Development of phage therapy to treat staphylococci bone and joint infections in France: isolation and characterization of seventeen novel anti-*Staphylococcus* bacteriophages

Camille Kolenda, Mathieu Medina, Leslie Blazere, Tiphaine Legendre, Mélanie Bonhomme, Tiphaine Roussel-Gaillard, Patricia Simoes-Martins, Anne Tristan, Tristan Ferry, Frédéric Laurent, PHAGE*in*Lyon consortium

Clinical Microbiologist, PharmD, PhD student,

Lyon University Hospital – Department of Bacteriology
CIRI Lyon – Team « Pathogenesis of Staphylococci Infections »

Programme PHAGE*in*LYON

HCL HOSPICES CIVILS DE LYON — FONDATION HCL HOSPICES CIVILS DE LYON

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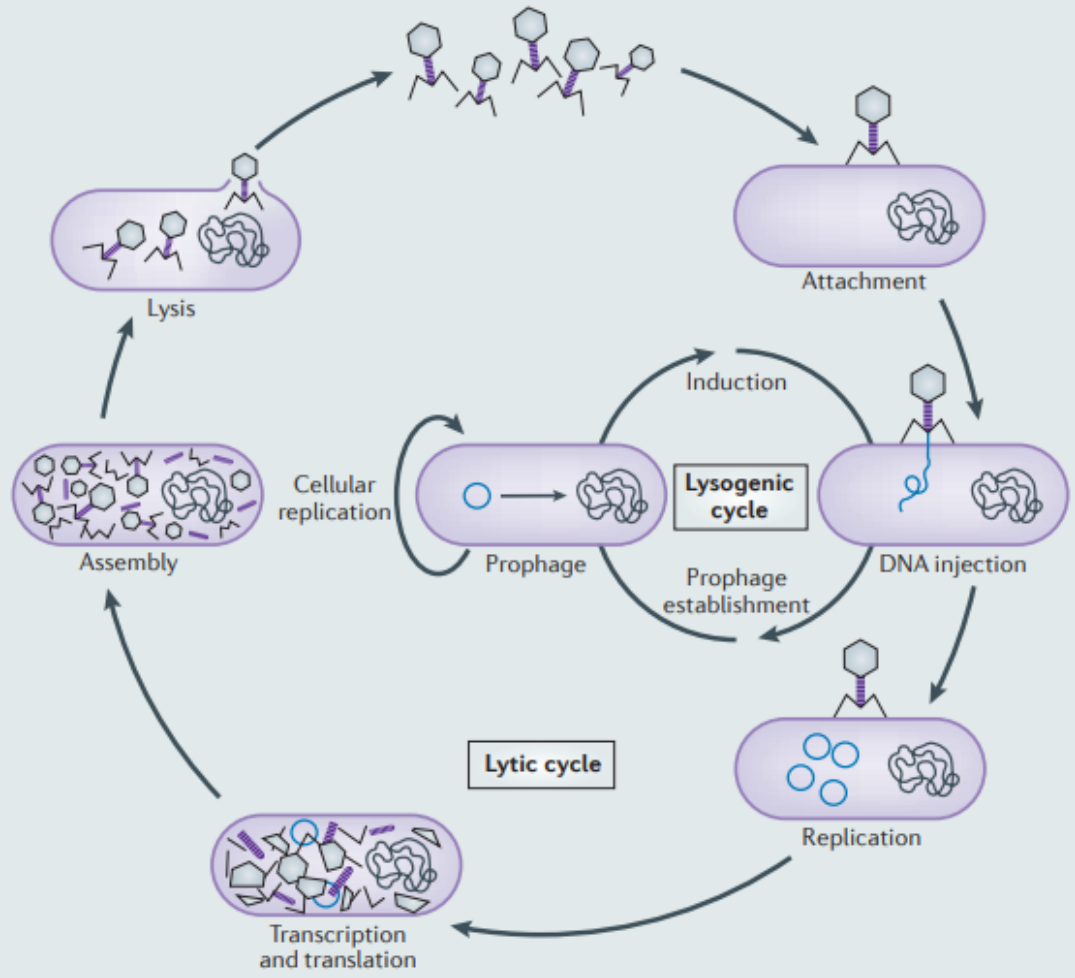
STAPHYLOCOQUES Centre National de Référence

Context

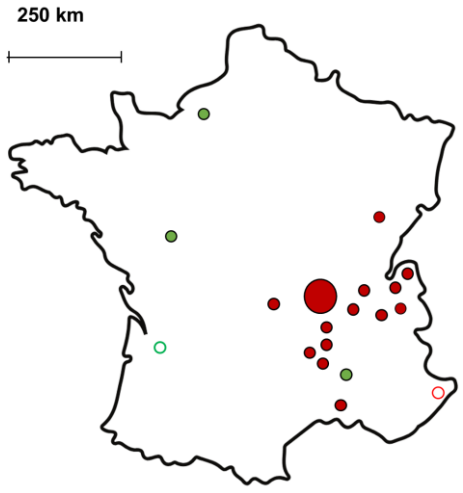
- Bacteriophages = **viruses specific** of bacteria



- Phage therapy : a promising **alternative and adjunctive** therapy to antibiotics:
  - Antimicrobial resistance
  - Difficult to treat/chronic infections:
    - Synergistic effects with antibiotics
    - Antibiofilm effect

Salmond *et al*. Nature 2015

Experience of phage therapy in Lyon, France



T. Ferry *et al*. EBJIS 2021
CMI Oct 2021

- **29 patients** since 2017 including 26 with BJI ● ○
  - Compassionate use
  - Under supervision of French Drug National Agency (ANSM)
- Origin of phages:
  - Pherecydes Pharma (France)
  - Queen Astrid Hospital (Belgium)

PHERECYDES PHARMA

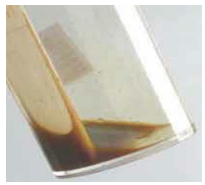
MHKA HMRA

- **Limits of phage therapy development:**
  - Limited number of phages/active against few bacterial species : mainly *S. aureus, P. aeruginosa*
  - Time required to perform the phagogram/obtain phages

➢ **ANSM supports the development of an academic platform for the production and validation of use of therapeutic phages**

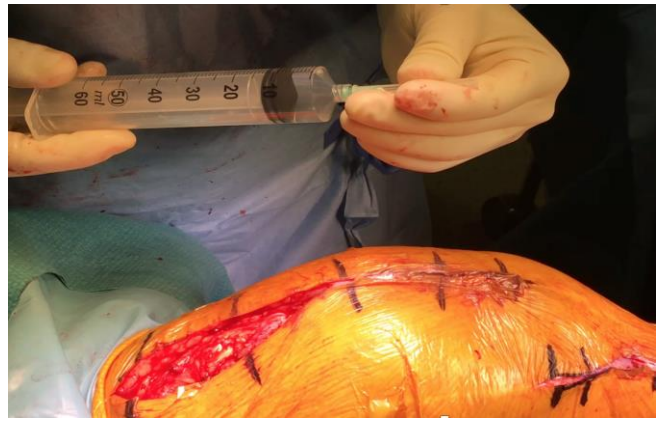
Development of an **academic production of therapeutic phages**




Our challenge





X billions of natural phages

>10^{10} PFU/mL purified phages

Isolate, characterize, produce and purify phages active against various pathogens for **human administration** according to drug agencies' requirements

PHAG-ONE

National research grant : 2.8 M€

Proof of concept: seventeen novel anti *S. aureus* phages

- *Myoviridae* : 14 *Kayvirus*/3 *Silviavirus*
- ***Silviavirus* more active than *Kayvirus* phages against *S. aureus* strains :**
  - Activity against 70 to 90% or 10 to 67% of strains respectively
  - Phage **V1SA20 with the widest activity spectrum**
- **Phages more active against MSSA than MRSA strains :**
  - Median activity against 76% *vs* 38% of MSSA and MRSA strains respectively

	Kayvirus														*Silviavirus*		
Phage	V1SA1	V1SA5	V1SA6	V1SA7	V1SA8	V1SA9	V1SA10	V1SA11	V1SA12	V1SA13	V1SA14	V1SA15	V1SA16	V1SA18	V1SA19	V1SA20	V1SA22
MSSA	82	41	76	82	71	76	71	76	88	18	76	12	47	65	82	94	82
MRSA	38	15	23	46	54	46	46	38	38	0	31	15	15	38	54	85	62
Total	**63**	**30**	**53**	**67**	**63**	**63**	**60**	**60**	**67**	**10**	**57**	**13**	**33**	**53**	**70**	**90**	**73**

% of activity of phages against a panel of S. aureus strains representative of clinical and genetic diversity

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Activity against coagulase negative staphylococci (CNS)

- ***Kayvirus*** **more active than** ***Silviavirus*** **phages against CNS strains**
  - Mainly *S. capititis, S. lugdunensis*
  - Low activity against *S. epidermidis*

Phage/ Species	n	*Kayvirus*														*Silviavirus*		
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S. epidermidis	10	0	0	0	1	2	4	0	1	0	0	0	0	0	0	1	1	1
S. capitis	5	4	3	3	4	4	4	4	4	4	3	3	4	3	3	0	0	0
S. lugdunensis	5	2	2	4	4	3	4	3	4	2	1	2	4	4	3	2	1	1
S. pseudintermedius	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
S. caprae	3	2	0	2	2	2	2	1	1	1	1	2	0	2	2	0	0	0
S. haemolyticus	3	1	0	0	1	1	1	0	0	0	0	0	1	0	0	1	0	0
S. warneri	3	1	0	0	1	0	0	1	0	0	1	0	2	2	0	0	0	0
Total	**33**	**10**	**5**	**9**	**13**	**12**	**15**	**9**	**10**	**7**	**6**	**7**	**11**	**11**	**8**	**5**	**2**	**2**

Activity of phages against a panel of CNS strains causing BJI

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Activity of phages against a panel of CNS strains causing BJI

Phage training

- **Natural coevolution** of phages/bacteria with **mutual adaptations** in environment
- Phage training : **force phage evolution** to increase activity/enlarge activity spectrum

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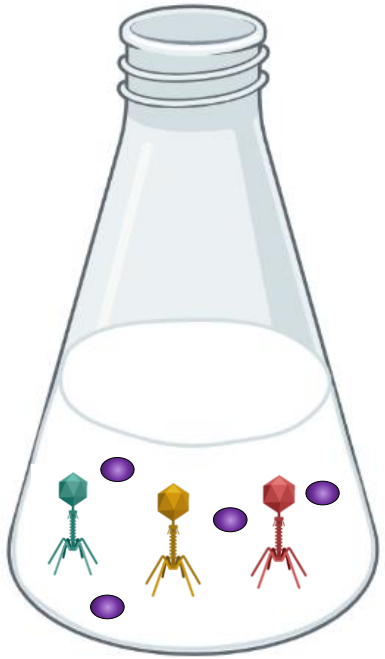
Ancestral phages +
Bacteria



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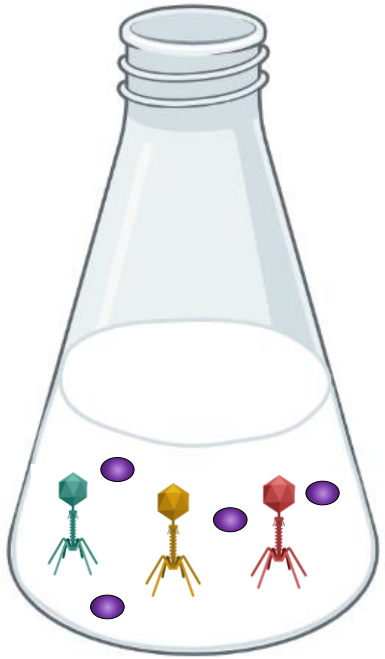
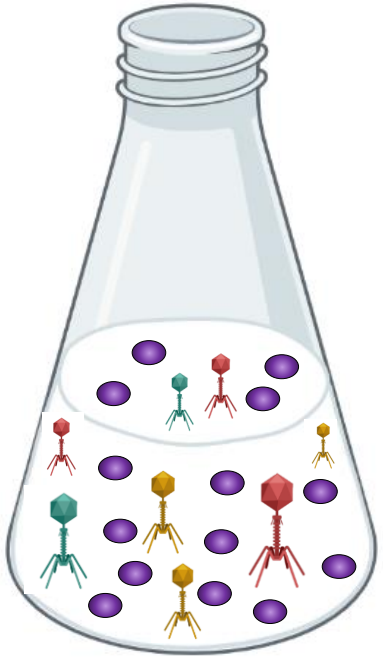


24h, 37°C

Co-multiplication of phages
and bacteria
Passage 1



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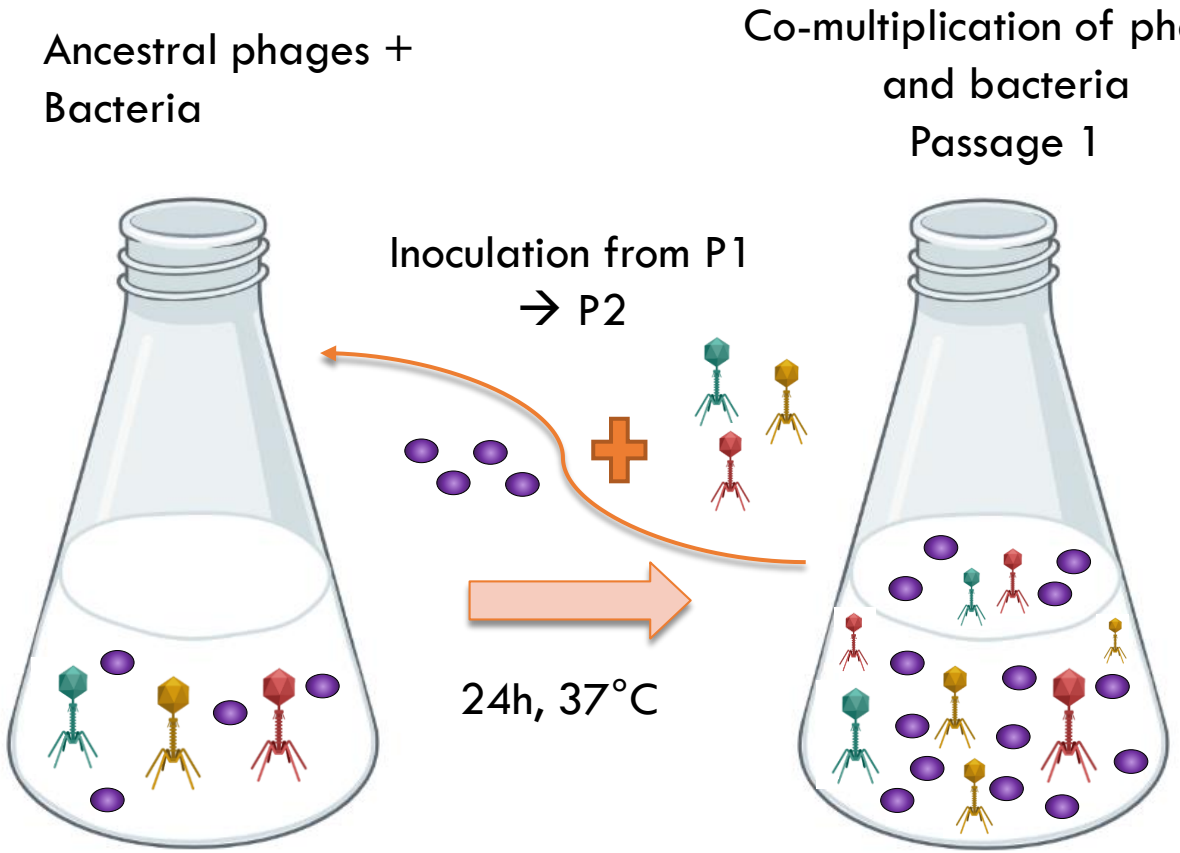

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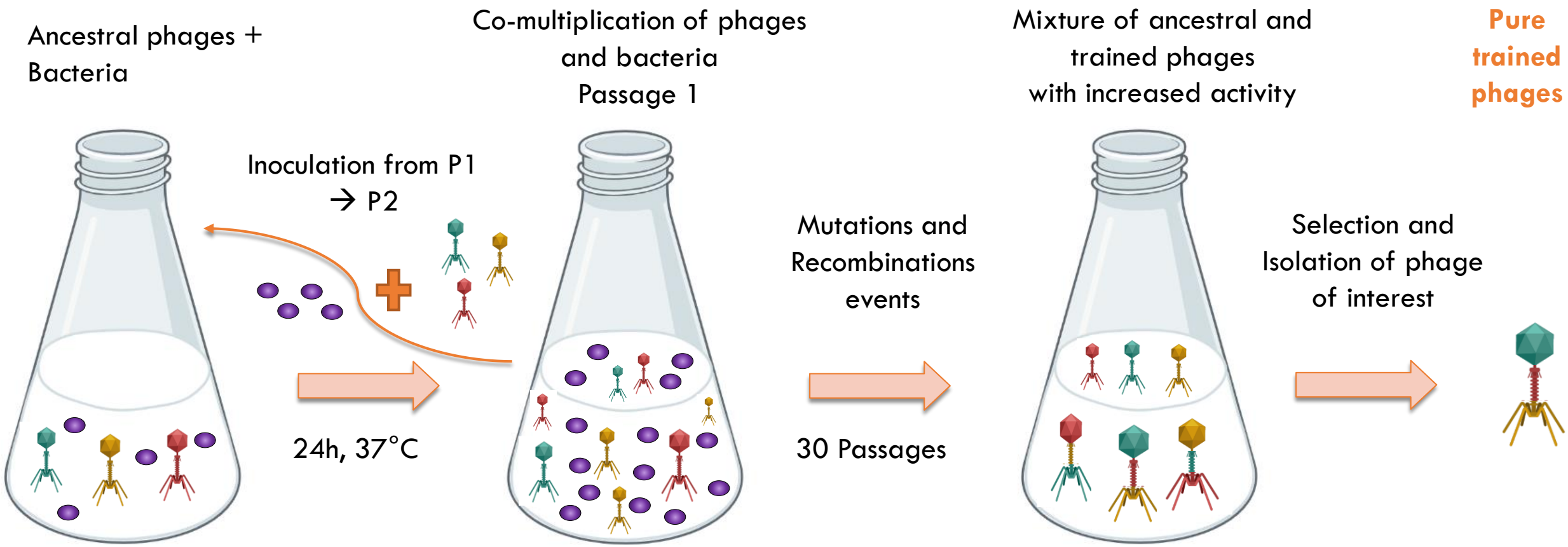


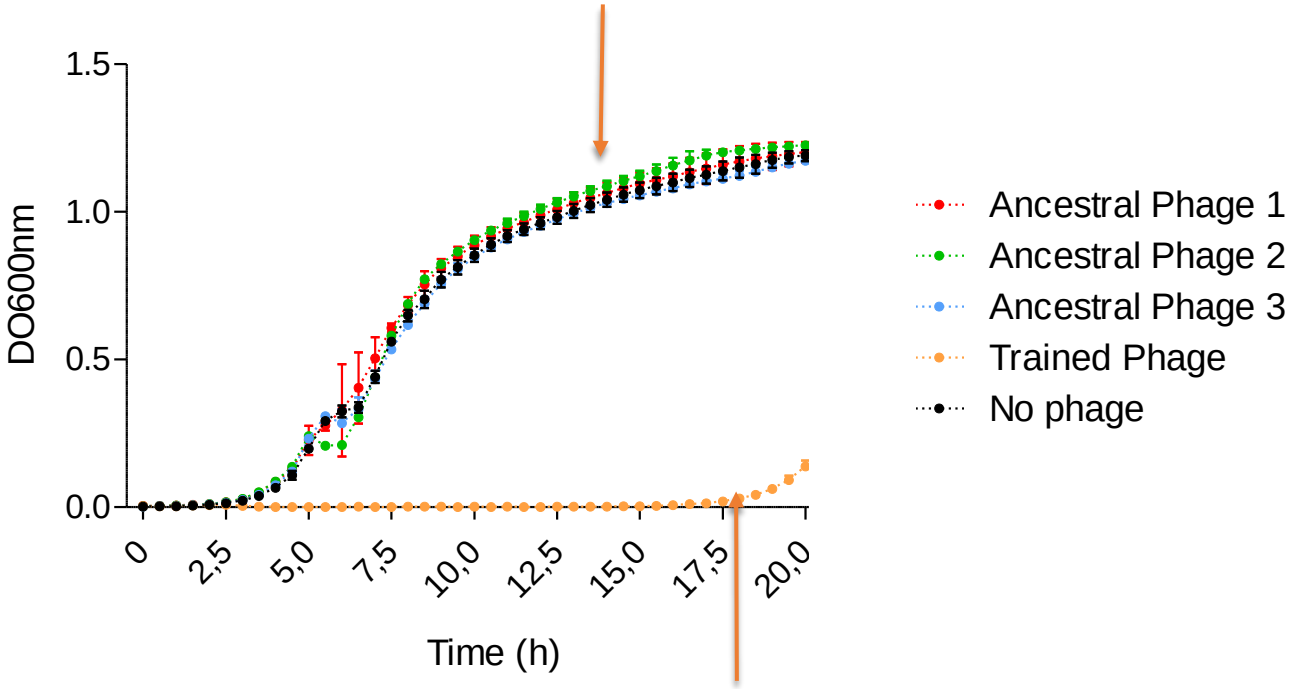
Phage training – *Staphylococcus epidermidis*

- 7 *S.epidermidis* strains of different sequence types responsible for BJI

- **Activity increase against 2/7 strains**
  - Including one strain belonging to **ST2**

  = major antibiotic resistant *S. epidermidis* clone causing BJI
  - Obtained trained phage was active against **60% of a collection of ST2 strains** (n=30) *versus* no activity of ancestral phages

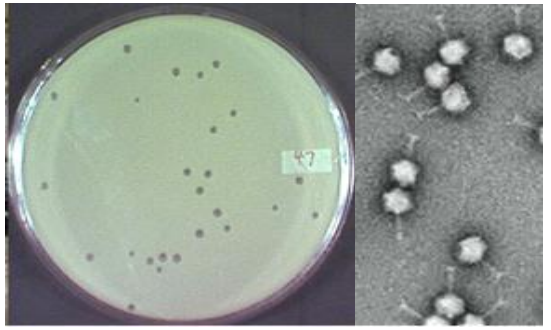
No inhibitition of bacterial growth by ancestral phages



Inhibitition of bacterial growth by trained phages

Conclusion

- Description of a **large collection of anti-staphylococci phages** with **complementary activities** against *S. aureus* and CNS
  - Activity against 90% of *S. aureus* strains
  - Activity against CNS species depended on bacterial species
  - Phage training to increase activity against *S. epidermidis* strains



- **On-going and future work …**
  - Isolation of other phages (*S. epidermidis*, *E. coli*) and pursuit of phage training
  - Set up of protocols for pharmaceutical production and purification of these phages according to ANSM requirements
  - Clinical cohorts of compassionate use and clinical trials

Acknowledgements - PHAGE*in*LYON team

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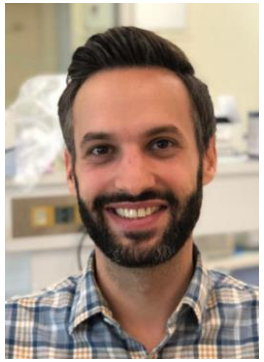
Pr. Fabrice PIROT

Dr. Camille MERIENNE

Microbiology lab

Pr. Frédéric LAURENT



Mathieu MEDINA

Dr. Floriane LAUMAY

Leslie BLAZERE

Tiphaine LEGENDRE

Emilie HELLUIN

Mélanie BONHOMME

Phage training – *Staphylococcus epidermidis*

- 8 strains of different ST responsible of BJI
- Activity increase against 2/8 strains
  - Including one strain belonging to **ST2**

  = major antibiotic resistant *S. epidermidis* clone causing BJI
  - Increased activity of obtained trained phage against **60% of a collection of ST2 strains**