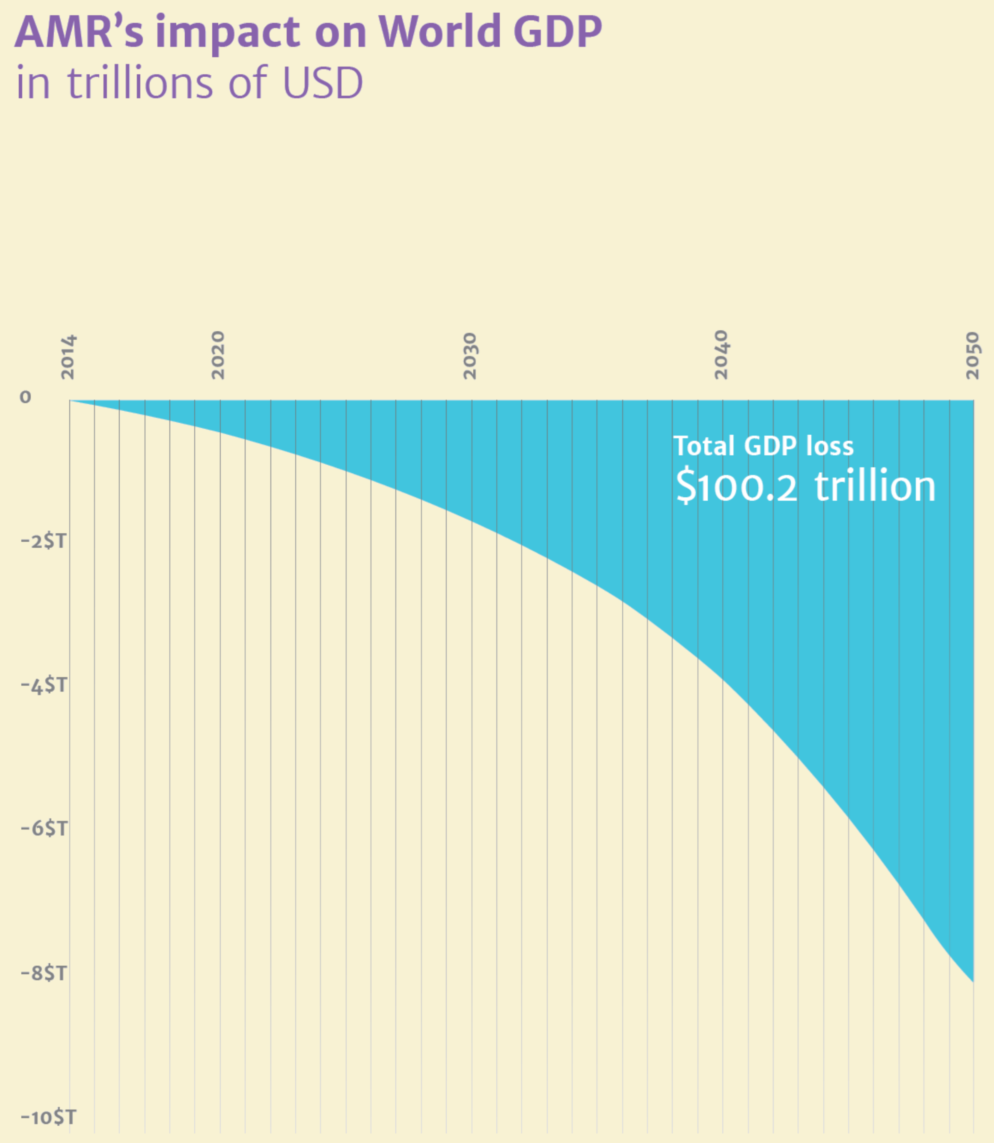
Alarming data indicate new scientific priorities

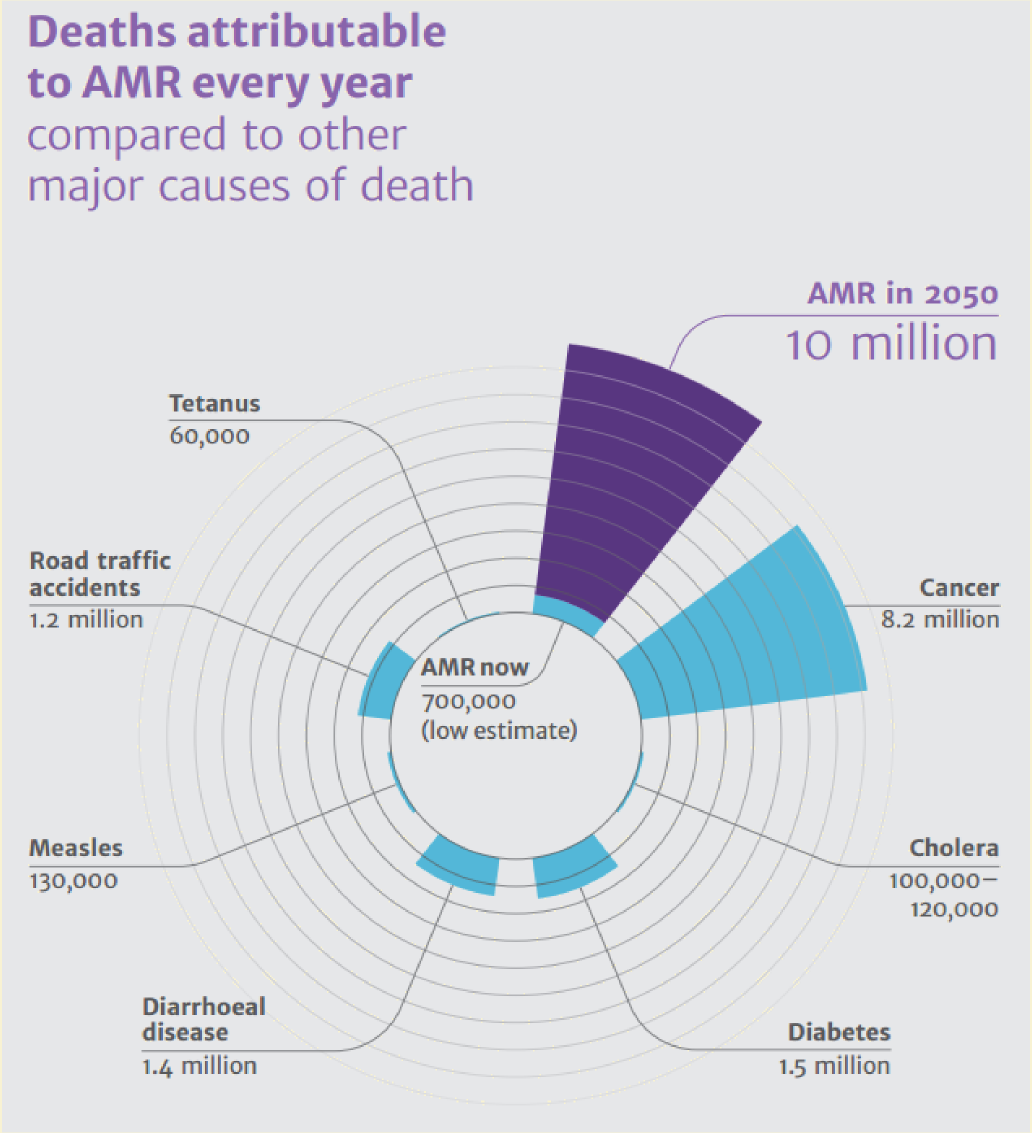
Why is Antimicrobial Resistance (AMR) a scientific priority?

Case Study - China, Shrimp

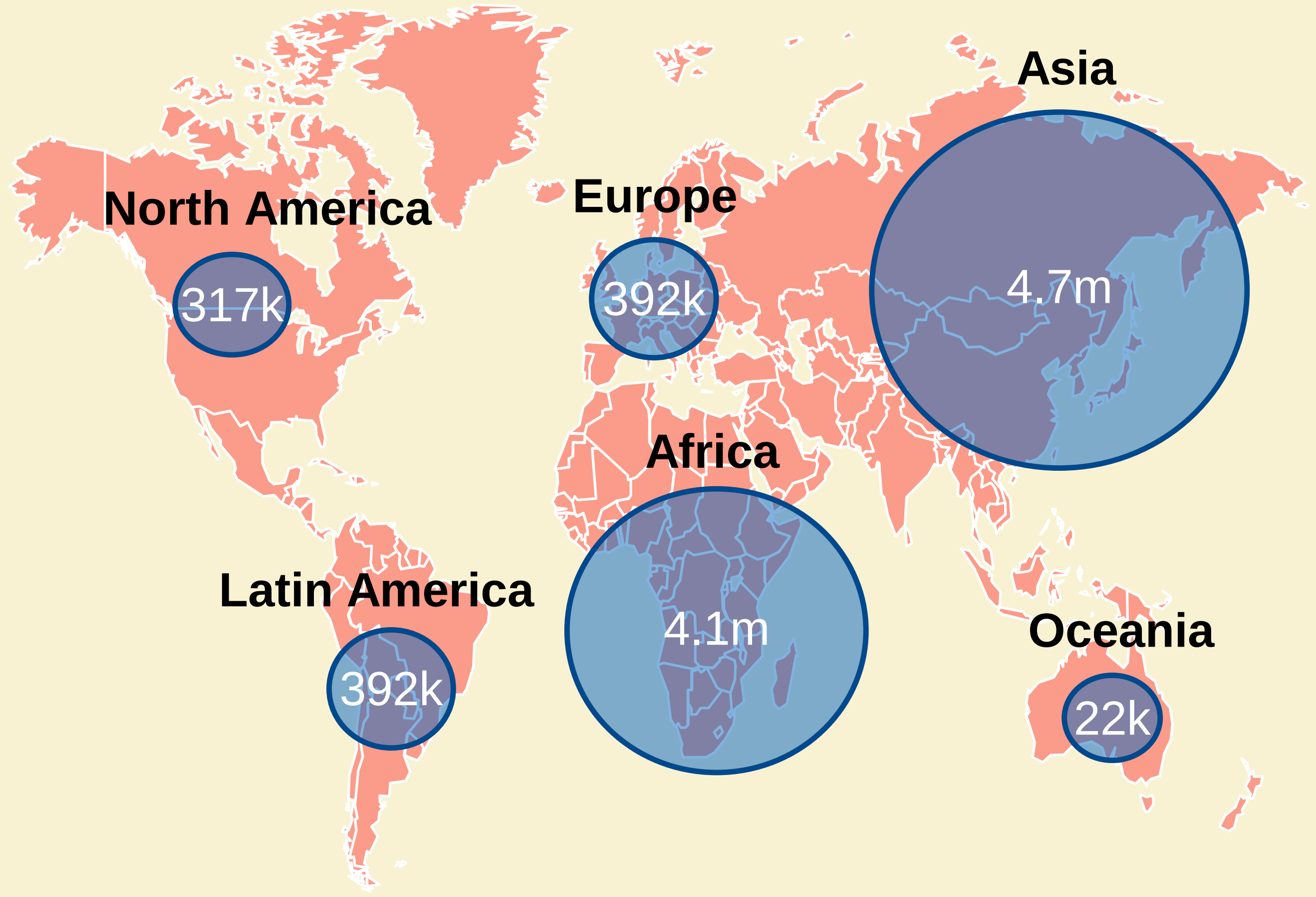
- It is common in China for the waste from other livestock (e.g. pigs) to be sluiced into aquaculture ponds as feed.
- As much as 90% of antibiotics administered to pigs pass un-degraded through their urine and faeces. This has a direct impact on farmed seafood.
- In a November 2015 study, resistant genes were found in China that can turn a dozen or more types of bacteria into superbugs. This gene has since been found in patients, food, and environmental samples in more than 20 countries, indicating that food can be a crucial vector for transmission.

Source: Bloomberg Businessweek (Dec 2016). "How antibiotic-tainted seafood from China ends up on your table.





Source: Research conducted by the UK Government, 2014



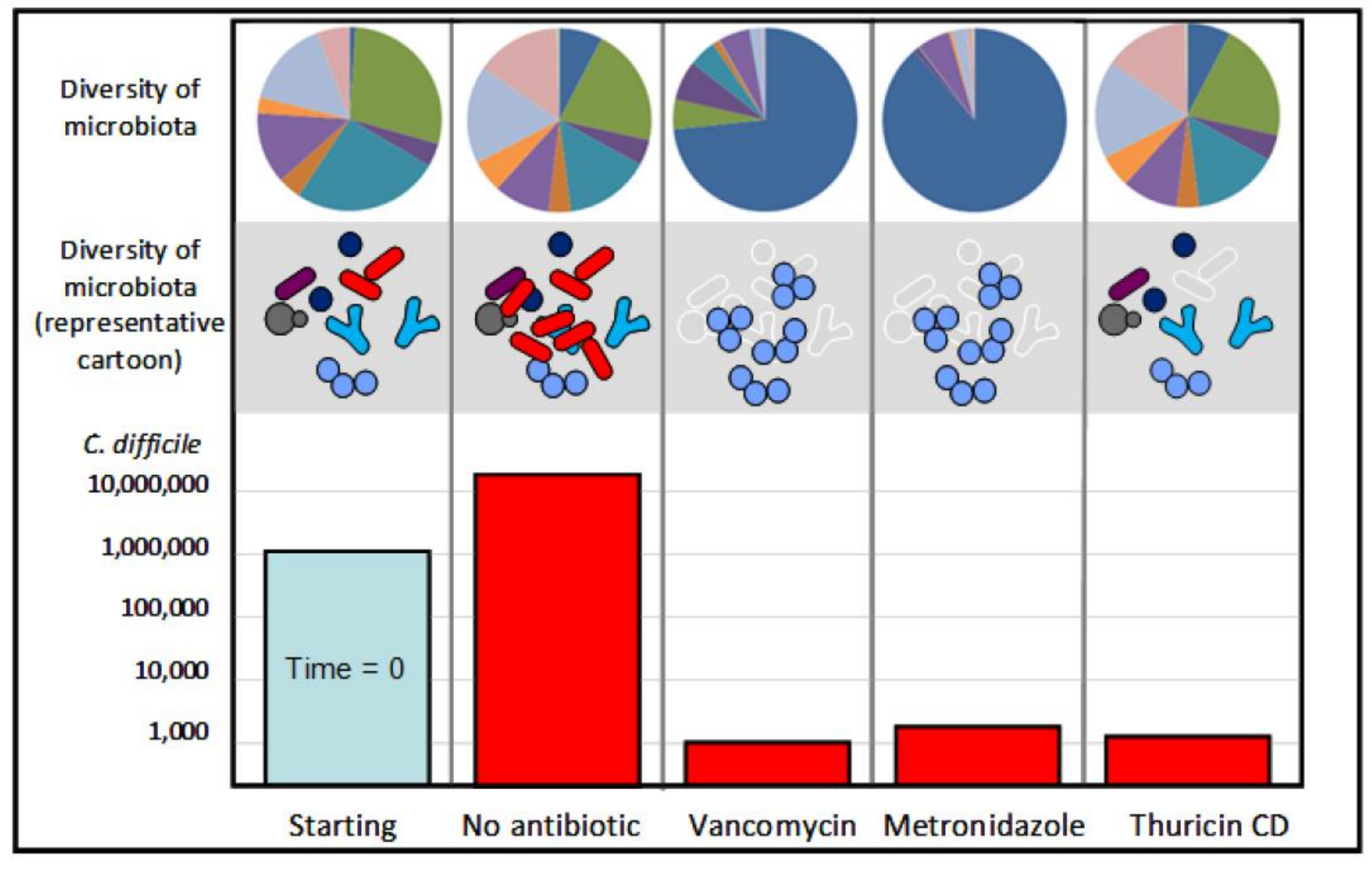
Number of deaths per year attributable to AMR by 2050 if current (2015) resistance rates increased by 40%

Source: European Centre for Disease Prevention and Control.

The rise of antibiotic resistance

Collateral damage
Decreasing the microbiota diversity

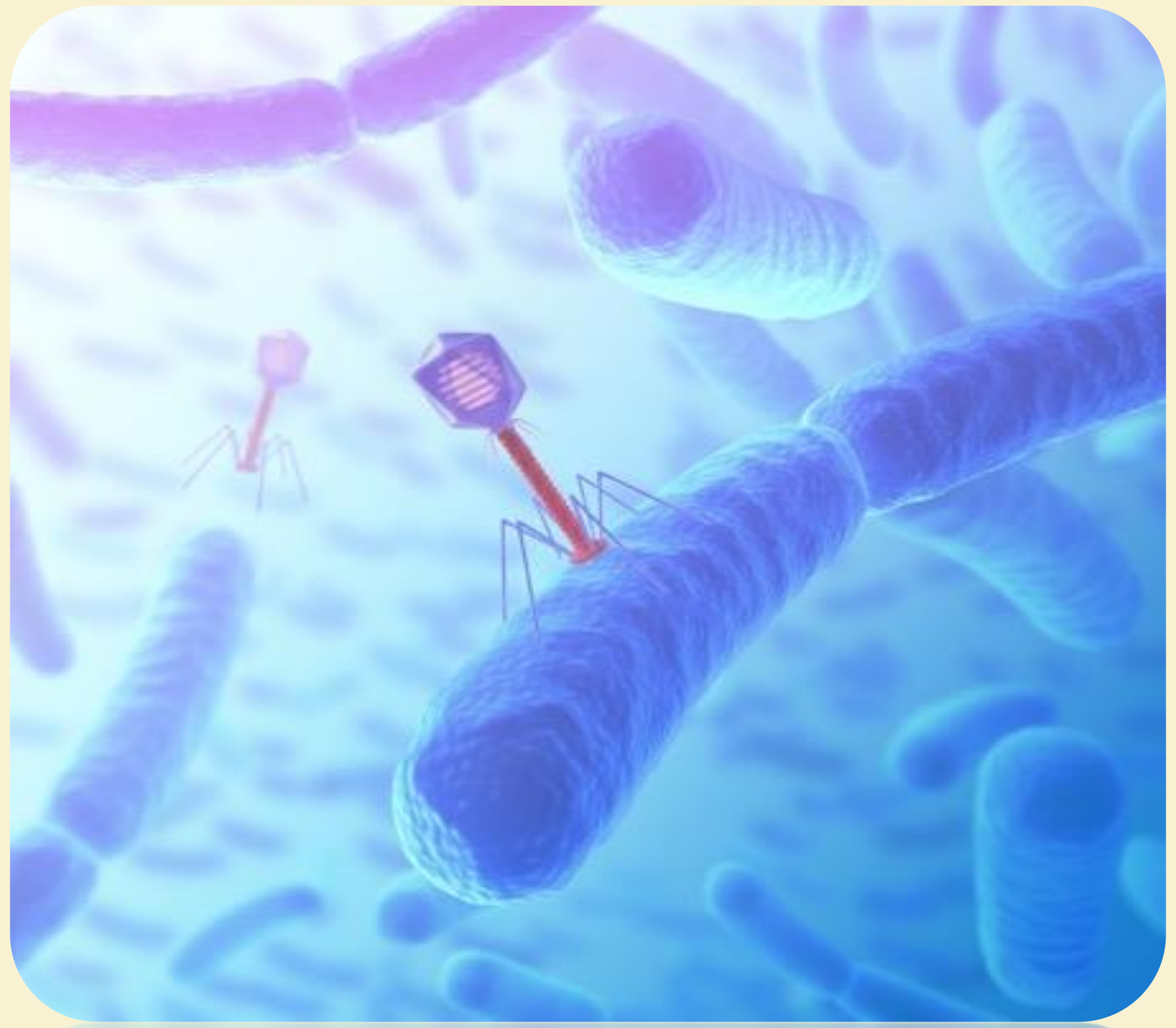
• V



Paul D Cotter, Discovery Medicine 2012

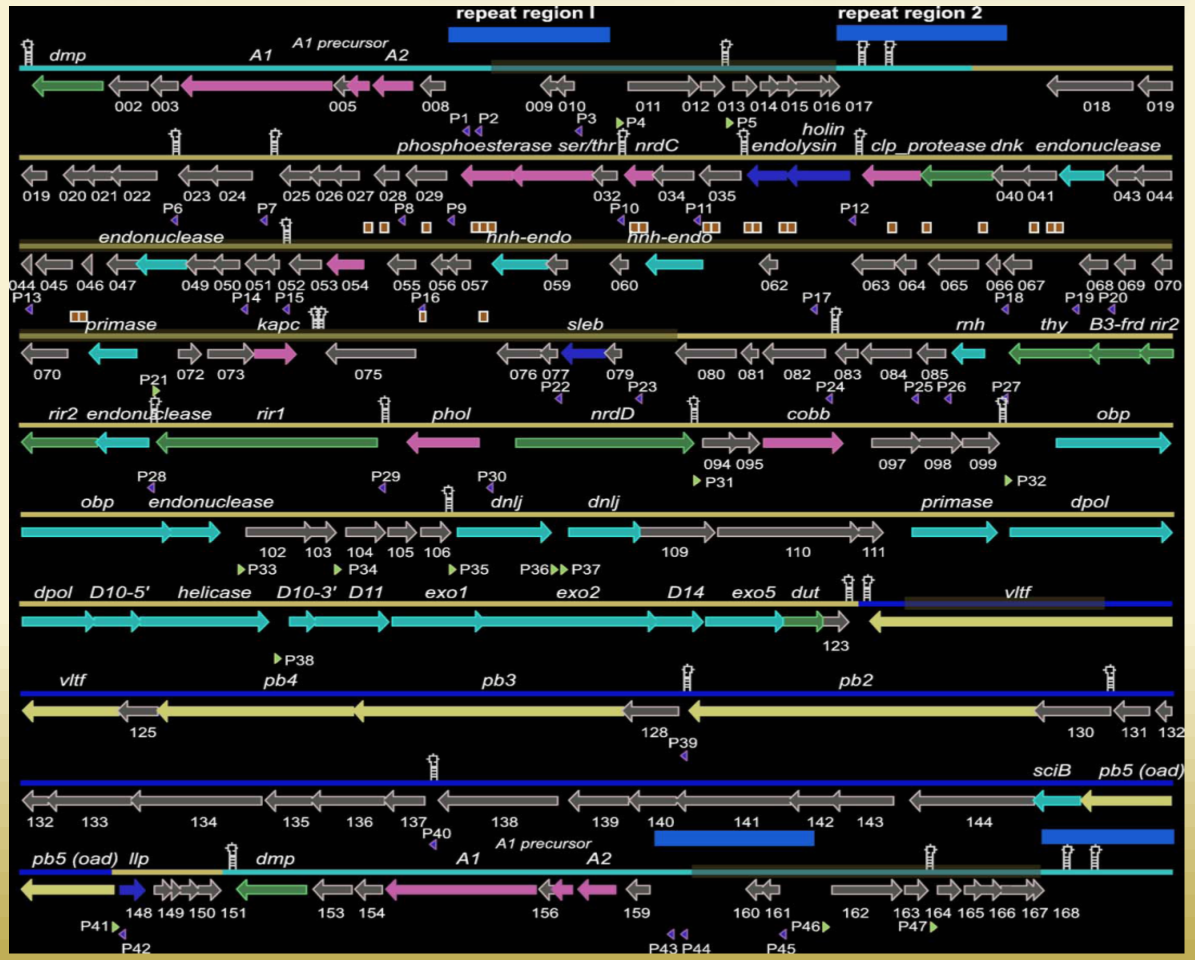
The comeback of bacteriophages

Getting molecular



Recent progress in molecular biology and technology helped to solve many initial problems related to production of bacteriophages on an industrial scale. The latest high throughput genome sequencing methods enable a precise characterization of both the infecting bacteriophages and the pathogenic bacterial hosts.

Available literature has confirmed success in the application of bacteriophages in the treatment of various human infections, especially in cases of prolonged infections from multi-drug bacteria.

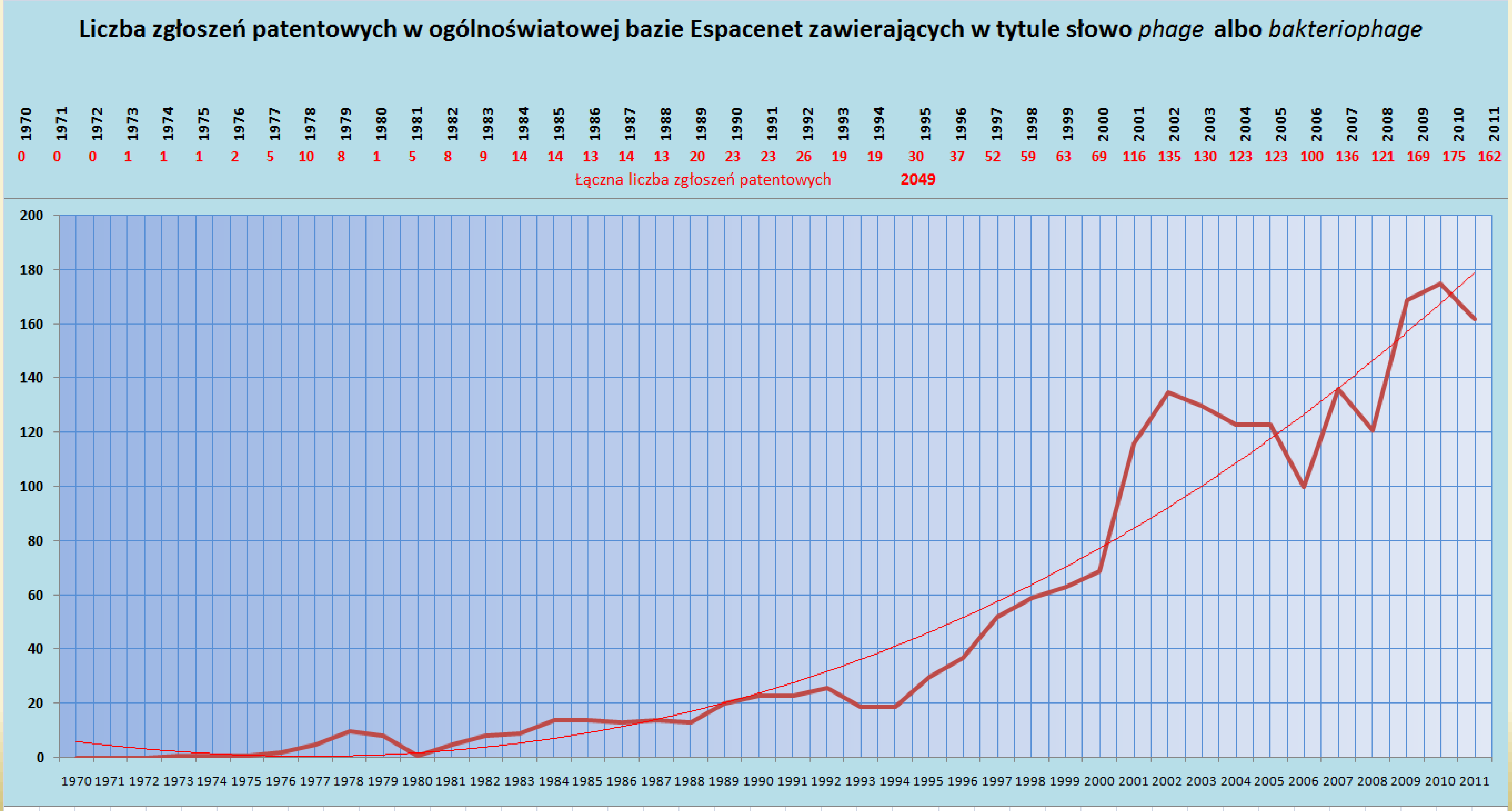


The powerful combination of new generation DNA sequencing and bioinformatics allows us to maximize the amount of information available for decision making process while designing bacteriophage based solutions. This could be ilustrated by presented genomic map of a T5 bacteriophage.

Wang et al. 2005

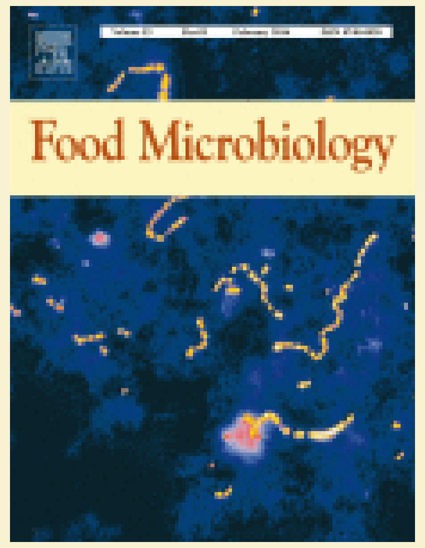
The comeback of bacteriophages

Increasing numbers of bacteriophage related patents



Bacteriophages for animal health

Application of bacteriophages as a biosecurity measure to improve food safety in poultry



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- Borie C, Albala I, Sánchez P, Sánchez ML, Ramírez S, Navarro C, Morales MA, Retamales AJ, Robeson J. **Bacteriophage treatment reduces Salmonella colonization of infected chickens**. *Avian Dis.* ***2008*** *Mar;52:64-7.*
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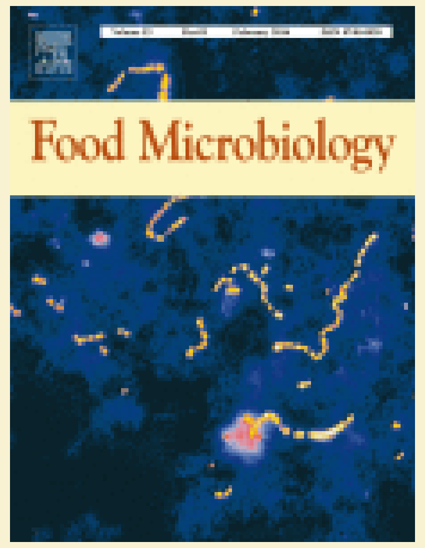
Bacteriophages for animal health



Application of bacteriophages to prevent mortality related to bacterial pathogens in aquaculture

- Richards GP. **Bacteriophage remediation of bacterial pathogens in aquaculture: a review of the technology.** Bacteriophage. **2014**, 20;4:e975540
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Bacteriophages for poultry

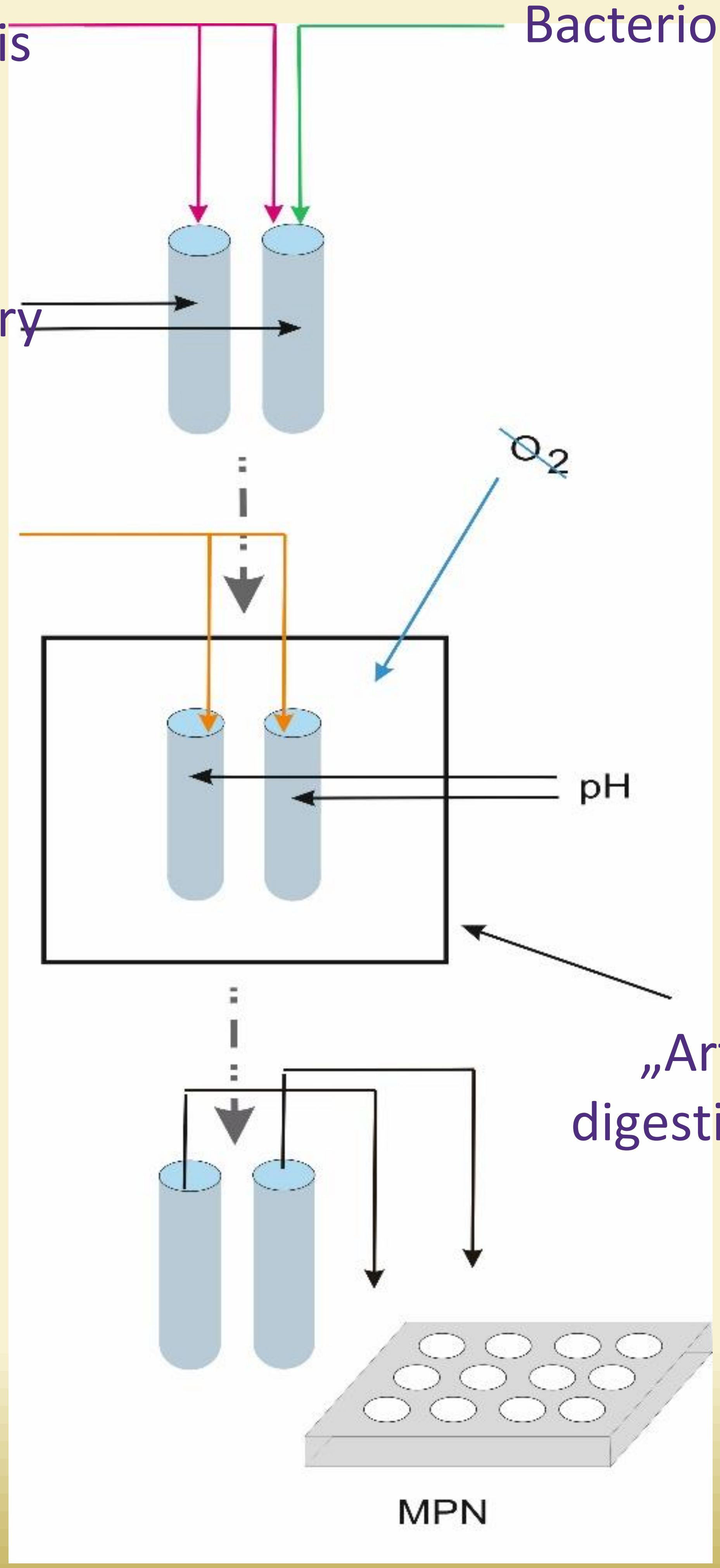
In vitro efficacy assay

Salmonella Enteritidis

Bacteriophage component

Luiquid supplementary feed

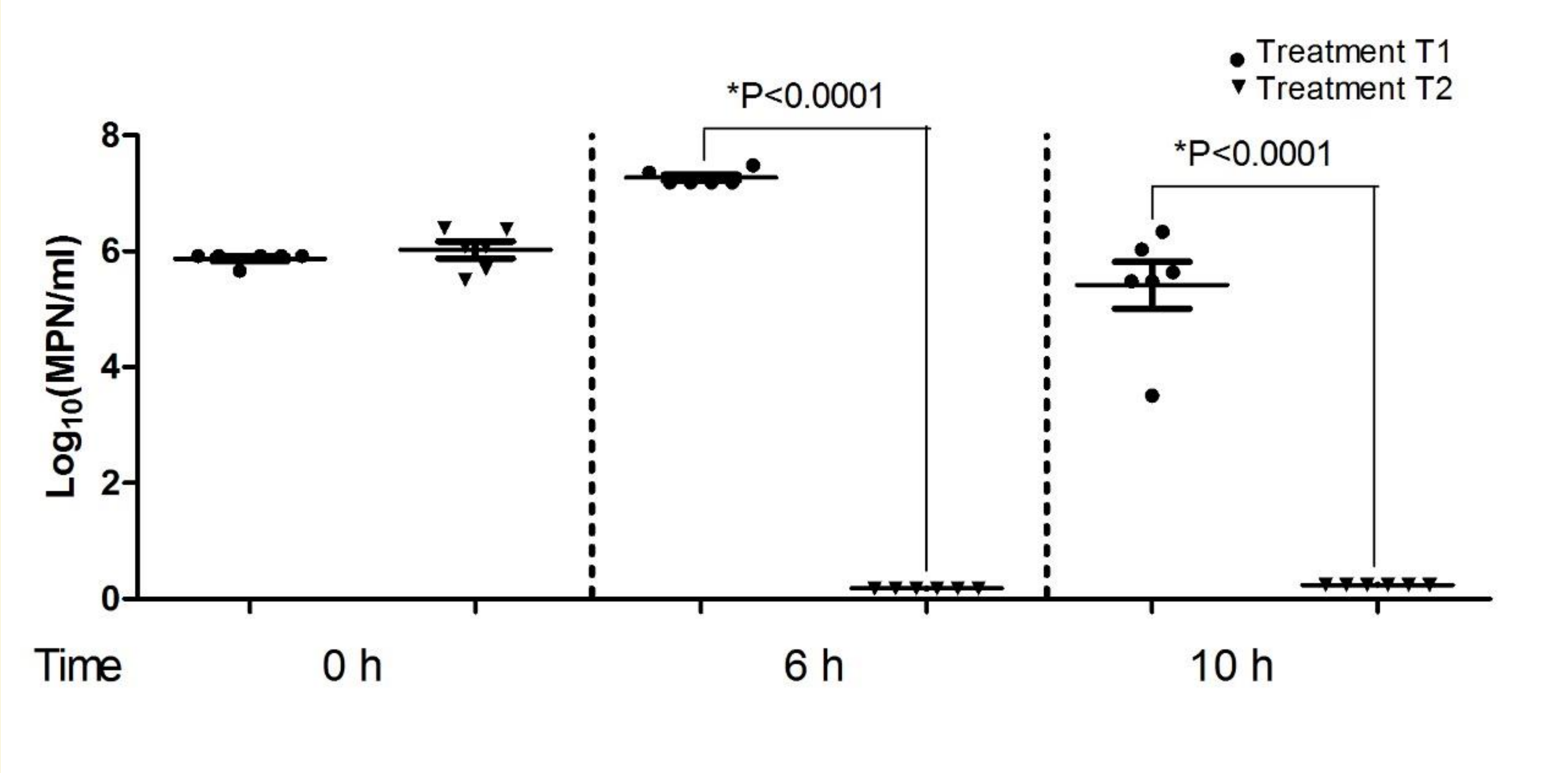
Digestive enzymes, HCL, bile salts



„Artificial digestive tract"

Determination of the number of Salmonella

Results



Stańczyk M. et al. Unpublished data

Bacteriophages for poultry

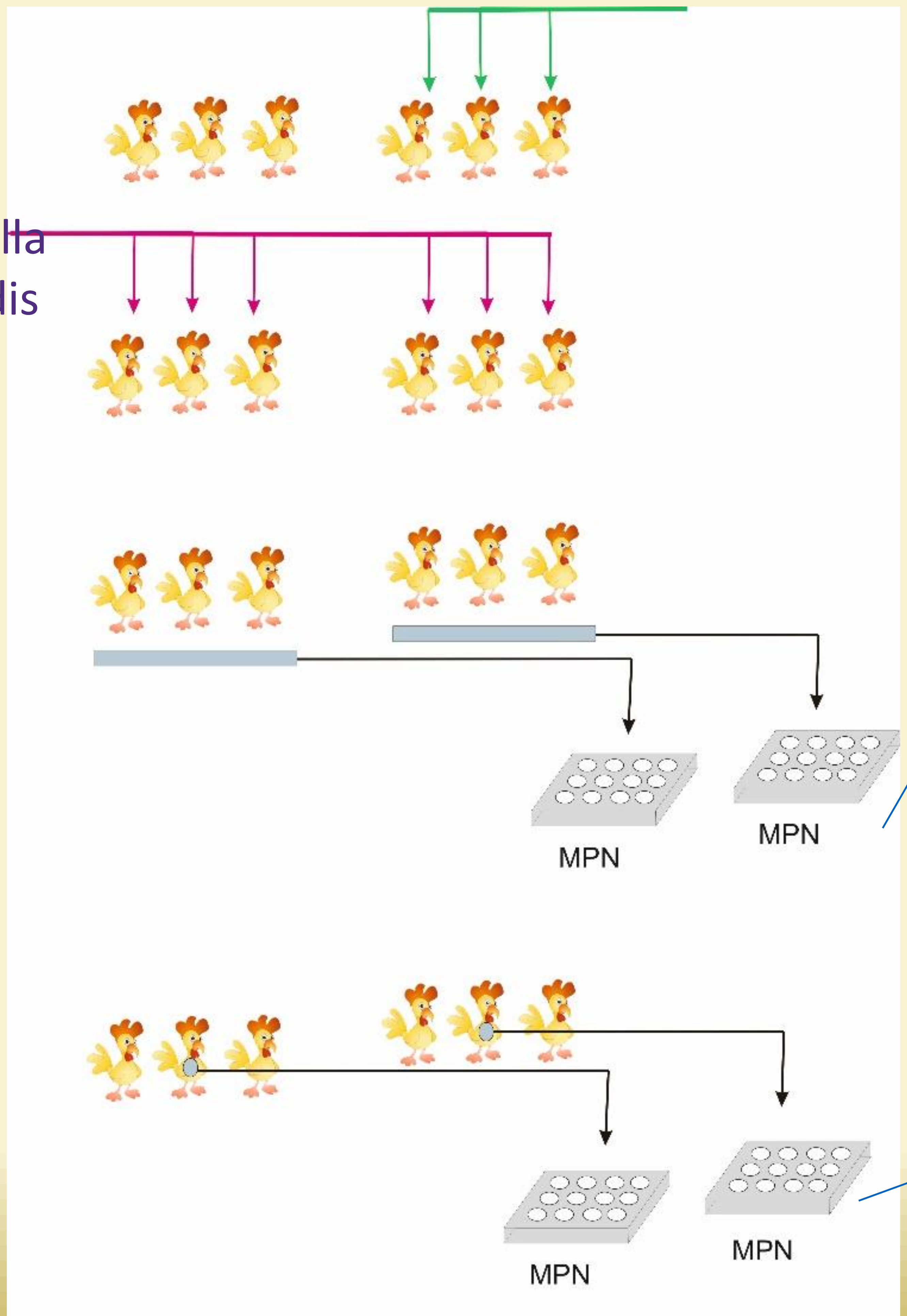
In vivo efficacy trial

Day 1

Bacteriophage component

Day 3

Salmonella Enteritidis



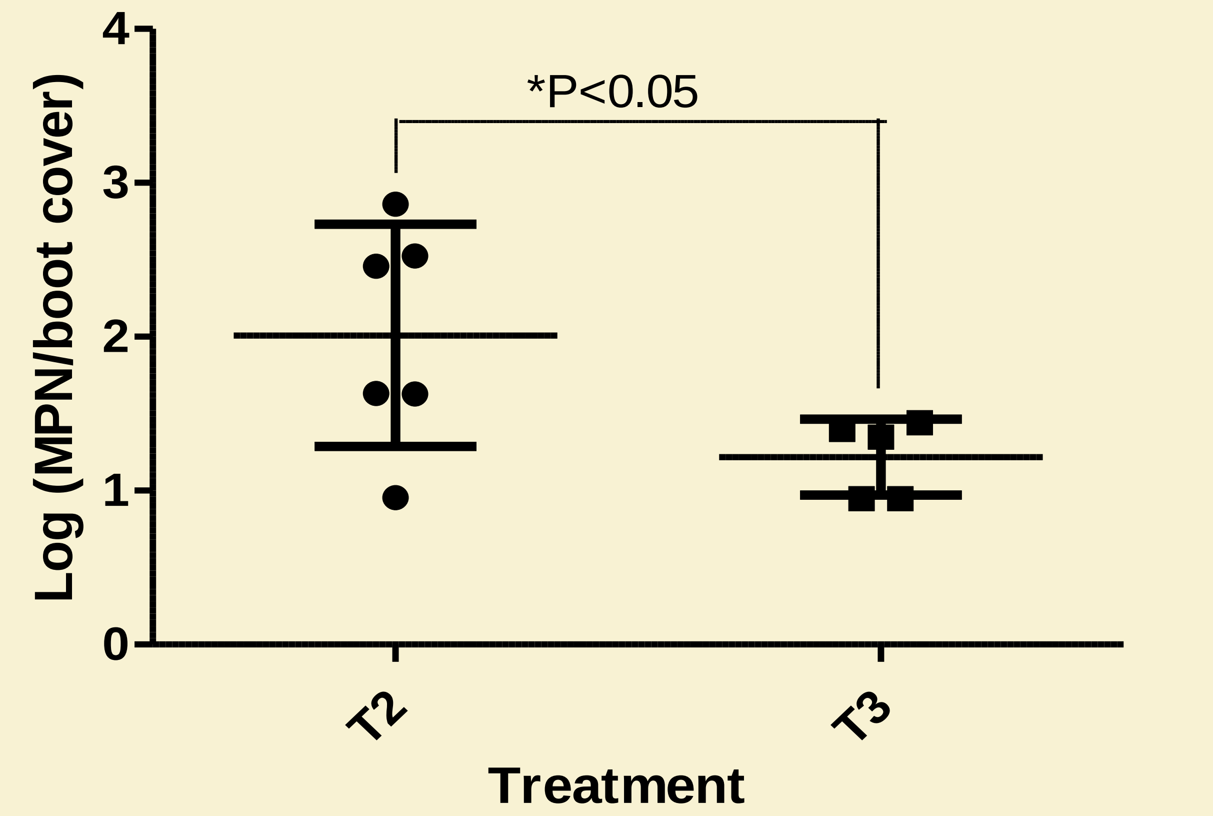
Day 21

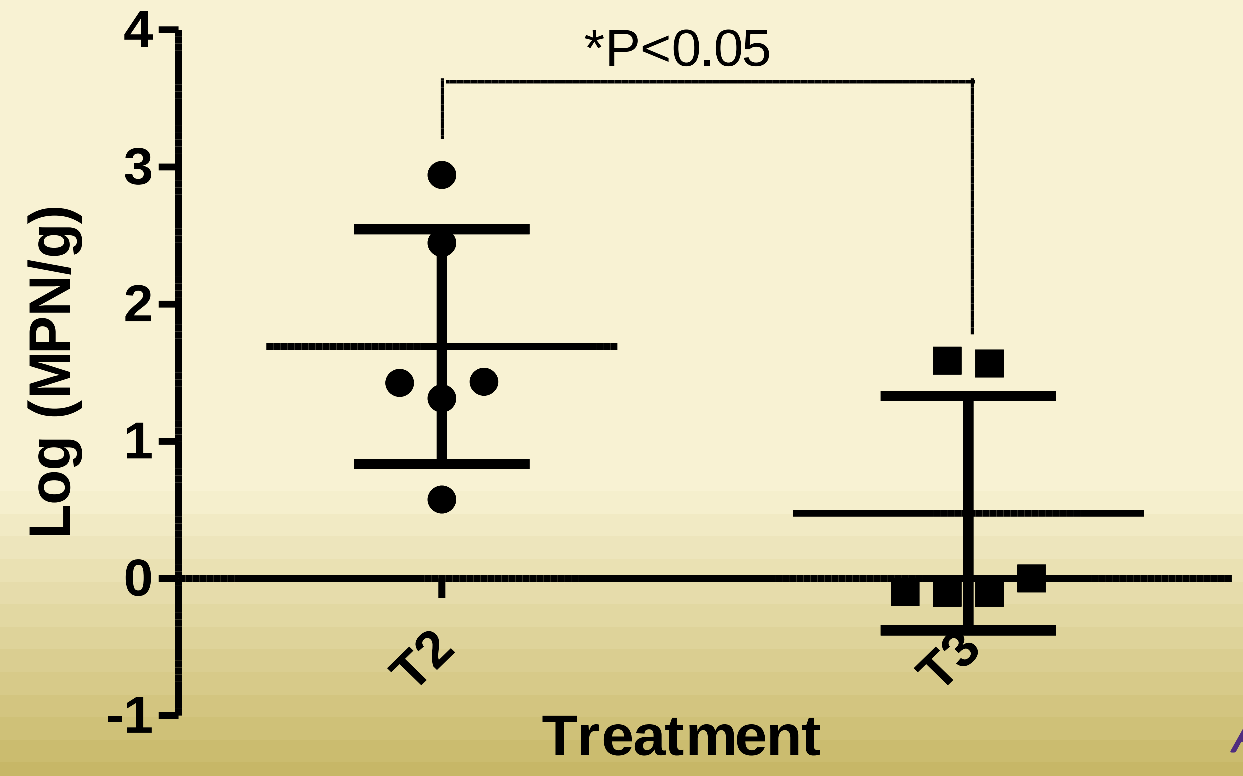
Determination of the number of Salmonella in bedding

Day 36

Determination of the number of Salmonella in caeca

Results





Awdosiewa I. et al. Unpublished data

Bacteriophages for poultry

Characterization of bacteriophages

Deep search for protein homology of ORFs in bacteriophage genomes

Protein	8Sent65	3Sent1	8Sent1748
deoxynucleoside-5'-monophosphatase			
A1 protein			
A2-A3 protein			
H-N-H-endonuclease			
H-N-H-endonuclease			
serine/threonine protein phosphatase			
serine/threonine protein phosphatase			
thioredoxin			
lysozyme			
holin			
ATP-dependent Clp protease			
deoxynucleoside-5'-monophosphate kinase			
acetyltransferase-related protein			
pi3 protein 38-like/T5.067			
DNA primase			
nicotinamide mononucleotide transporter			
nicotinamide-nucleotide adenylyltransferase			
cAMP-dependent protein kinase catalytic subunit			
putative transmembrane protein			
spore cortex-lytic enzyme/cell wall hydrolase			
putative metallopeptidase			
ribonuclease H			
thymidylate synthase			
dihydrofolate reductase			
ribonucleoside diphosphate reductase, small unit			
H-N-H-endonuclease			
ribonucleoside diphosphate reductase, large unit			
phosphate starvation-inducible/PhoH-like protein			
anaerobic ribonucleoside triphosphate reductase			
NAD-dependent deacetylases SIR2/transferase			
replication origin binding protein			
H-N-H-endonuclease			
D2 protein			
D3 protein			

EM morphology

Wojtasik A. et al. Unpublished data

Bacteriophages for aquaculture

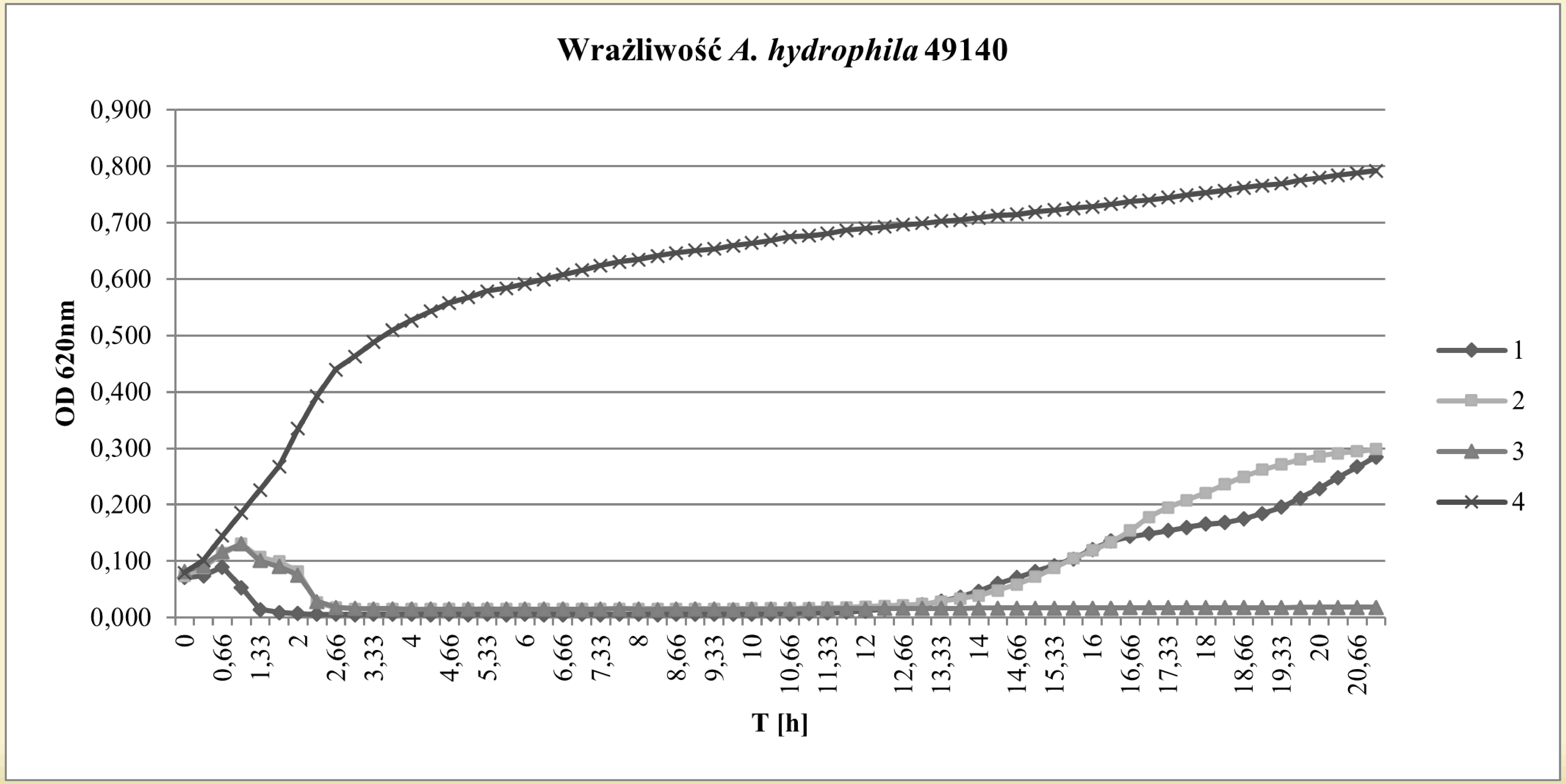
Screening for bacteriophages against *Aeromonas hydrophila*

Bacteriophages	11AhydR10PP	13AhydR10PP	14AhydR10PP	19AhydR15PP	25AhydR2PP	50AhydR13PP	53AhydR13PP	60AhydR15PP	62AhydR11PP	80AhydR10PP	82AhydR10PP	85AhydR10PP	86AhydR10PP	72AsobR5PP	75AsobR5PP	76AsobR5PP
A. hydrophila																
R2	-	-	-	-	cl	-	-	-	-	-	-	-	-	-	-	-
R6	-	-	-	-	-	cl	-	+	-	-	-	-	-	-	-	-
R9	-	-	-	-	cl	+	-	+	-	-	-	-	-	-	-	-
R10	-	cl	cl	-	-	-	-	-	-	-	-	-	-	-	-	-
R11	-	-	-	-	-	-	-	-	cl	-	-	-	-	-	-	-
R12	-	cl	cl	-	-	-	-	-	-	-	-	cl	-	-	-	-
R13	-	-	-	-	-	cl	-	cl		-	-	-	-	-	-	-
R14	-	cl	-	-	-	cl	-	cl	cl	-	-	-	-	-	-	-
R15	-	cl	-	-	-	+	-	-	-	-	-	-	-	-	-	-
R21	-	-	-	-	-	cl	-	-	-	-	-	-	-	-	-	-
R22	-	-	-	-	-	cl	-	-	-	-	-	-	-	-	-	-
R23	-	-	-	-	-	cl	-	-	-	-	-	-	-	-	-	-
R24	-	-	-	-	-	cl	-	cl	cl	-	-	-	-	-	-	-
R25	-	-	-	-	-	cl	-	cl	cl	-	-	-	-	-	-	-
R26	-	-	-	-	cl	-	-	-	-	-	-	-	-	-	-	-
R40	cl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R41	-	-	-	-	-	-	cl	-	-	-	-	-	-	-	-	-
R48	-	cl	cl	-	-	-	-	-	-	-	cl	cl	cl	-	-	-
R52	-	cl	cl	-	-	-	-	-	-	-	cl	cl	cl	+	-	-
R53	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
R55	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-
R59	-	-	-	-	-	-	-	-	cl	-	-	-	-	-	-	-
R65	-	cl	-	-	-	-	-	cl	-	-	-	-	-	-	-	-
R71	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-

Dastych J. et al. Unpublished data

Bacteriophages for aquaculture

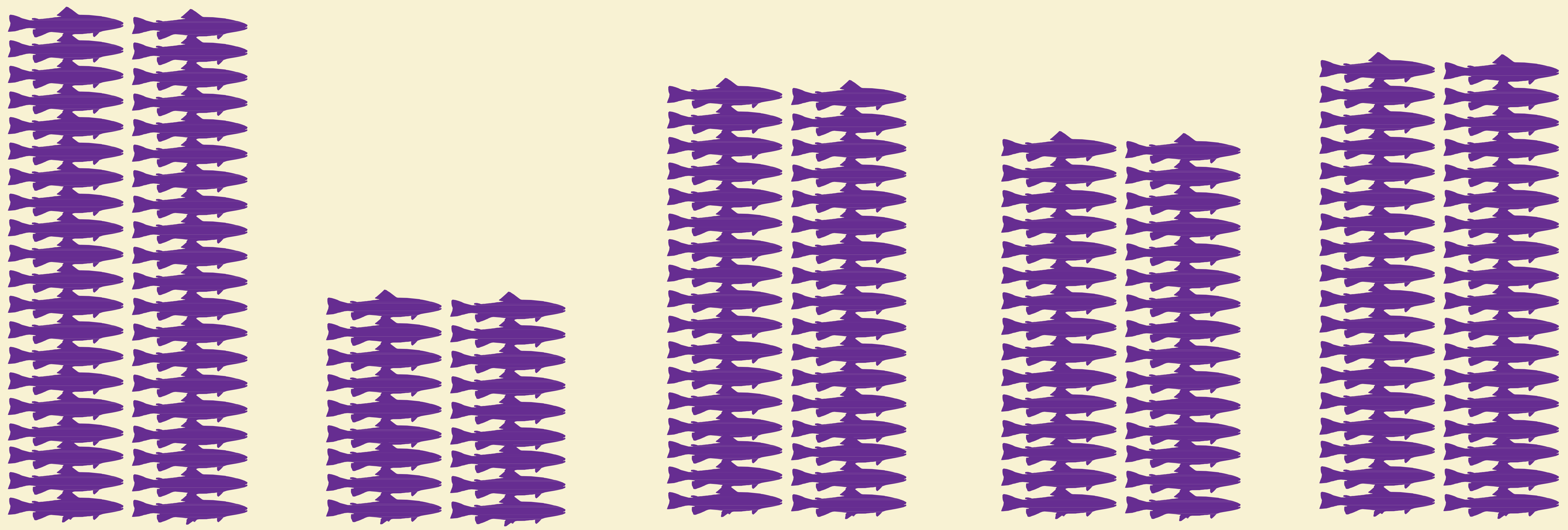
In vitro efficacy determination of bacteriophages by kinetics of bacterial growth



Dastych J. et al. Unpublished data

Bacteriophages for aquaculture

Efficacy study in a closed containment system for carp (*Cyprinus carpio*)



Challenge with *Pseudomonas fluorescens*	No	Yes	Yes	Yes	Yes
Bacteriophage treatment by immersion	No	No	24 hrs after challenge	48 hrs after challenge	24 and 48 hrs after challenge
Mortality	0%	55%	15%	25%	10%

Siwicki A. et al. Unpublished data

Regulatory and technological challenges

Safety

- Safety of bacteriophage application has been discussed in scientific literature and assessed at several occasions by regulatory bodies such as FDA and EFSA. There is a general agreement on a high level of safety of bacteriophage application.

- Based on the current state of scientific knowledge the major if not the only safety concern is related to observations that certain bacteriophages as a part of microbiological ecosystem participate in mechanism of horizontal gene transfer (HTS). Particularly bacteriophages act as shuttles transferring genes from one bacterial genome to another one. This is related to process known as lysogeny resulting in integration of bacteriophage DNA into bacterial chromosome.

Regulatory and technological challenges

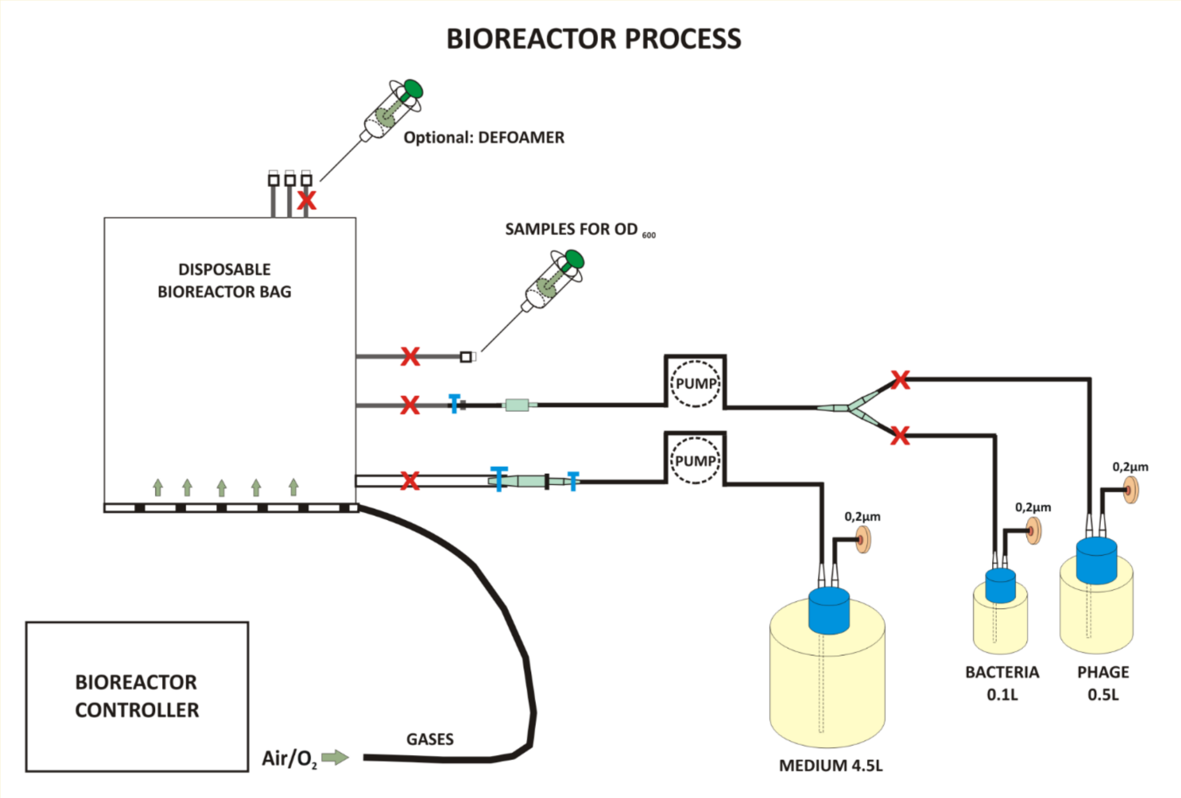
Current regulatory situation

FDA	G.R.A.S. for human food preservatives Phase I/II clinical trials for topical application in human
EFSA	Positive scientific opinion on safety Pending application for feed additive
EMA	Internal discussion Phase I/II clinical trials for topical application in human

Georgia	Human medicine
Russia	Human medicine
Ukraine	Feed additive
New Zealand	Food preservative
Korea	Feed additive

Regulatory and technological challenges

Technological challenges



Defined chemical composition of active substance

Stability of active substststance

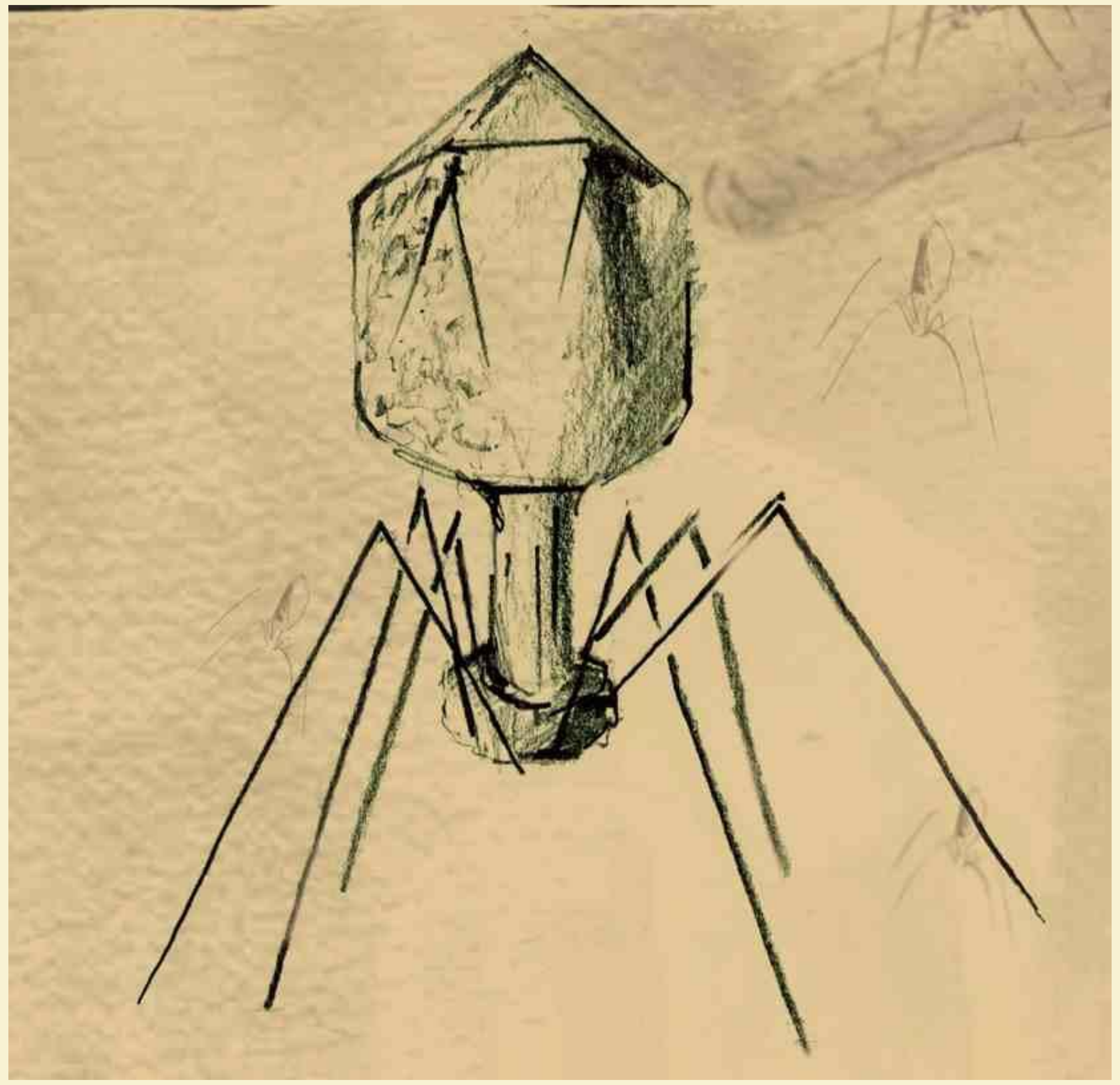
Genetic stability of phages

Antigenic variability in bacterial pathogens

Production under control environment (GMP)

Effective purification

Difficulties in in vitro culture of bacterial pathogens



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Piotr Kwieciński, Brudzew, Poland
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Thank you for your attention!