BACTERIOPHAGES AS AN ALTERNATIVE TO ANTIBIOTICS

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PROTEON PHARMACEUTICALS S.A.

TALLINN, 7.06.2017
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 beginning

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.
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.

Tbilisi 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

1. Phage DNA (double stranded) attaches to host cell and injects DNA.
2. Phage DNA circularizes and enters lytic cycle or lysogenic cycle.
4. New phage DNA and proteins are synthesized and assembled into virions.
5. Occasionally, the prophage may excise from the bacterial chromosome by another recombination event, initiating a lytic cycle.
7. Phage DNA integrates within the bacterial chromosome by recombination, becoming a prophage.
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

- 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


Source: Credit: Joana Ricou / Steven H. Lee / Studio Graphiko
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 undegraded 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: Research conducted by the UK Government, 2014

The rise of antibiotic resistance

Alarming data indicate new scientific priorities

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.

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

Collateral damage
Decreasing the microbiota diversity

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 illustrated 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


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
**Bacteriophages for poultry**

**In vitro efficacy assay**

**Results**

- **Salmonella Enteritidis**
- Luid supplementary feed
- Digestive enzymes, HCL, bile salts
- Bacteriophage component

**Determination of the number of Salmonella**

- "Artificial digestive tract"
- **MPN**

**Stańczyk M. et al. Unpublished data**
Bacteriophages for poultry
In vivo efficacy trial

Results

Determination of the number of Salmonella in bedding

Determination of the number of Salmonella in caeca

Salmonella Enteritidis

Day 1

Day 3

Day 21

Day 36

Bacteriophage component

Log (MPN/boot cover)

Log (MPN/g)

*A<0.05

Awdosiewa I. et al. Unpublished data
# Bacteriophages for poultry

## Characterization of bacteriophages

Deep search for protein homology of ORFs in bacteriophage genomes

### EM morphology

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<th>Bacteriophage A1</th>
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Wojtasik A. et al. Unpublished data
# Bacteriophages for aquaculture

Screening for bacteriophages against *Aeromonas hydrophila*

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*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*)

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*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 substance
- Genetic stability of phages
- Production under control environment (GMP)
- Effective purification
- Antigenic variability in bacterial pathogens
- Difficulties in in vitro culture of bacterial pathogens
Credit to:

Ewelina Wójcik, Lodz, Poland
Arkadiusz Wojtasik, Lodz, Poland
Małgorzata Stańczyk, Lodz, Poland
Andrzej Krzysztof Siwicki, Olsztyn, Poland
Jan Jankowski, Olsztyn, Poland
Piotr Kwieciński, Brudzew, Poland
Irina Kornilowna Awdosiewa, Lviv, Ukraine

Thank you for your attention!