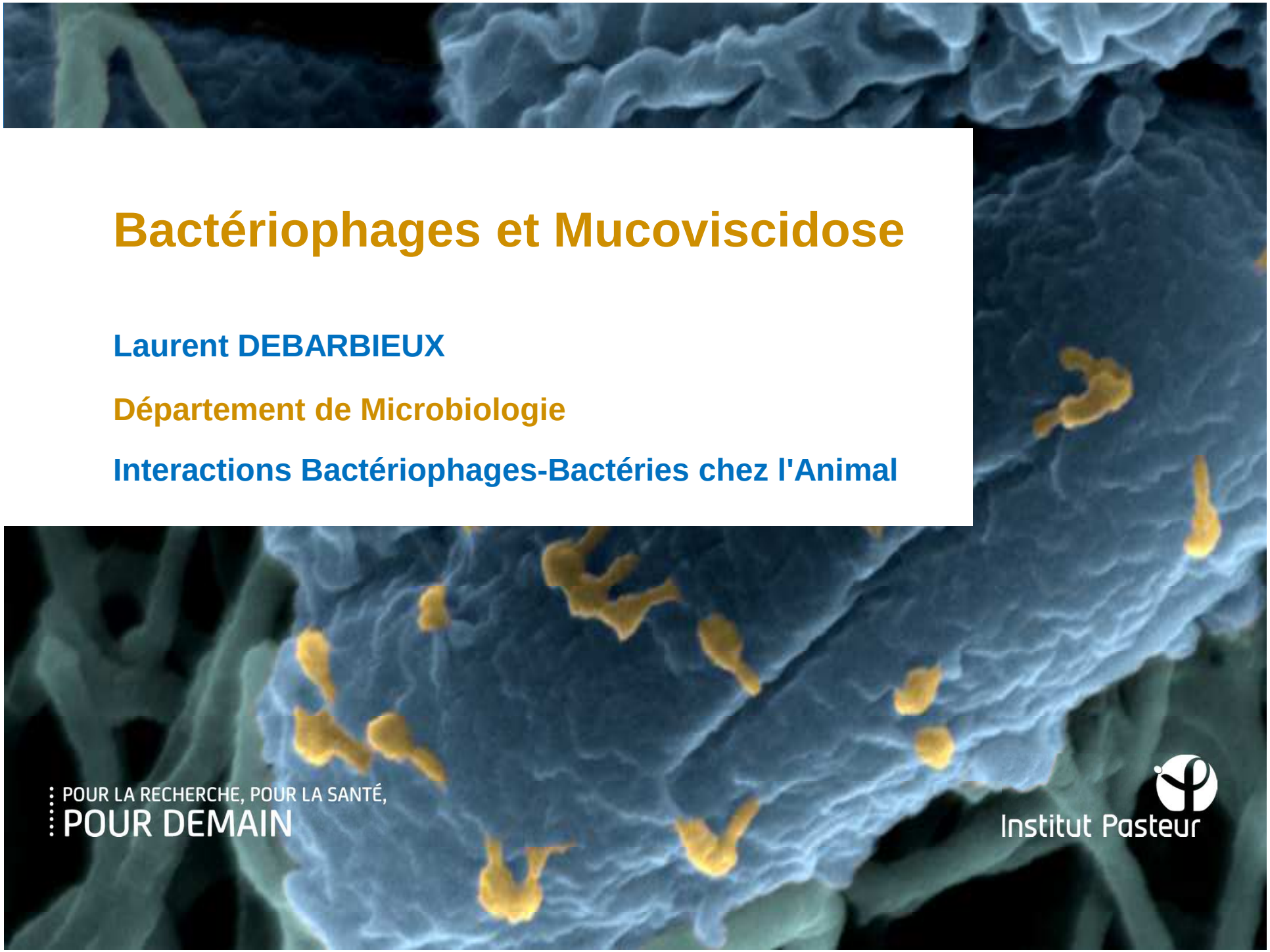


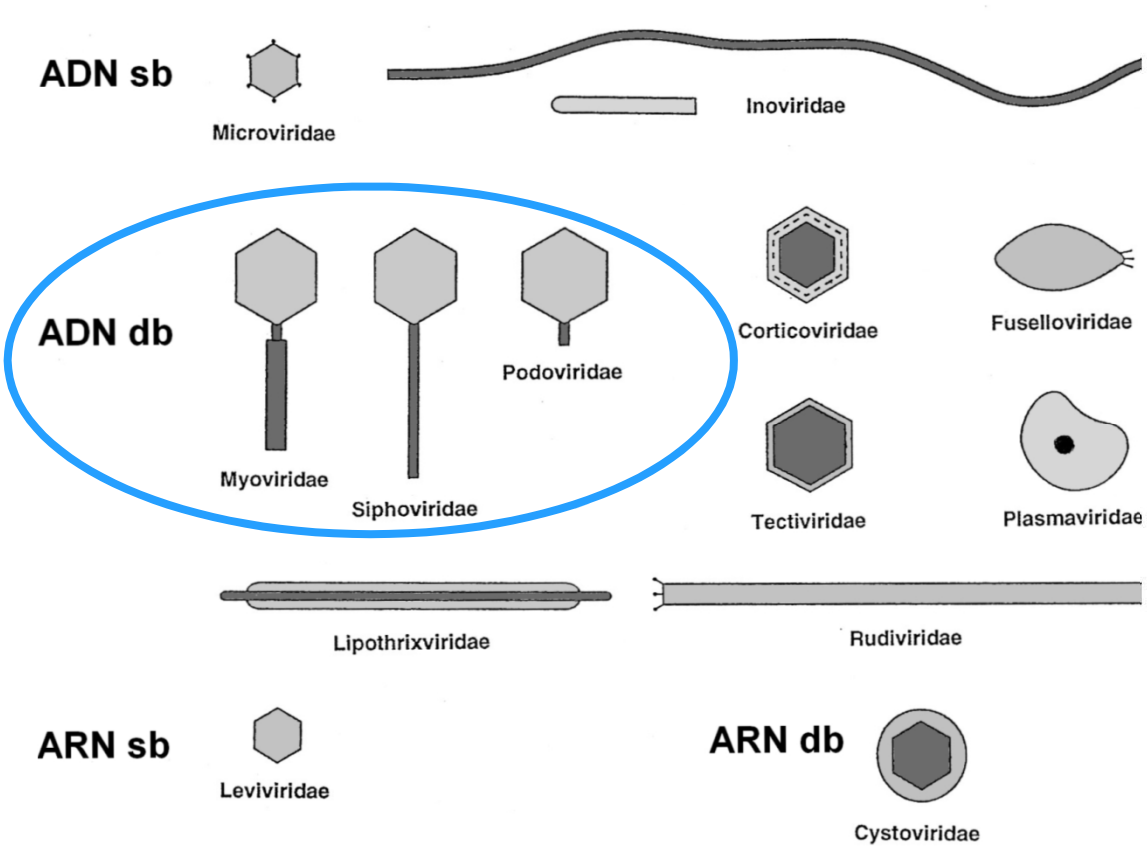
Bactériophages et Mucoviscidose

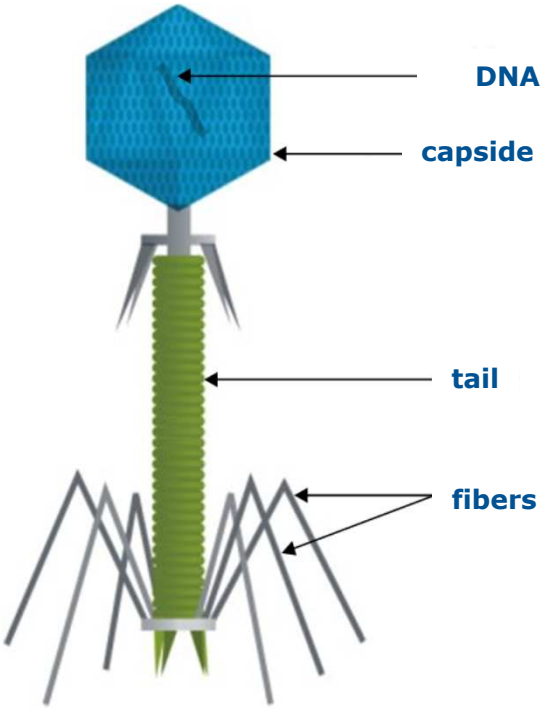
Laurent DEBARBIEUX

Département de Microbiologie

Interactions Bactériophages-Bactéries chez l'Animal

Bacteriophages belong to viruses infecting microbes





The T4 phage model

96% of bacteriophages reported belong to *Caudovirales* family of viruses

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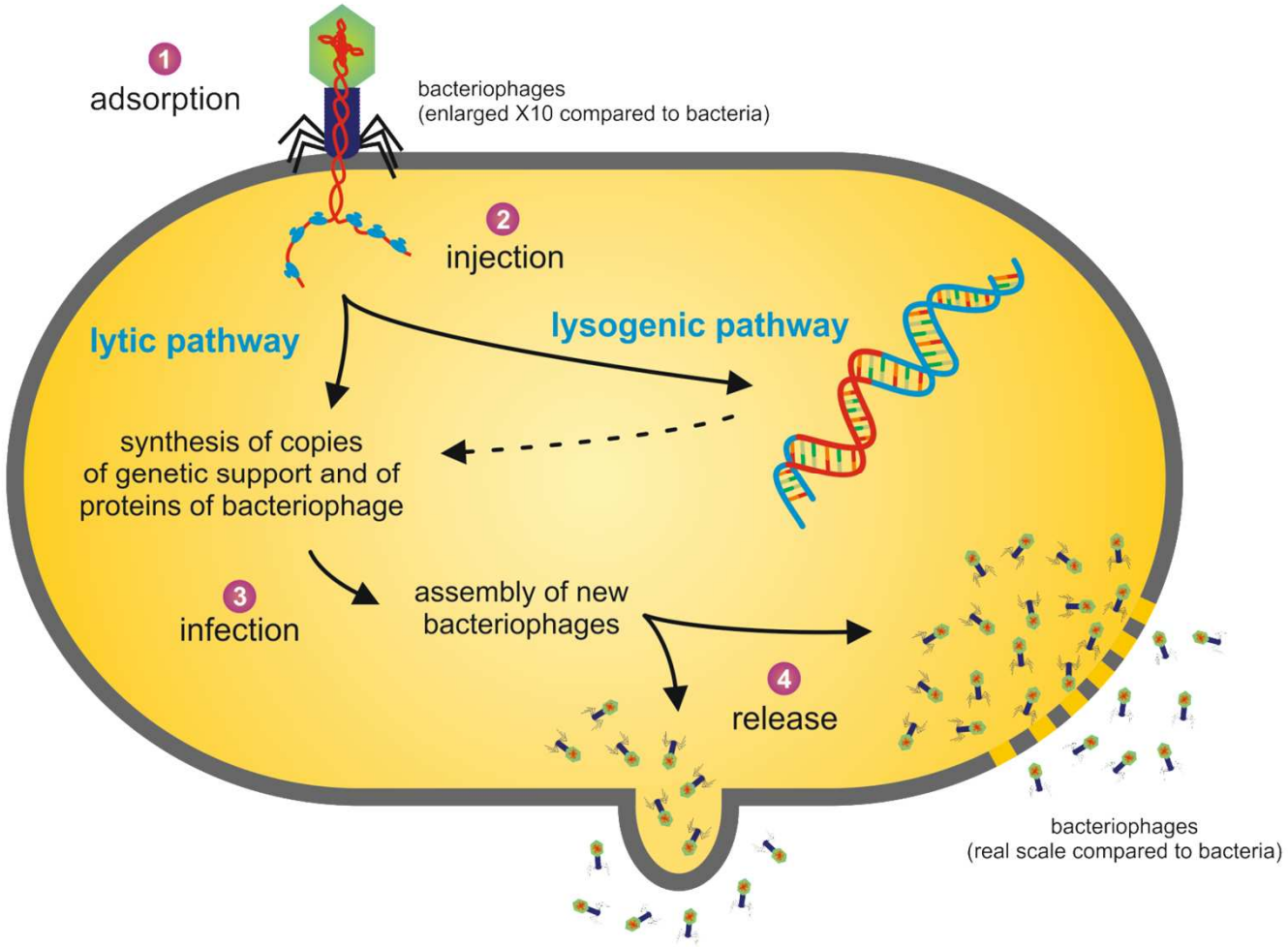
Bacteriophages: virulents vs. temperate



World's population of viruses is estimated to 10^{30} particles

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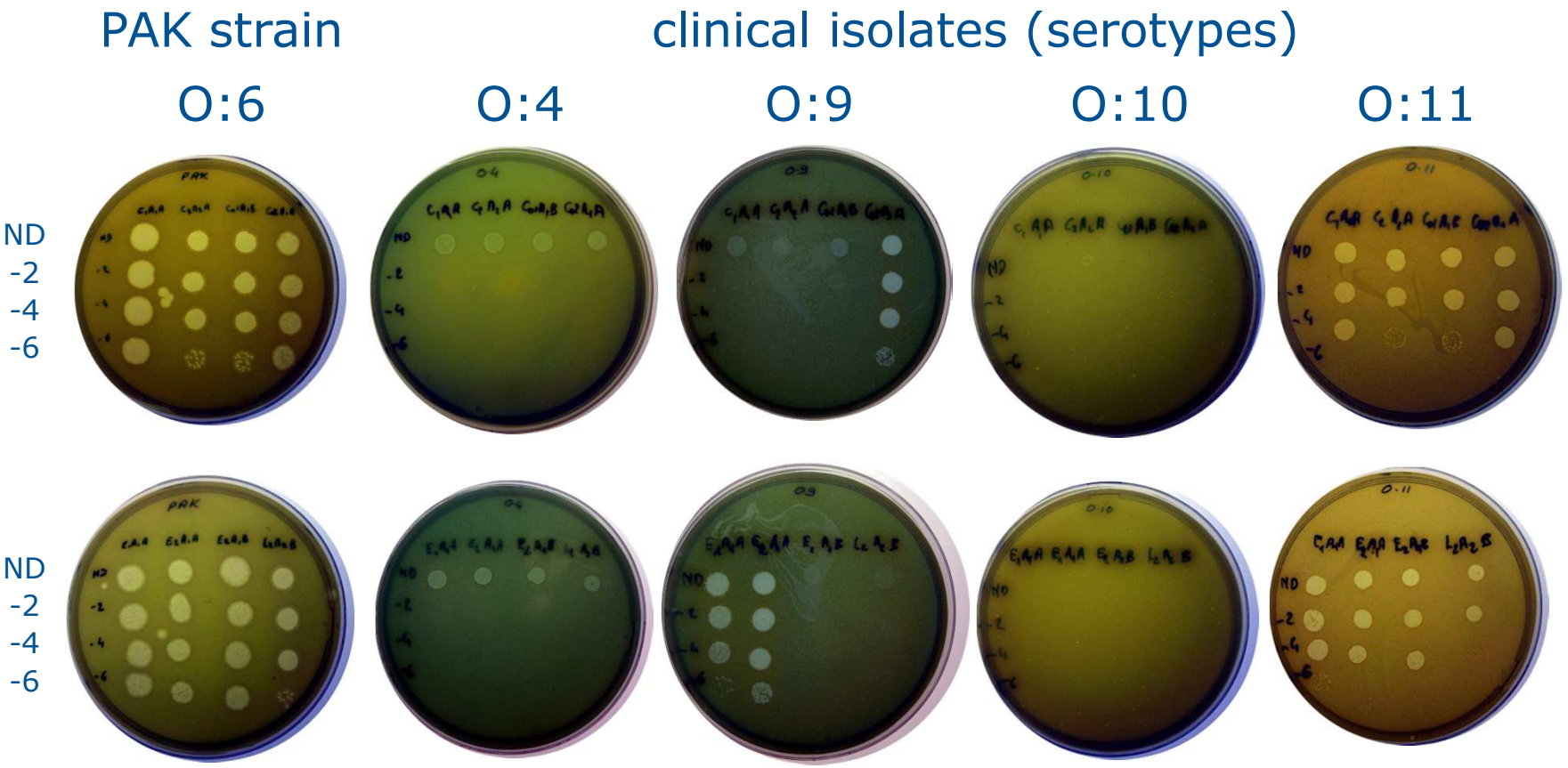
Bacteriophages: host range characterization



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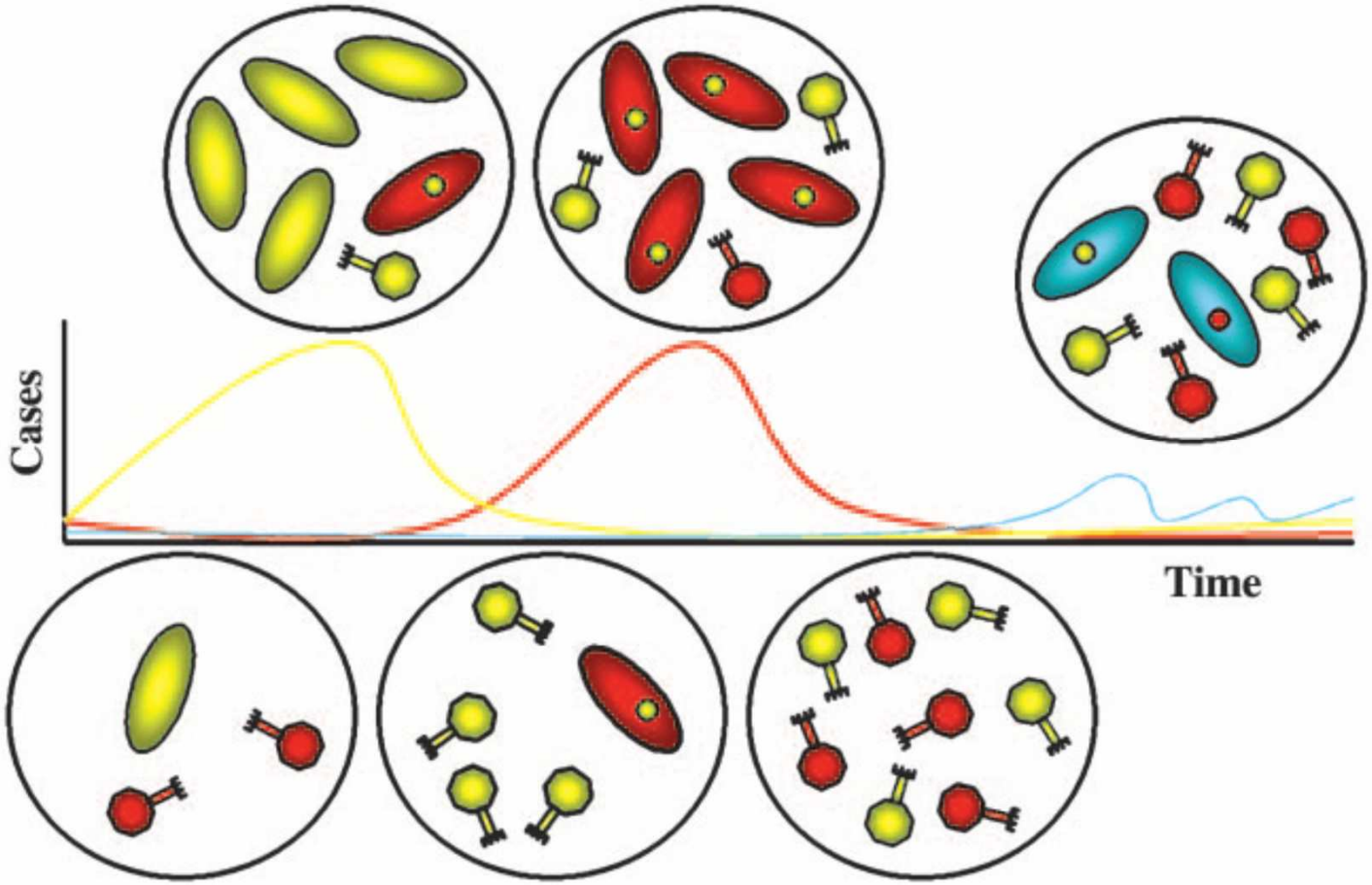
Coevolution bacteriophages/bacteria



Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages
Faruque et al., 2005, PNAS, vol 102, p1702

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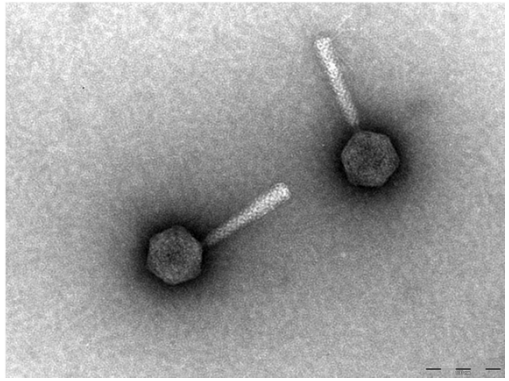
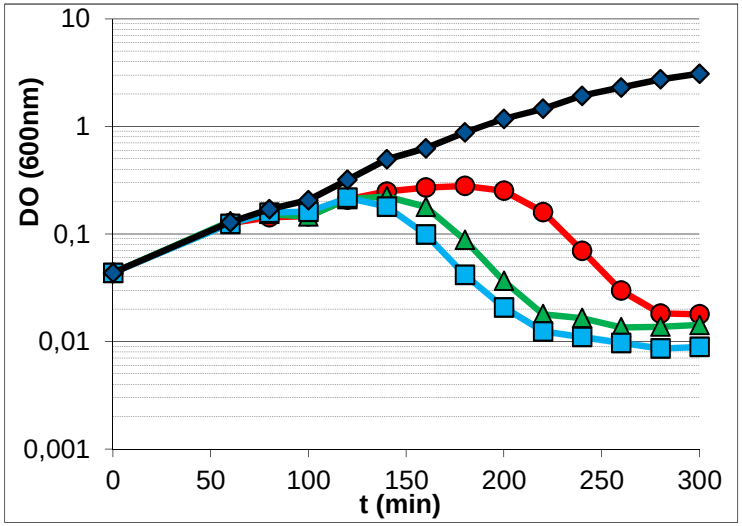
Bacteriophages isolation and characterization

Plaques

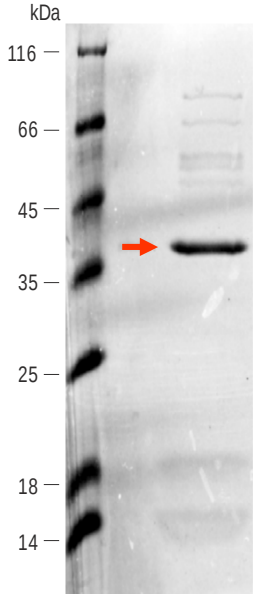
EM



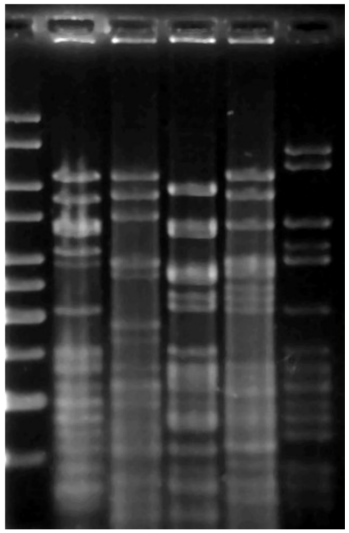
Kinetics parameters



Mass Spec



RFLP



Genome sequencing

↓

Virus classification

↓

Molecular studies

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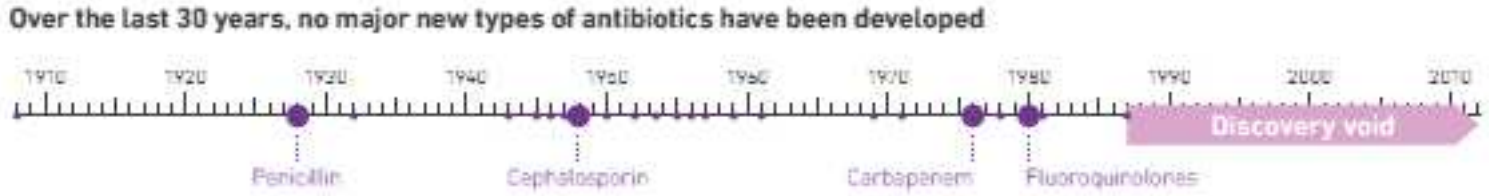
WHO report on antimicrobial resistance (March 30, 2014)

World Health Organization

WHO's first global report on antimicrobial resistance, with a focus on antibiotic resistance, reveals that it is no longer a prediction for the future. Antibiotic resistance - when bacteria change and antibiotics fail - is happening right now, across the world.

Without urgent action we are heading for a post-antibiotic era, in which common infections and minor injuries can once again kill.



The evolving threat of antimicrobial resistance - Options for action
April 2014, 125 pages

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5 JUNE 2014 VOL 150 NATURE

NEWS IN FOCUS

CLIMATE CHANGE Grasslands experiments aim to fix holes in vegetation knowledge **p.16**

REGENERATIVE MEDICINE Salvaged stem-cell trial offers fresh hope for spinal injuries **p.18**

POLICY Constitutional changes free up German science funding **p.19**

ENERGY Race to build an artificial leaf in the lab heats up **p.22**

Bacteriophages could be a resource for fighting drug-resistant bacterial infections.

MICROBIOLOGY

Phage therapy gets revitalized

The rise of antibiotic resistance rekindles interest in a century-old virus treatment.

BY SARA REARDON

For decades, patients behind the Iron Curtain were denied access to some of the best antibiotics developed in the West. To make do, the Soviet Union invested them to treat patients for nearly a century.

Now, faced with the looming spectre of antibiotic resistance, Western researchers and governments are giving phages a serious look. In March, the US National Institute of Allergy and Infectious Diseases listed phage therapy Phagoburn: the first large, multi-centre clinical trial of phage therapy for human infections, funded by the European Commission.

Ryland Young, a virologist at Texas A&M University in College Station, attributes the previous lack of Western interest to clinicians' preference for treating unknown infections with broad-spectrum antibiotics that kill many types of bacterium. Phages, by contrast, kill just one species or strain. But researchers now realize that they need more precise ways to target pathogenic bacteria, says microbiologist Michael Schmidt of the Medical University of South Carolina in Charleston. Along with the rising tide of strains resistant to last-resort antibiotics, there is growing appreciation that wiping out the human body's beneficial microbes along with disease-causing ones can create a niche in which antibiotic-resistant bacteria can thrive. "Antibiotics are a big hammer," Schmidt says. "You want a guided missile."

Finding a phage for a bacterial target is relatively easy, Young says. Nature provides an almost inexhaustible supply: no two identical phages have ever been found. As a bacterium becomes resistant to one phage — by shedding the receptor on the cell surface that the virus uses to enter — the Eliava Institute researchers simply add more phages to the viral cocktails that patients receive. Kutateladze says that they update their products every eight months or so, and do not always know the exact combination of phages that make up the cocktail.

Resch, who is one of the leaders of the Phagoburn study, says that regulatory agencies would need to figure out how to oversee such a rapidly evolving product before the therapy could progress beyond clinical trials. He hopes that phage therapy will be treated in a similar way to the seasonal influenza vaccine, for instance, which is updated every year as new flu strains emerge.

The fact that the European Union (EU) is contributing €3.8 million (US$5.2 million) to the Phagoburn study shows that it is willing to consider the approach, Resch says. Beginning in September, researchers in France, Belgium and the Netherlands plan to recruit 220 burn

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What is Phage Therapy ?

The use of bacteriophages to kill pathogenic bacteria

Date: 1920's

Father: Félix d'Herelle (1873-1949)

Location: Institut Pasteur, Paris, France

Particular signs: first specific antibacterial treatment

1920's-1940's: world expansion (Brazil, Egypt, Georgia,…)

1950's-1990's: world decline (except Eastern Europe)

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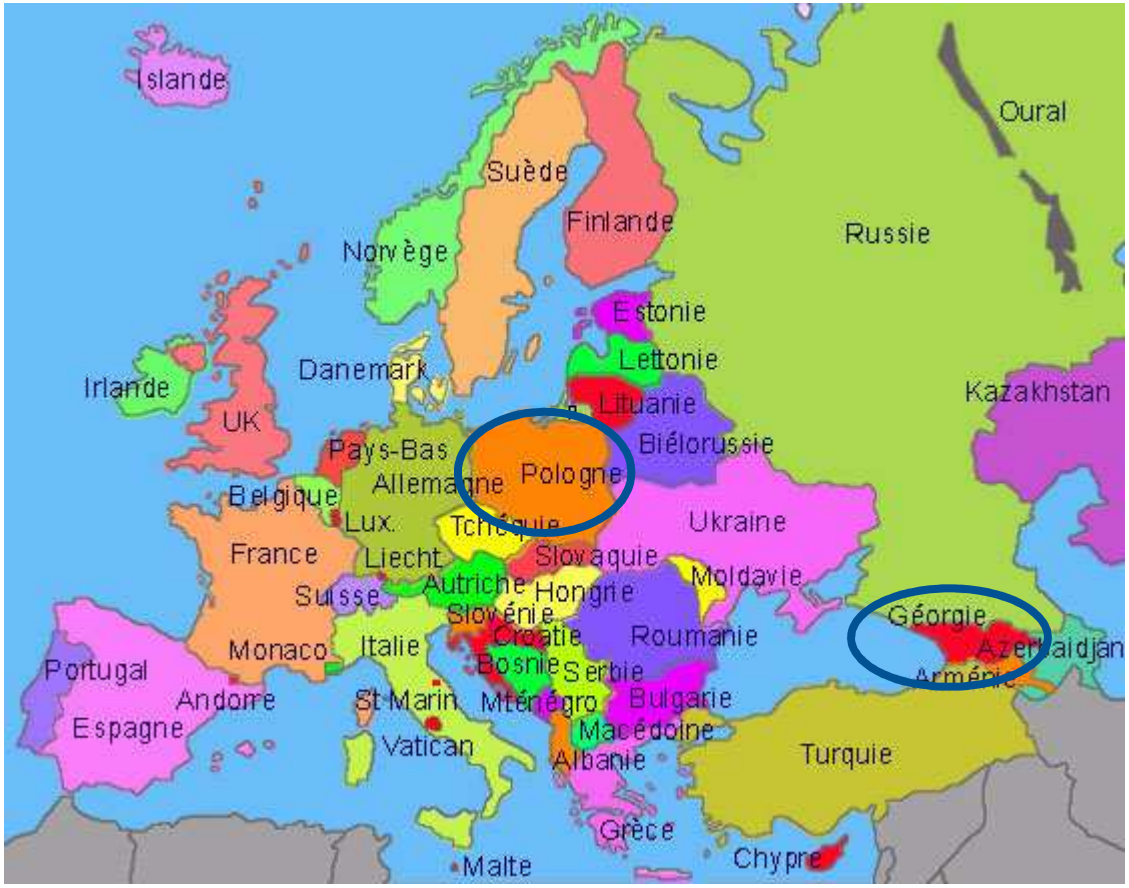
Phage Therapy, the forgotten cure

In Eastern Europe, several countries developed phage therapy



… and today patients are still being treated in Georgia and Poland

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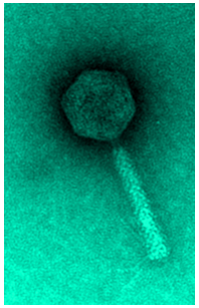
Phage Therapy, how does it work ?

1) Animal models

2) Clinical strains

3) Bacteriophages



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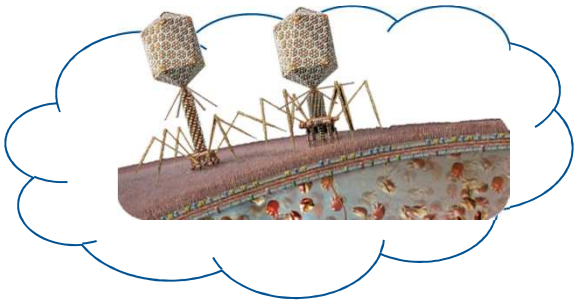

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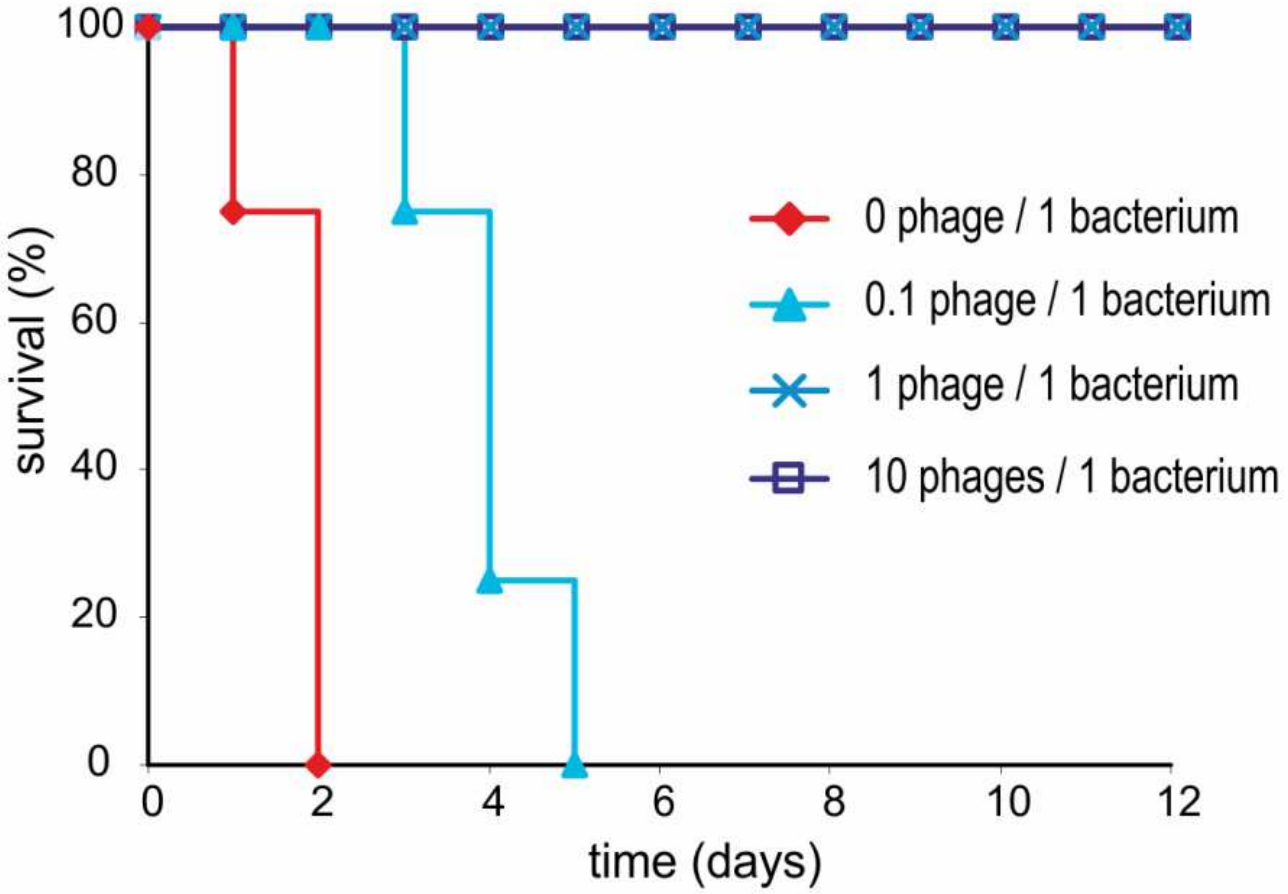
Bacteriophages treatment of lung infection in mice

Infection by $1.0x10^7$ bacteria and 2H later different doses of phages



PAK_P1

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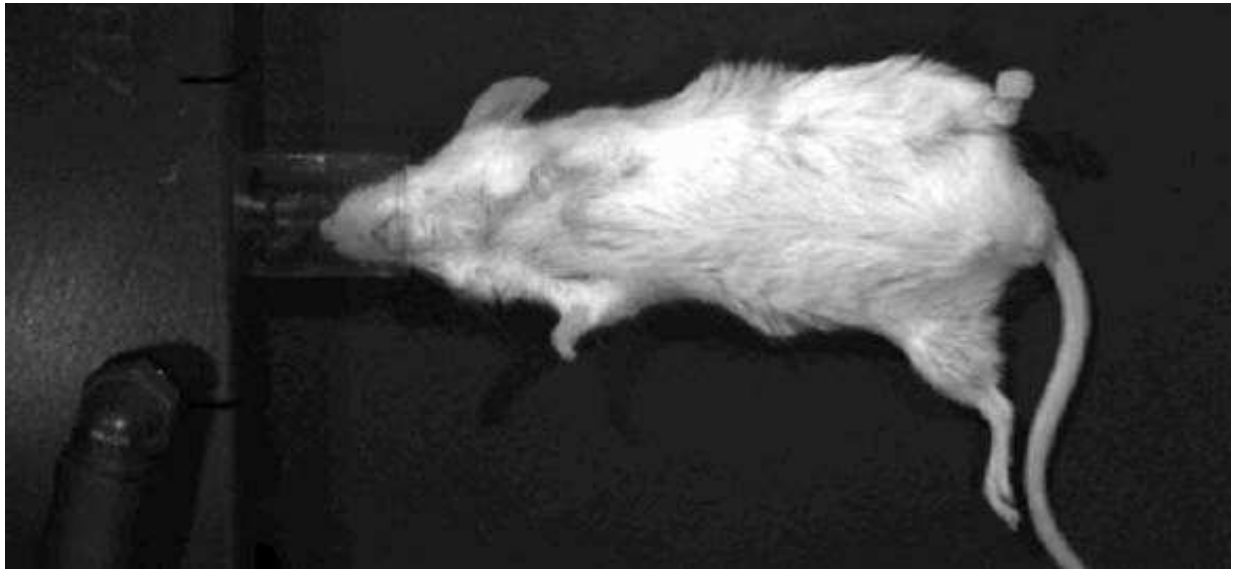
Elightening bacteriophage treatment

Bioluminescent bacteria allow to follow-up the kinetics of infection and treatment.

Advantages of bioluminescence:

- monitoring individual animals (no euthanasia)
- quantification of the infection



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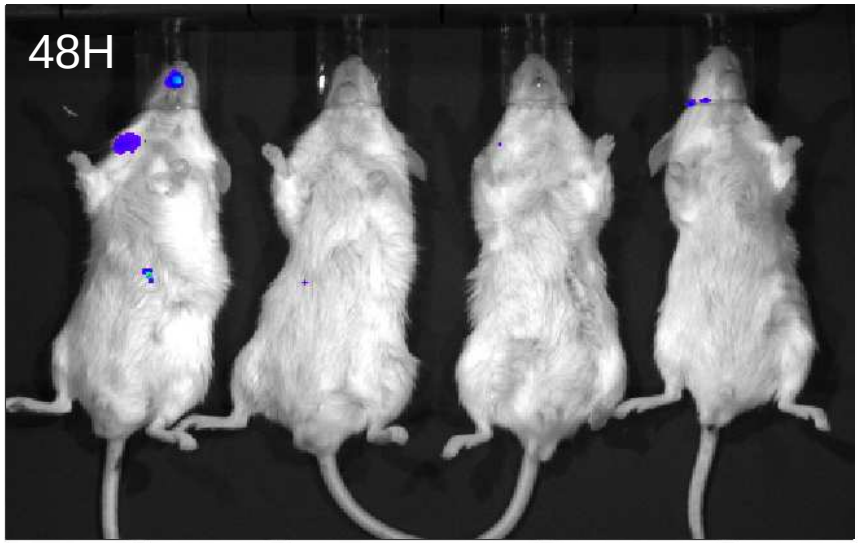
Kinetics of bacteriophage treatment

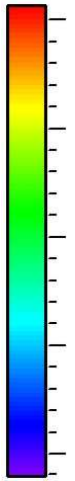
Infection at time 0
by $1.0x10^7$ bacteria

48H

100% death

Infection at time 0
by $1.0x10^7$ bacteria
At time 2H
treatment with
$1.0x10^8$ bacteriophages





Debarbieux, J. Infect. Dis. 2010, 201: 1096-1104

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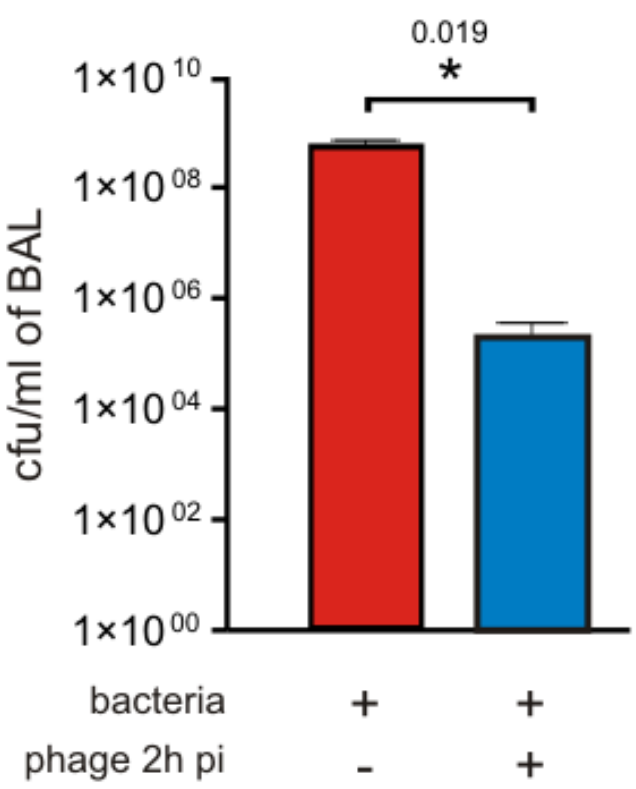
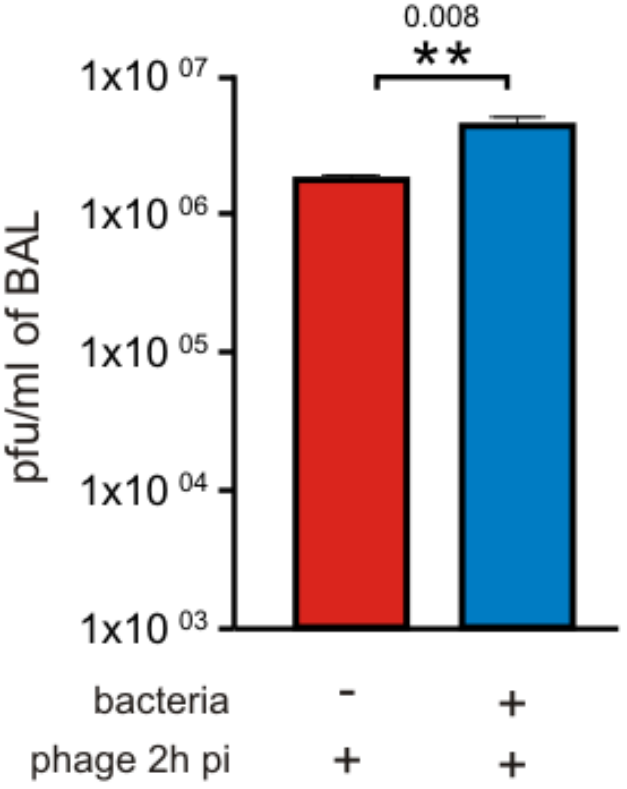
Lungs content 20H post-infection



Bacteria



Bacteriophages

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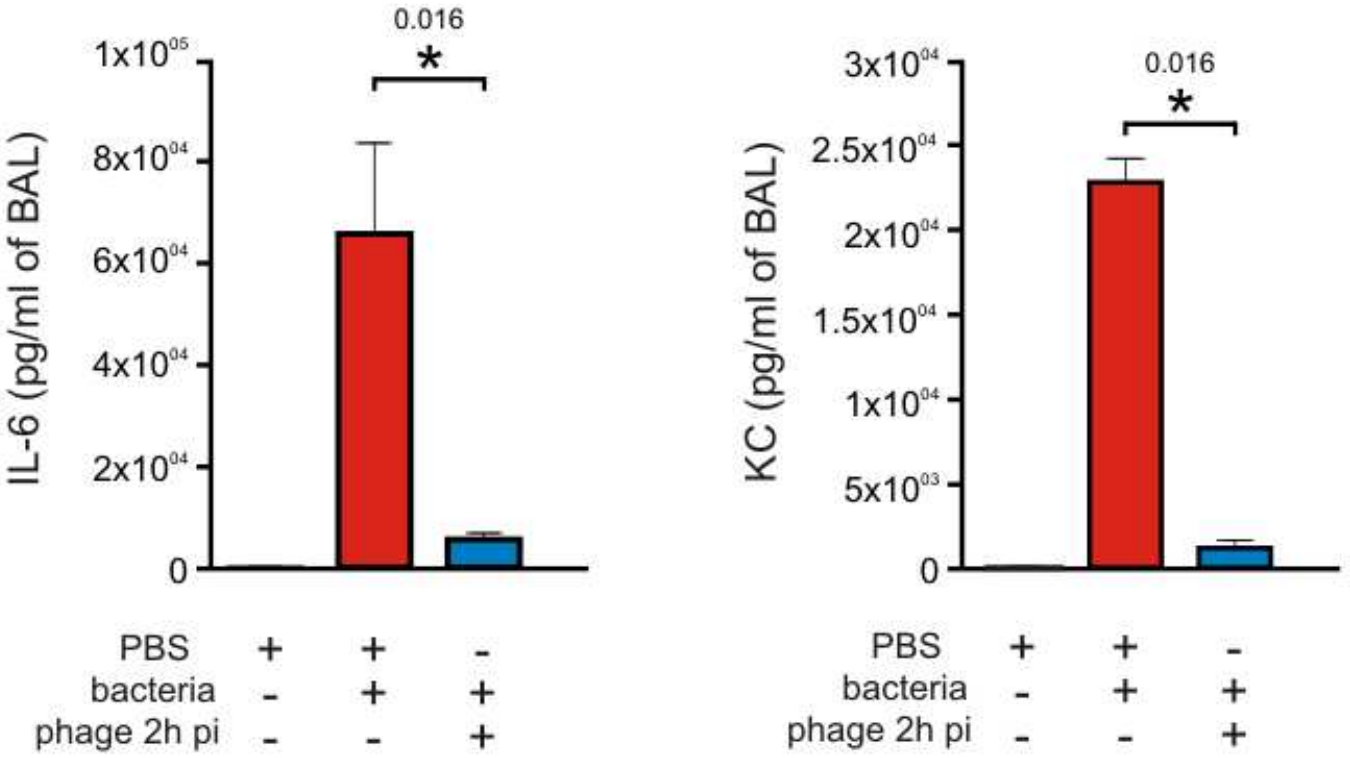
Lungs content 20H post-infection

inflammatory markers



IL-6 **KC**

Bacteriophage treatment reduces inflammatory response

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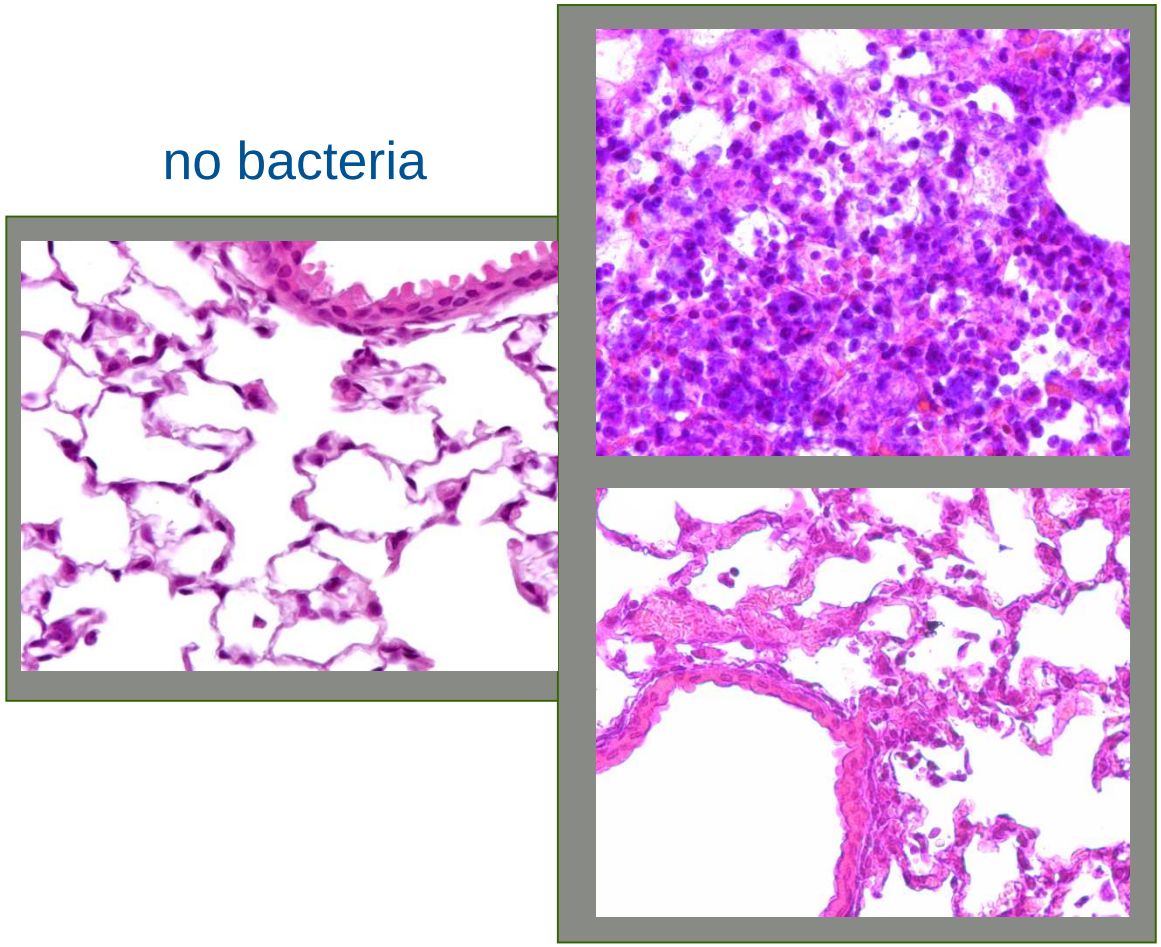
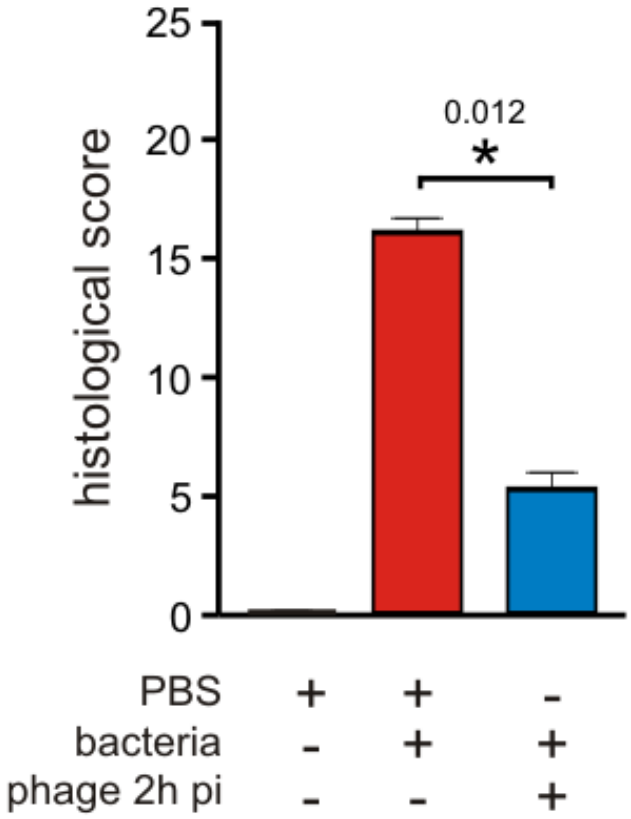
Histology on bacteriophage-treated lungs

bacteria

no bacteria



bacteria + phage



(PMNs, Lymphocytes, Infiltration

Alveolitis, Brochitis, Necrosis)

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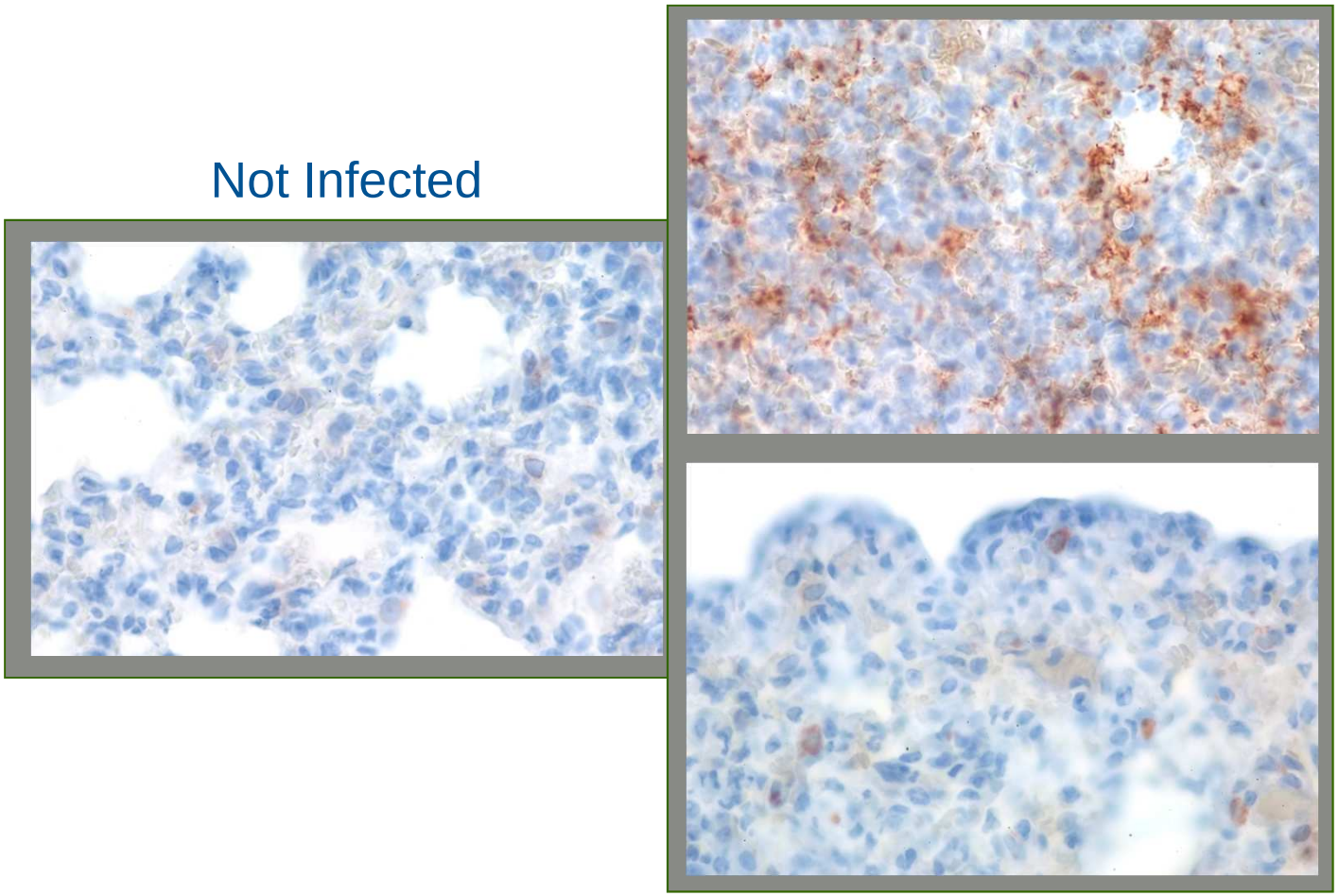
Immuno-histochemistry on bacteriophage-treated lungs

Infected

Not Infected



Phage treated

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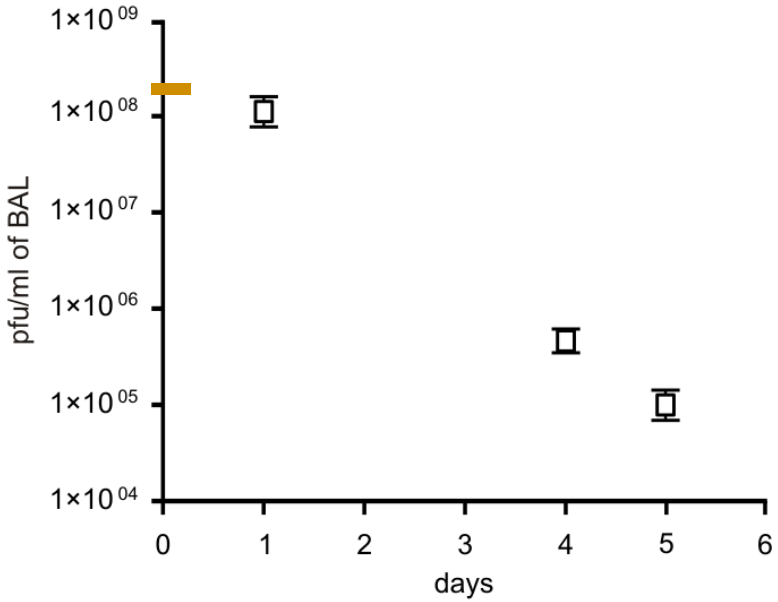
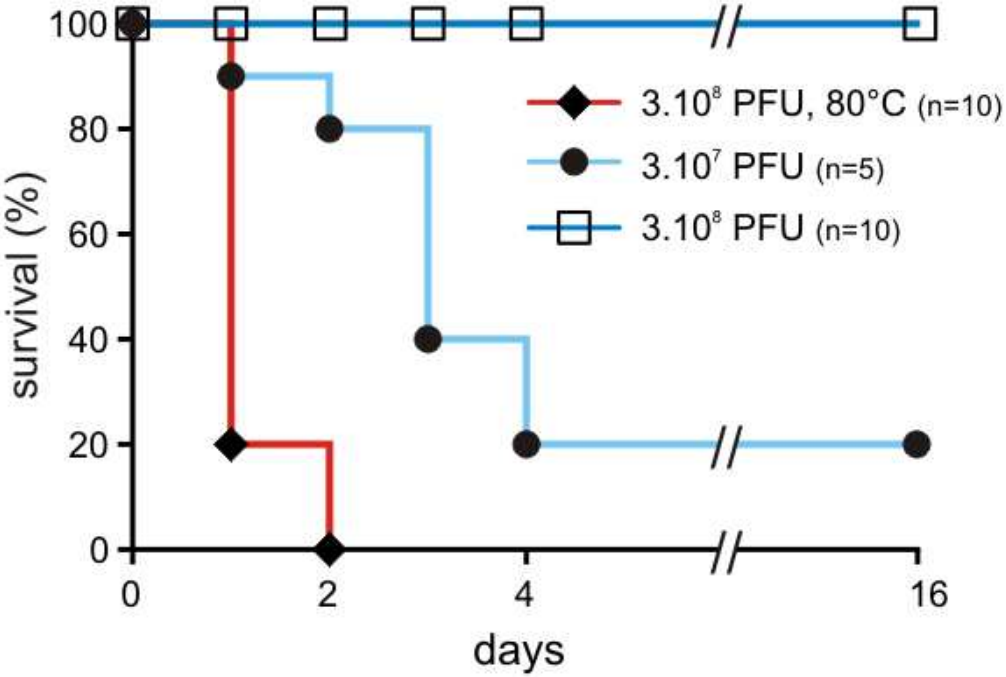
Can bacteriophage prevent lungs infection ?



decay of bacteriophages in the lungs



4 days pre-treatment led to full protection

(bact. loads, histo; immunohisto)

Morello, *PLoS ONE* 2011, 6: e16963

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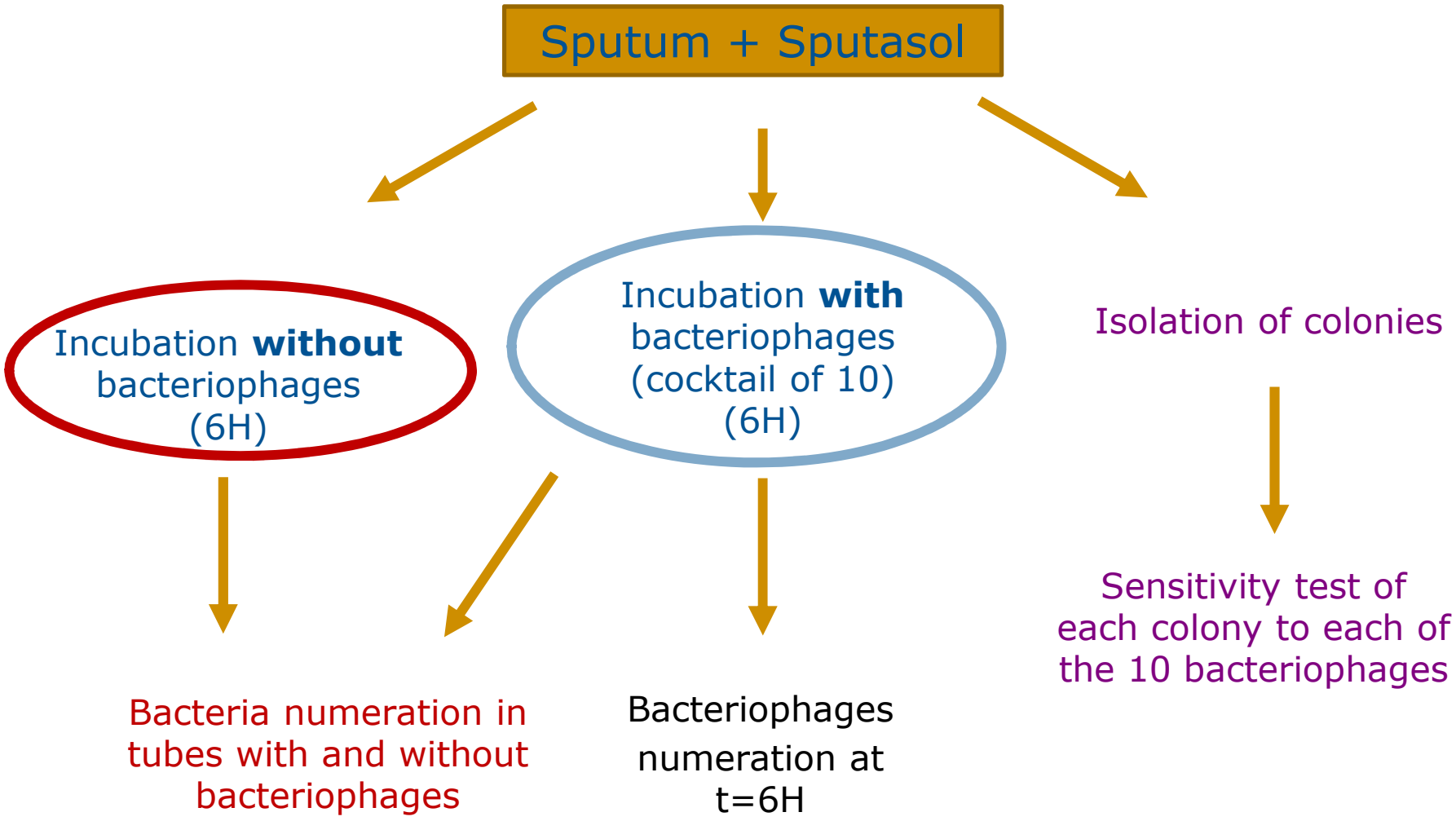
Efficacy of bacteriophages on CF sputum samples



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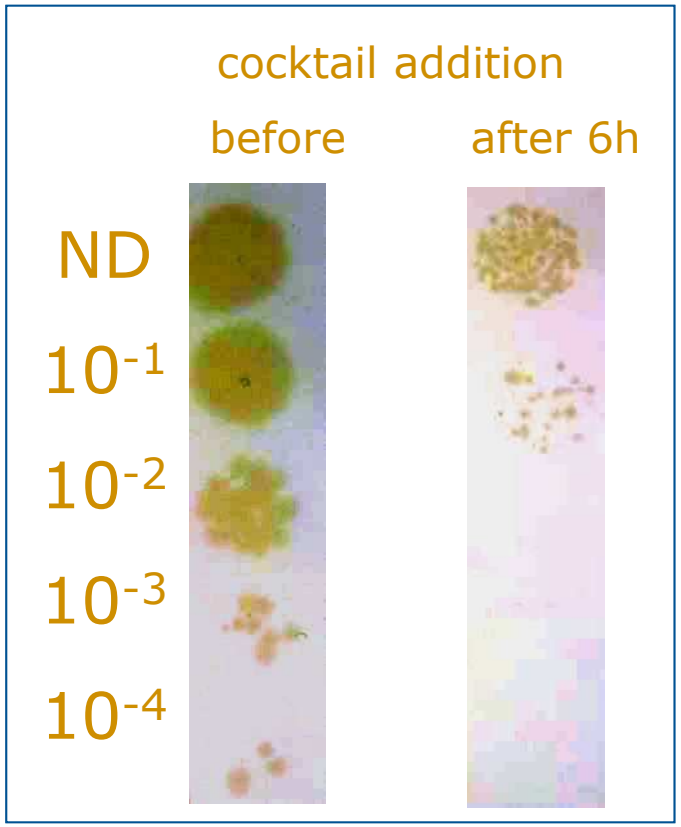
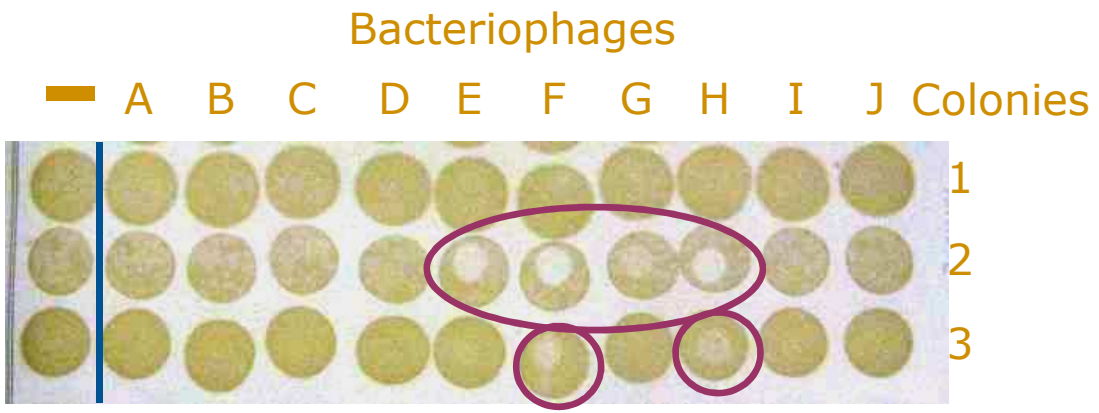
Efficacy of bacteriophages on CF sputum samples

- Bacterial numeration



- Sensitivity tests (>8.000 tests)



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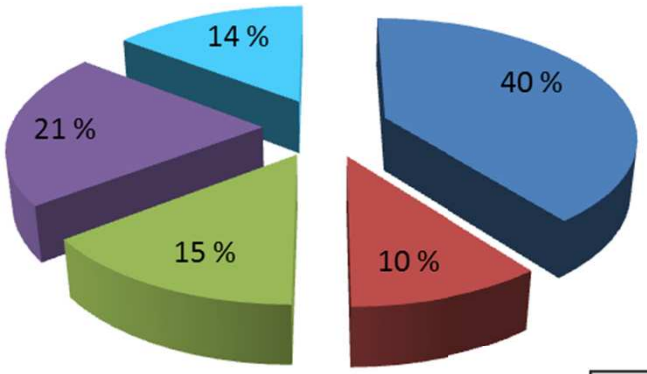
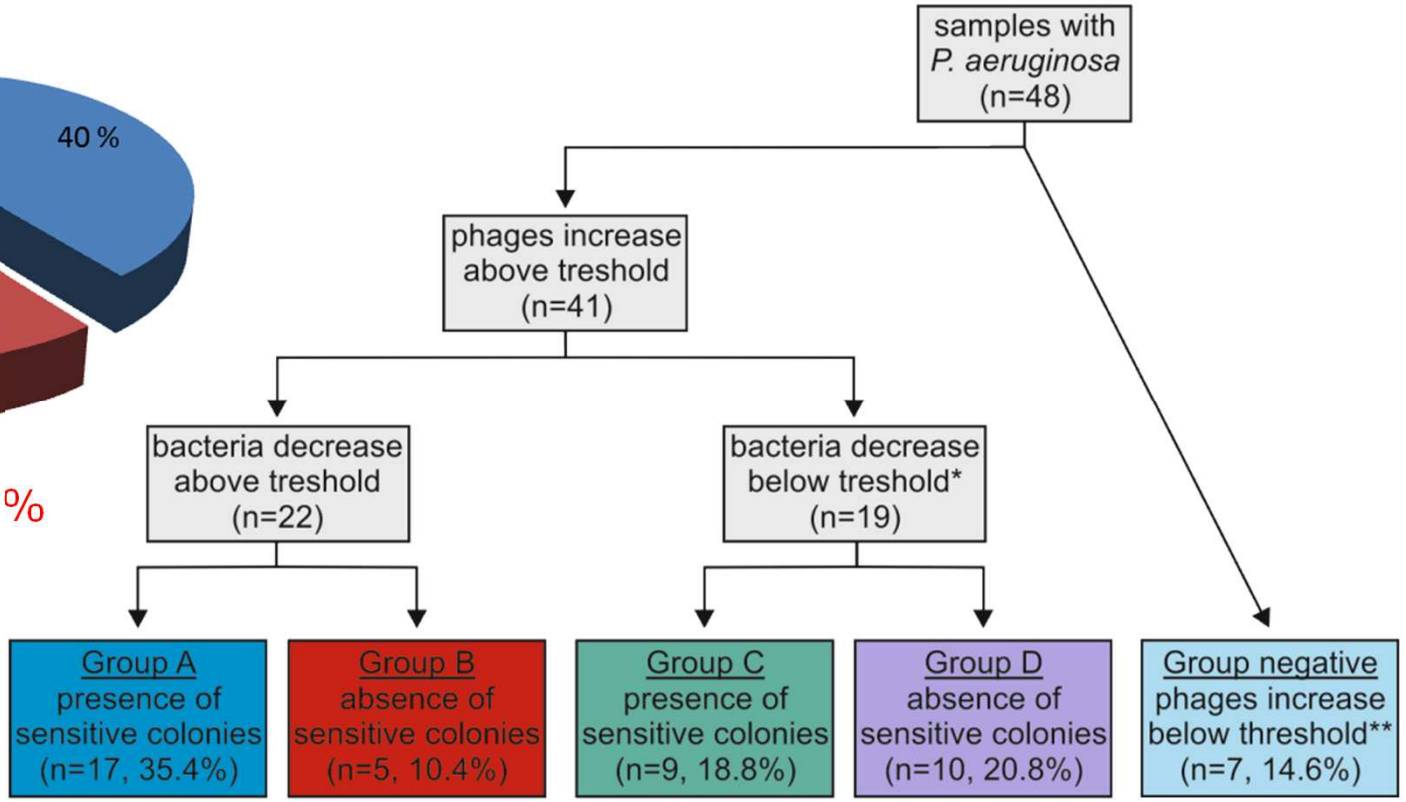
Efficacy of bacteriophages on CF sputum samples

Bacteriophages increase / Bacteria reduction / colonies +/- phages



Groups A+B = 50%



Saussereau, *Clinical Microb. Infect.*, 2014

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Correlation with clinical data ?

Variable	Enrolled	*P. aeruginosa*-positive	p value (groups)
Patients	58	48	NS
Sex (female / male)	26 / 32	23 / 25	NS
CFTR genotype	n (%)	n (%)	
dF508/dF508	26 (45)	23 (48)	NS
dF508/other	20 (34)	16 (33)	NS
other/other	12 (21)	9 (19)	NS
Age, yrs (mean ± SD)	26 ± 10	27 ± 10	NS
Baseline FEV1 (% predicted, mean ± SD)	52 ± 20	52 ± 19	NS
Length of colonization (months)		147 ± 56	NS
Antibiotics treatment (%)	93	94	NS

NS: p>0.05

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Take home message: don't miss this oppotunity !

Do bacteriophages work *in vivo* ?	**YES**
Does CFTR background matter ?	**NO**
Can bacteriophages work in complex media ?	**YES**
Does patient background matter ?	**NO**

What are we waiting for clinical trials ? What about regulations ?

First european clinical trial (www.phagoburn.eu) is ongoing.
More trials to be launched soon (bone infections, pneumonia…)
What about cystic fibrosis ?

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IBBA project: People and Funding

Current Lab members

Anne CHEVALLEREAU

Luisa De SORDI

Nicolas DUFOUR

Dwayne ROACH

Former Lab members

Matthieu GALTIER

Damien MAURA

Eric MORELLO

Emilie SAUSSEREAU

Collaborators:

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Rob Lavigne (K.U. Leuven)

James Di Santo (I. Pasteur)

Spencer Shorte (I. Pasteur)

Isabelle Sermet-Gaudelus (H. Necker)

Jean-Damien Ricard (H. L. Mourier)

Raphaël Chiron (H. Montpellier)

Isabelle Vachier (H. Montpellier)

Stephen Lory (Harvard Medical School)

Guy Schoehn (IBS Grenoble)

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