PHAGES AS FRIENDS AND ENEMIES IN FOODS

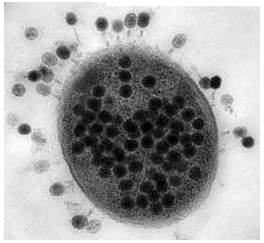
Prof. Sylvain Moineau OC, OQ, PhD, FRSC
Canada Research Chair in Bacteriophages
Dept. Biochemistry, Microbiology, and Bioinformatics
Faculty of Sciences & Engineering

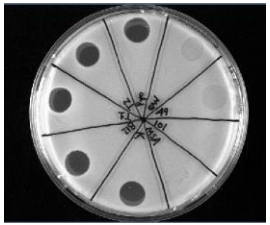
25 - 28 MAY 2025
BRUSSELS PALACE OF THE ACADEMIES
ONE HEALTH IN FOCUS
AS2AMR Advancing Solutions to Antimicrobial Resistance CONFERENCE
2nd Bacteriocin International Conference BIC2025

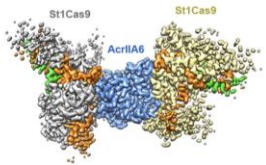
May 25th, 2025

@smoineau

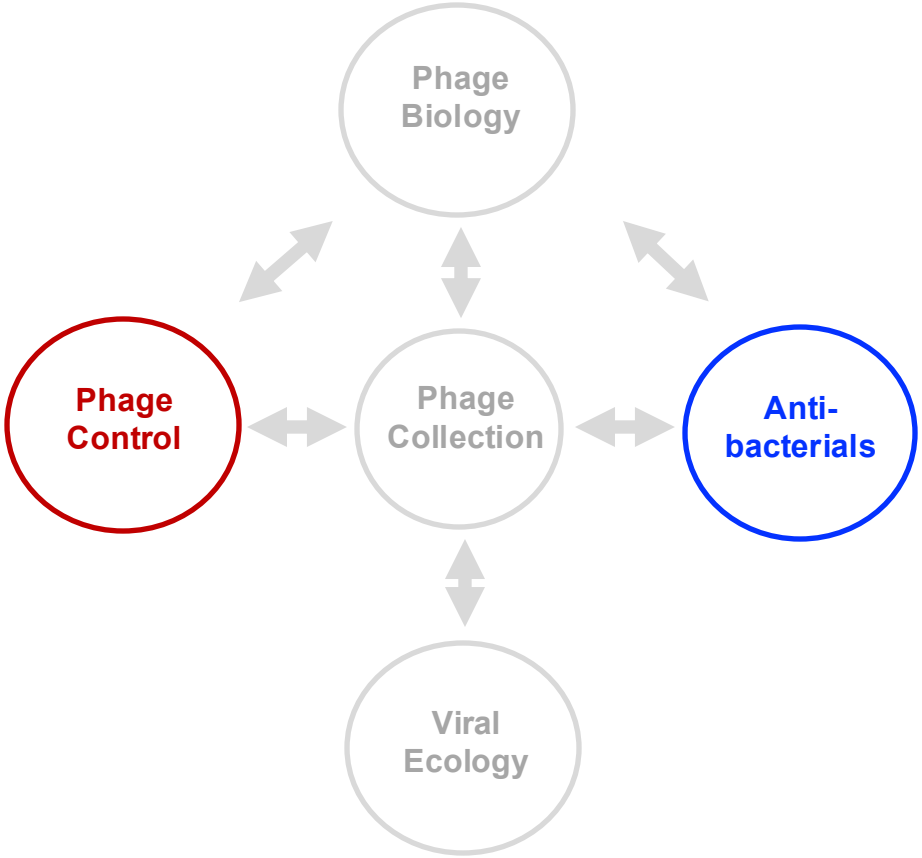
UNIVERSITÉ LAVAL

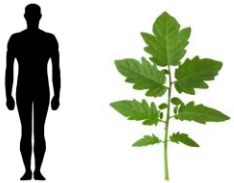






www.moineau.bcm.ulaval.ca
www.phage.ulaval.ca



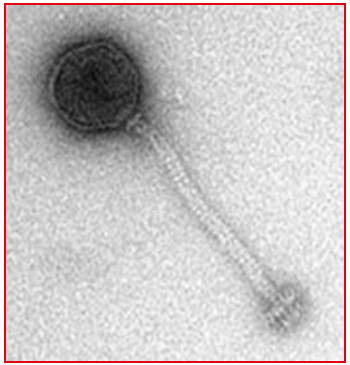


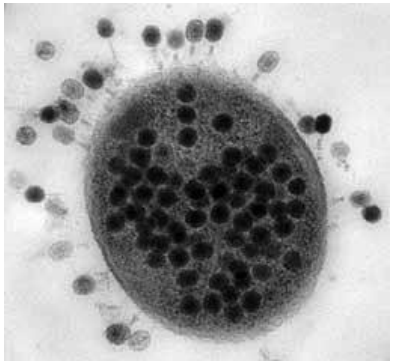
Plan

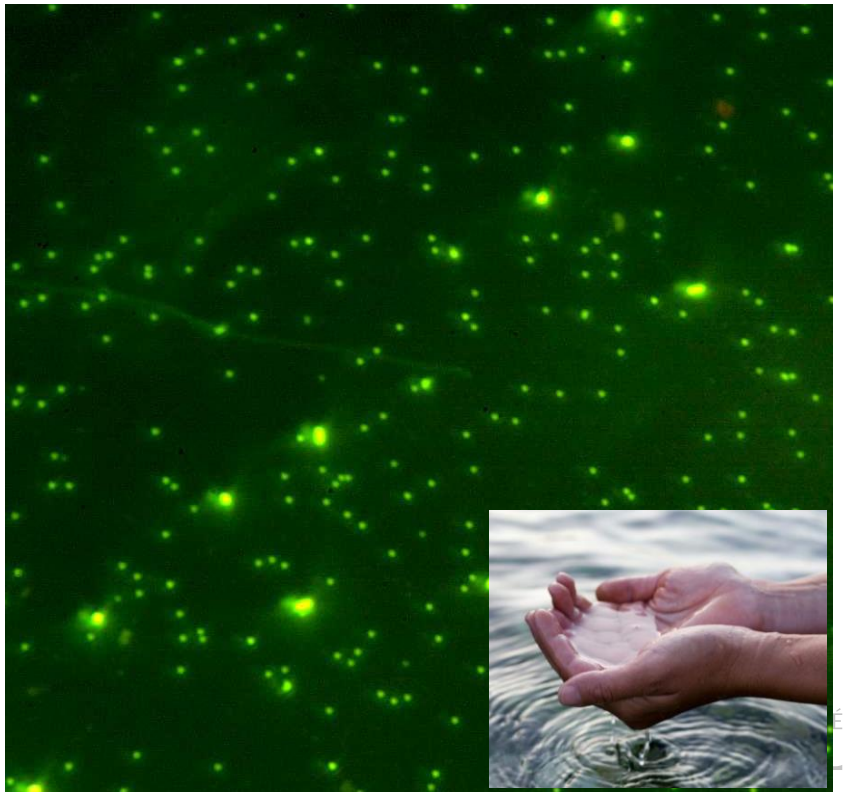
- **Bacteriophages**
  - ***Enemies***
    - **Control phages**
  - ***Friends***
    - **Phages as biocontrol agents**
- **Conclusions & perspectives**

Bacteriophages, bacterial viruses

- Most abundant biological entities
- Highly diverse
- Found with bacteria

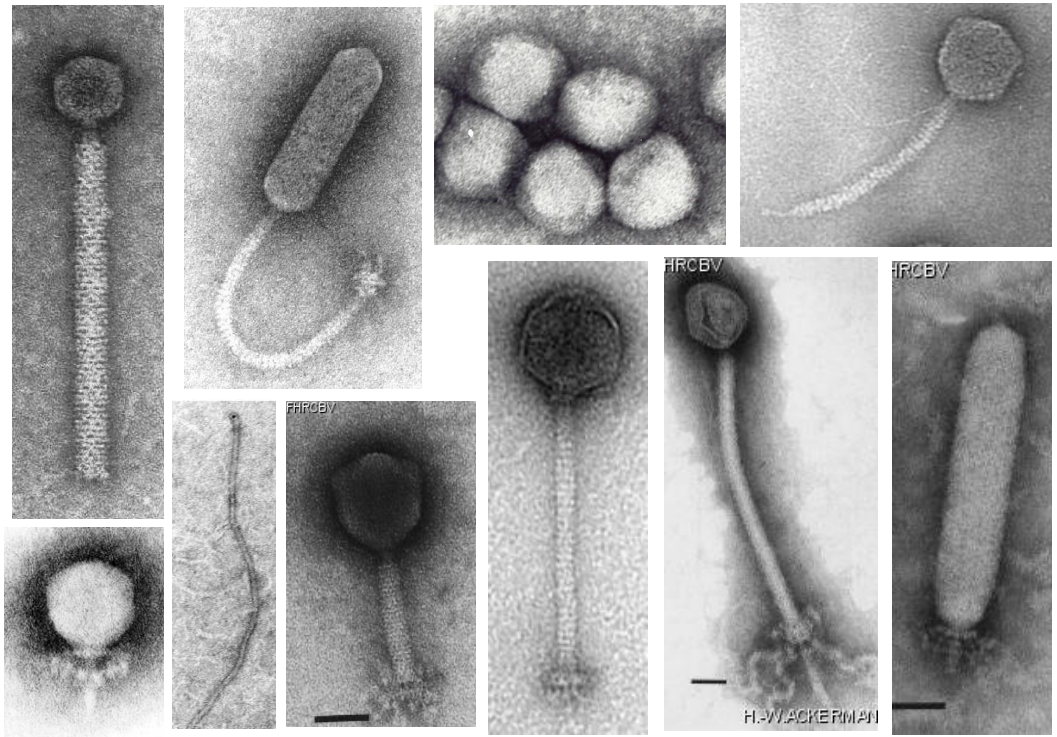


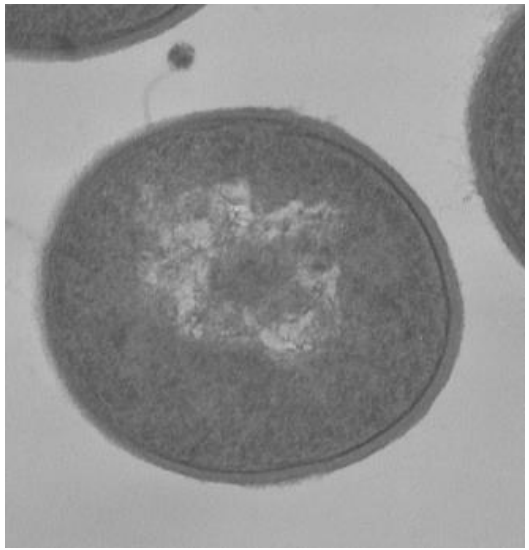




A. Culley

Diversity

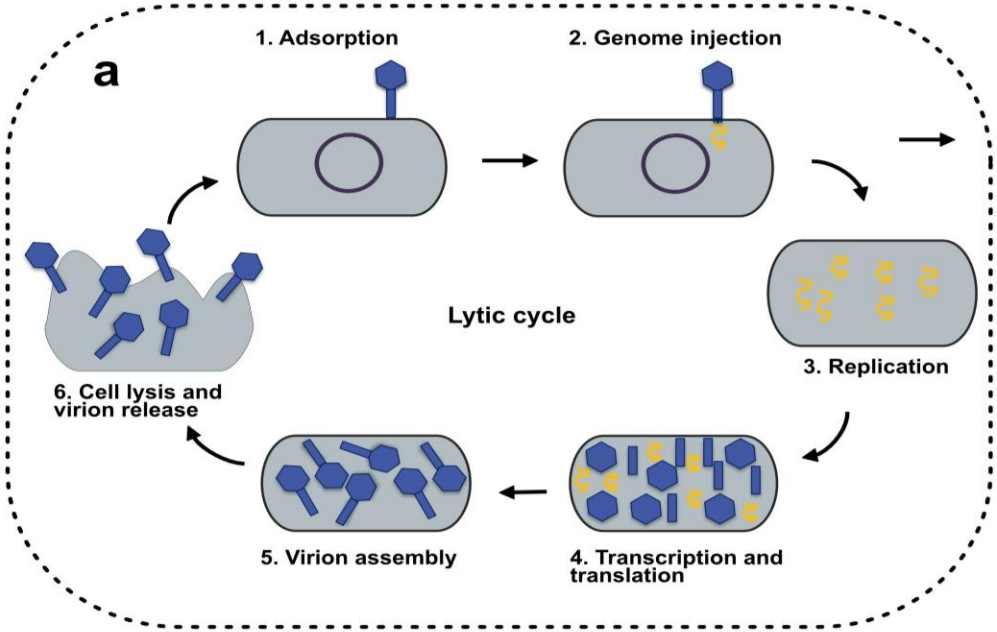




www.phage.ulaval.ca

UNIVERSITÉ LAVAL

Lytic Phages (dsDNA)

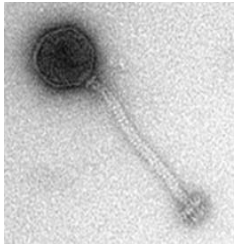


- Regulated
- Coordinated
- Cycle: < 1 hour
- > 100 new phages/cell.

Bact: 1 → 2 → 4 → 8

Phage: 1 → 100 → 10^4 → 10^6

UNIVERSITÉ LAVAL



Roles of lytic phages

Enemies

- **Block or slowdown bacterial fermentations**
  - **Low-quality products**

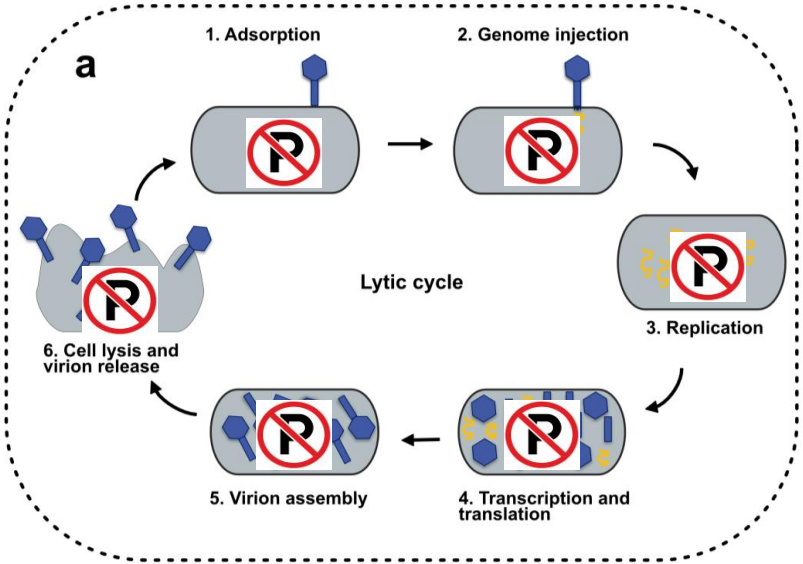
Friends

- **Control of bacterial populations**
- **Nutrient cycling**
- **Phage therapy / biocontrol**

UNIVERSITÉ LAVAL

Bacteria: Several natural defense systems against phages

Abi
BREX
Gabija
CBASS
Retron
Theoris
Etc.



Block adsorption

Block DNA entry

Cut nucleic acids

- R-M
- ***CRISPR-Cas***

Prokaryotic Antiviral Defence LOCator

Payne et al. (2021) NAR

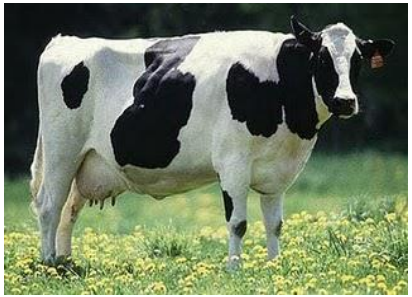
DefenseFinder

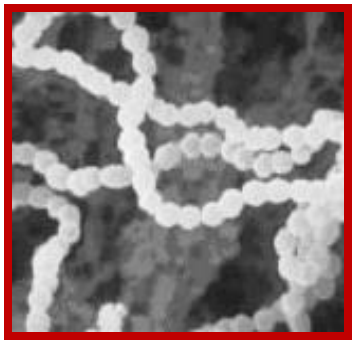
Tesson et al. (2022) Nature Comm.

Plan

- Bacteriophages
  - ***Enemies :*** **Any process relying on bacteria**
    - **Control phages**
  - *Friends*
    - Phages as biocontrol agents
- Conclusions & perspectives

Cheese 101 !





Cheese 101 !

Isolates
- Basic physiology
- Microbiology, genetic ID
- Phage (Lyso) typing

- Physiologic selective propagation
- Screening against phage library
- Battery of rapid tests for physiology, microbiology, genetics, taxonomy, . . .

Characterization
- Acidification
- Flavor / Texture
- Synergy / Antagonism
- Safety

- CINAC acidification (temperature, salt)
- Off flavor – GC, HPLC, sensory
- Compatibility testing
- QPS (Qualified Presumption of Safety)
- Genome sequencing

Development
- Process optimization
- Culture formulation
- Application trials

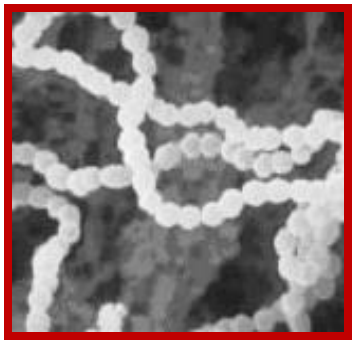
- Bench pre-pilot scale fermentation (Yield, concentration, activity)
- Culture formulation
- Application bench testing
- Phage; commercial whey testing

Commercialization
- Pilot manufacture
- Validation
- Customer Field trial

- Pilot scale fermentation
- Full downstream processing
- QC control
- Final culture format test
- Field Trials

- **Natural Microflora**
- **Undefined Mixed-Strain**
- **Defined Strains**
  - Single – Paired
  - Multiple-Strains
  - Multiple-Mixed-Strains

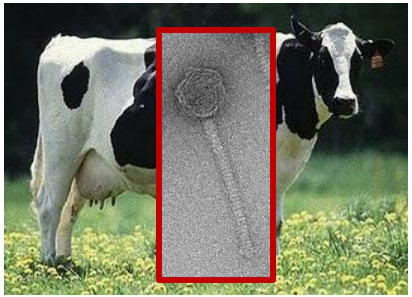
Repeated use

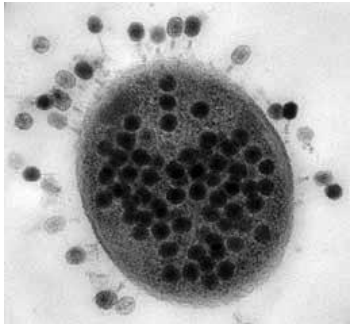


10^7 cfu / ml
Lactic acid bacteria

Dennis Romero

Cheese 101 !





Phage analysis and critical control point (PACCP)

1. Sources of phage contamination

- Raw ingredients
- Equipment and surfaces
- Staff
- Air
- Bacterial strains

2. Phage detection and characterization methods

Microbial	Microscopy	PCR-based methods	DNA analysis	Others
• Plaque assay • Growth assay	• Electron microscopy • Epifluorescence microscopy	• RAPD • Multiplex PCR • MLST • qPCR	• Genome sequencing • RFLP	• Flow cytometry • Biosensors • Immunodetection

3. Phage control strategies

Factory design	Industrial cleaning	Microbial
• Adequate ventilation system and airflow • Closed vessels • Pressure room • Products and personnel flow	• Raw ingredient treatments • Adequate sanitizers (CIP) • Efficient cleaning procedures • Staff training	• Monitoring phage populations • Bacterial rotation • Phage-resistant strains

Samson & Moineau. 2013. Annu. Rev. Food Sci. Technol.

UNIVERSITÉ LAVAL

Phage analysis and critical control point (PACCP)

2. Detection and characterization: *Know your enemies*

Microbial	Microscopy	PCR-based methods	DNA analysis	Others
• Plaque assay • Growth assay	• Electron microscopy • Epifluorescence microscopy	• RAPD • Multiplex PCR • MLST • qPCR	• Genome sequencing • RFLP	• Flow cytometry • Biosensors • Immunodetection

Samson & Moineau. 2013. Annu. Rev. Food Sci. Technol.

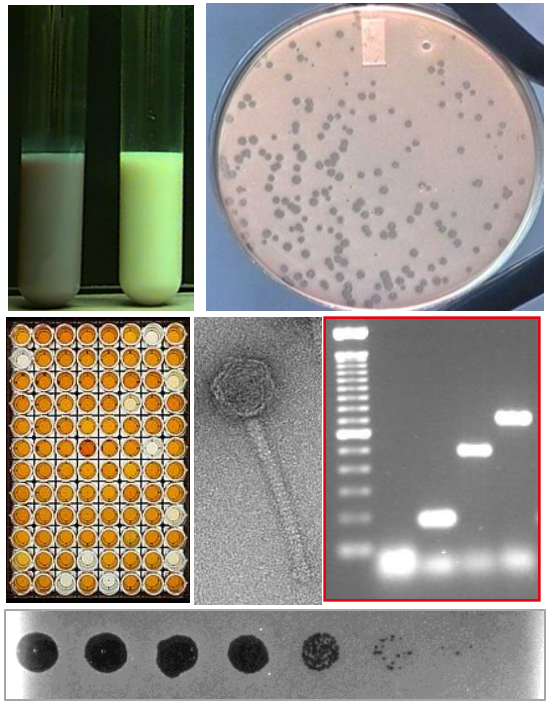
UNIVERSITÉ LAVAL

Phage monitoring program

- Biochemical assays
- Plaque assays

- PCR / qPCR
- Microscopy

- Innovations ?
- In-process detection ?



Phage analysis and critical control point (PACCP)

1. Sources

Raw ingredients	Equipment and surfaces	Staff	Air

- **Milk**
- **Whey**

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Whey powders are a rich source and excellent storage matrix for dairy bacteriophages

Natalia Wagner [a], Erik Brinks [a], Meike Samtlebe [b], Jörg Hinrichs [b], Zeynep Atamer [b], Witold Kot [c], Charles M.A.P. Franz [a], Horst Neve [a,*], Knut J. Heller [a]

Equipment and surfaces

Dissemination

Staff

936

Surface description	No. of genomes/cm^2	Positive/total assays[a]
Door handle	9,106 ± 789	3/3
Rough floor	6,115 ± 785	3/3
Top of paper towel dispenser	6,079 ± 800	3/3
Cleaning material	1,854 ± 271	3/3
Smooth floor	448 ± 145	15/15
Wall	407 ± 169	3/3
Push for hand sanitizer	266 ± 126	3/3
Office table	60 ± 28	3/3
Top of electrical panel	53	1/4
Top of stainless pipe	30 ± 7	3/3
Stair ramp	11 ± 10	4/6
Top of closed tank	3 ± 1	3/3
Vertical panel	2	1/3
Vertical surface (electrical)	0	0/3

UNIVERSITÉ LAVAL

Verreault et al. AEM, 2011

Phage analysis and critical control point (PACCP)

3. Control strategies

Factory design

- Adequate ventilation system and airflow
- Closed vessels
- Pressure room
- Products and personnel flow

Industrial cleaning

- Raw ingredient treatments
- Adequate sanitizers (CIP)
- Efficient cleaning procedures
- Staff training

Microbial

- Monitoring phage populations
- Bacterial rotation
- Phage-resistant strains

Samson & Moineau. 2013. Annu. Rev. Food Sci. Technol.

Cleaning

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Inactivation of dairy bacteriophages by commercial sanitizers and disinfectants

Céline Campagna [a,1], Manuela Villion [a,2], Simon J. Labrie [a], Caroline Duchaine [a,b], Sylvain Moineau

Phage inactivation protocol : European standard EN 13610:2002 (European Committee for Standardization)

- **0.13% & 0.25%, peracetic & acetic acids, H_2O_2, 1-octanesulfonic acid, octanoic acid))**
- **Using it in our lab !**
- **Lab cleaning three times a year**

Phage analysis and critical control point (PACCP)

3. Control strategies

Factory design

- Adequate ventilation system and airflow
- Closed vessels
- Pressure room
- Products and personnel flow

Industrial cleaning

- Raw ingredient treatments
- Adequate sanitizers (CIP)
- Efficient cleaning procedures
- Staff training

Microbial

- Monitoring phage populations
- Bacterial rotation
- Phage-resistant strains

Samson & Moineau. 2013. Annu. Rev. Food Sci. Technol.

Longitudinal Study of *Lactococcus* Phages in a Canadian Cheese Factory

AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

2023

Alice P. Jolicoeur,[a,b] Marie-Laurence Lemay,[a,b,c] Elyse Beaubien,[a,b] Jessy Bélanger,[a,b] Claudia Bergeron,[a,b] Françoise Bourque-Leblanc,[a,b] Laurie Doré,[a,b] Marie-Ève Dupuis,[a,b] Audrey Fleury,[a,b] Josiane E. Garneau,[a,b] Simon J. Labrie,[a,b*] Steve Labrie,[a,b§] Geneviève Lacasse,[a,b] Marianne Lamontagne-Drolet,[a,b] Roxanne Lessard-Hurtubise,[a,b] Bruno Martel,[a,b◊] Rym Menasria,[a,b] Rachel Morin-Pelchat,[a,b] Gabrielle Pageau,[a,b] Julie E. Samson,[a,b] Geneviève M. Rousseau,[a,b] Denise M. Tremblay,[a,b,d] Manon Duquenne,[e∞] Maryse Lamoureux,[e‡] Sylvain Moineau[a,b,d]

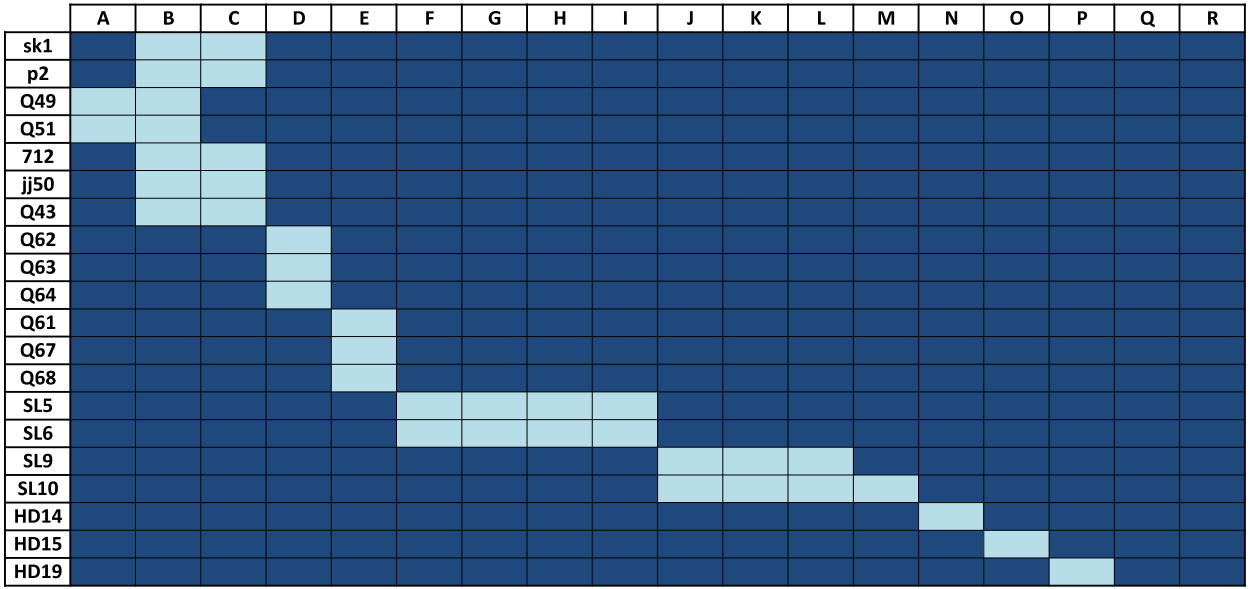
- **One** cheese factory
  - Use mostly their own starters
- For 20 years (2001-2020), phage testing + isolation
  - 241 unique lactococcal phages were isolated
    - 97% *Skunavirus* genus
    - 2% to the P335 group
    - 1% to the *Ceduovirus* genus
- 93 of them were isolated more than once
  - Phage GL 7 isolated 132 times !

Dairy phages are specialists

(almost strain specific)

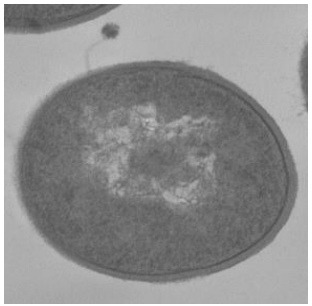
Lactic Acid Bacteria

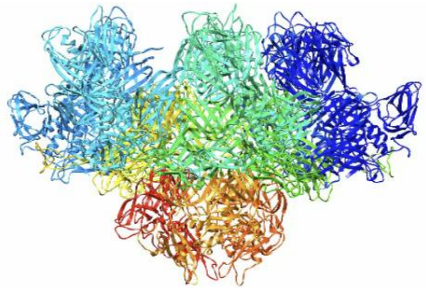
Phages

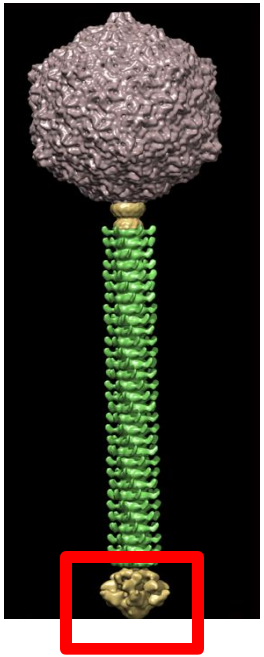


Phage receptor-binding proteins

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
sk1																		
p2																		
Q49																		
Q51																		
712																		
jj50																		
Q43																		
Q62																		
Q63																		
Q64																		
Q61																		
Q67																		
Q68																		
SL5																		
SL6																		
SL9																		
SL10																		
HD14																		
HD15																		
HD19																		



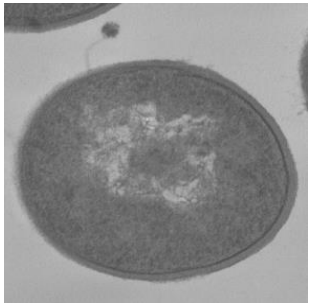


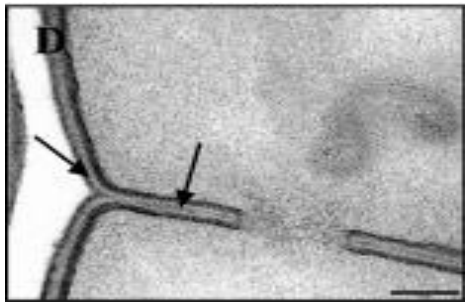


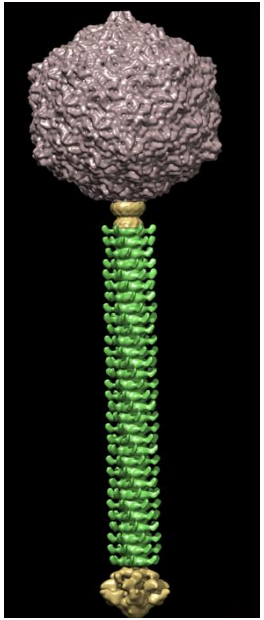
- Spinelli et al., 2006, Nature Struct. Mol. Biol.
- Veesler et al, 2009, J. Biol. Chem.
- Scaltriti et al., 2009, Mol. Microbiol.
- Sciara et al., 2010, PNAS
- Chapot-Chartier et al. 2010, JBC
- Bebeacua et al., 2013, J. Virol.
- Spinelli et al., 2014, J. Virol.

Phage receptors

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
sk1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
p2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Q49	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Q51	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
712	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
jj50	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Q43	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Q62	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Q63	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Q64	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Q61	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Q67	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Q68	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
SL5	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
SL6	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
SL9	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
SL10	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
HD14	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
HD15	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
HD19	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■







Cell Surface of *Lactococcus lactis* Is Covered by a Protective Polysaccharide Pellicle*

Received for publication, November 6, 2009, and in revised form, January 15, 2010 Published, JBC Papers in Press, January 27, 2010, DOI 10.1074/jbc.M109.082958

Marie-Pierre Chapot-Chartier[‡], Evgeny Vinogradov[§], Irina Sadovskaya[¶||], Guillaume Andre[**], Michel-Yves Mistou[‡ ‡‡], Patrick Trieu-Cuot[‡‡], Sylviane Furlan[‡], Elena Bidnenko[‡], Pascal Courtin[‡], Christine Péchoux[§§], Pascal Hols[¶¶1,2], Yves F. Dufrêne[1,3], and Saulius Kulakauskas[‡,4]**

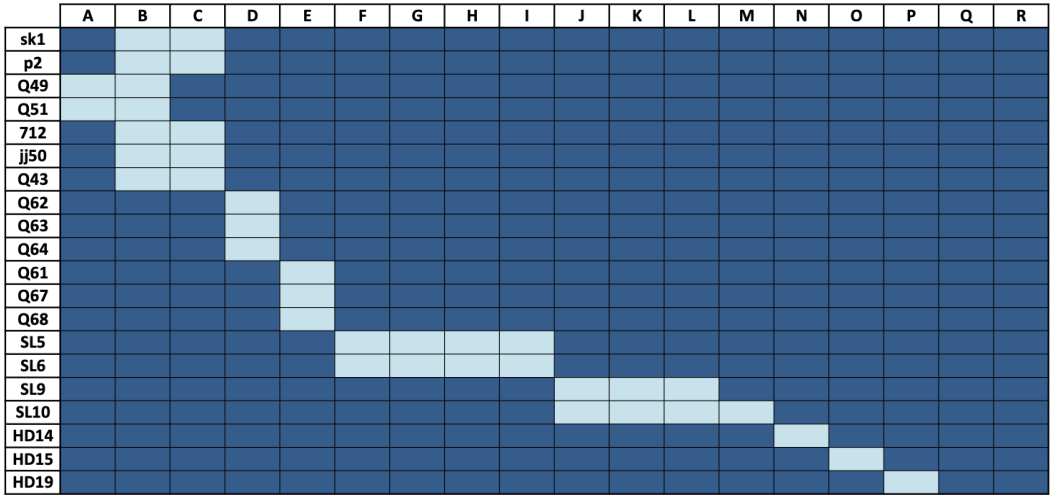
The CWPS Rubik's cube: Linking diversity of cell wall polysaccharide structures with the encoded biosynthetic machinery of selected *Lactococcus lactis* strains

Jennifer Mahony ✉, Cyril Frantzen, Evgeny Vinogradov, Irina Sadovskaya, Ilias Theodorou, Philip Kelleher, Marie-Pierre Chapot-Chartier, Christian Cambillau, Helge Holo, Douwe van Sinderen ✉

Dairy phages are specialists

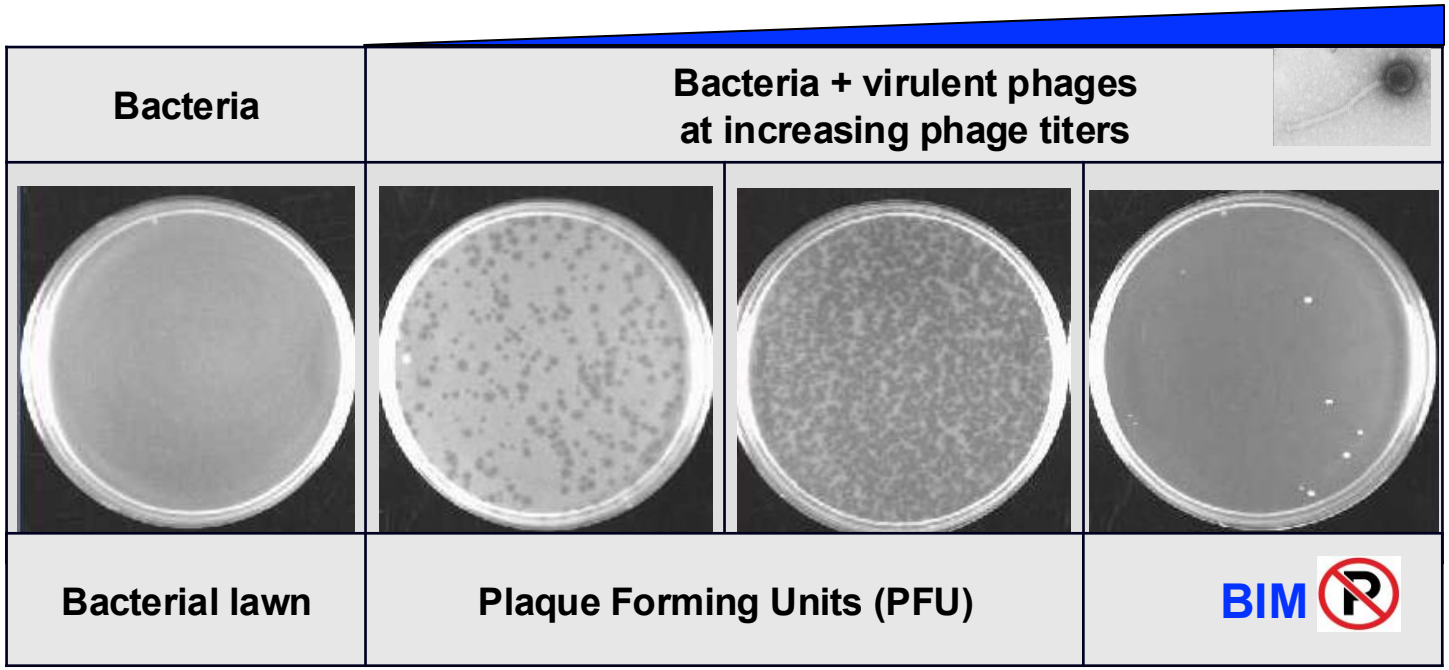
Lactic Acid Bacteria

Phages

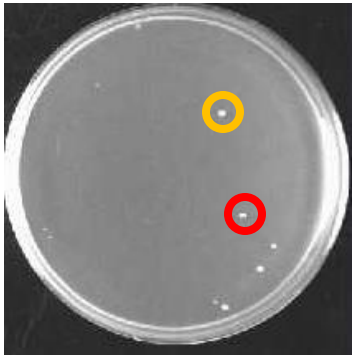


- ✓Change / rotate starter cultures
- ✓Change bacterial strains
- ✓Modify bacterial strains

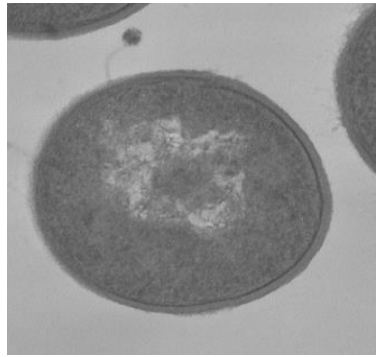
Selection of Bacteriophage Insensitive Mutants (BIMs)

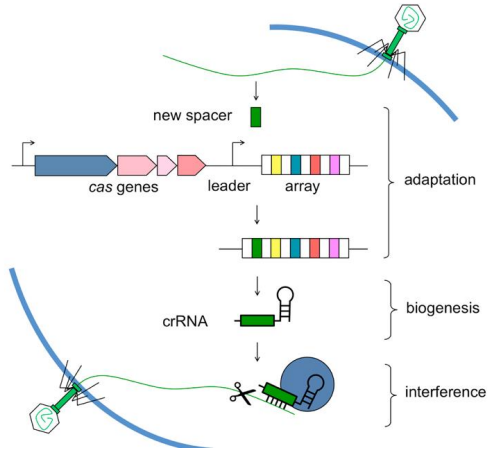


Streptococcus thermophilus



BIMs





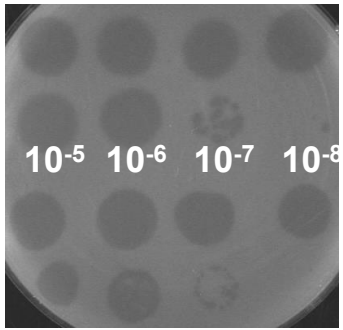
CRISPR-Cas

10^{-1} 10^{-2} 10^{-3} 10^{-4}

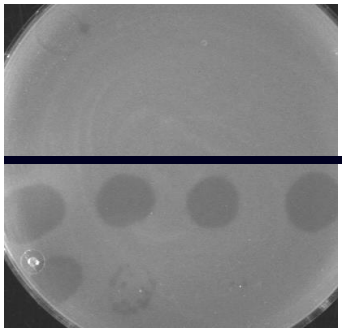
A

B

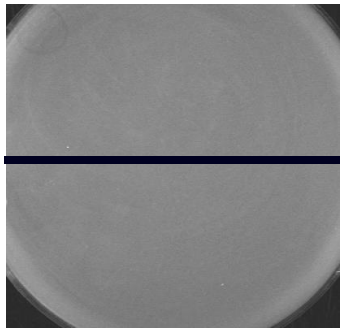
10^{-5} 10^{-6} 10^{-7} 10^{-8}



Wild-type



BIM #1



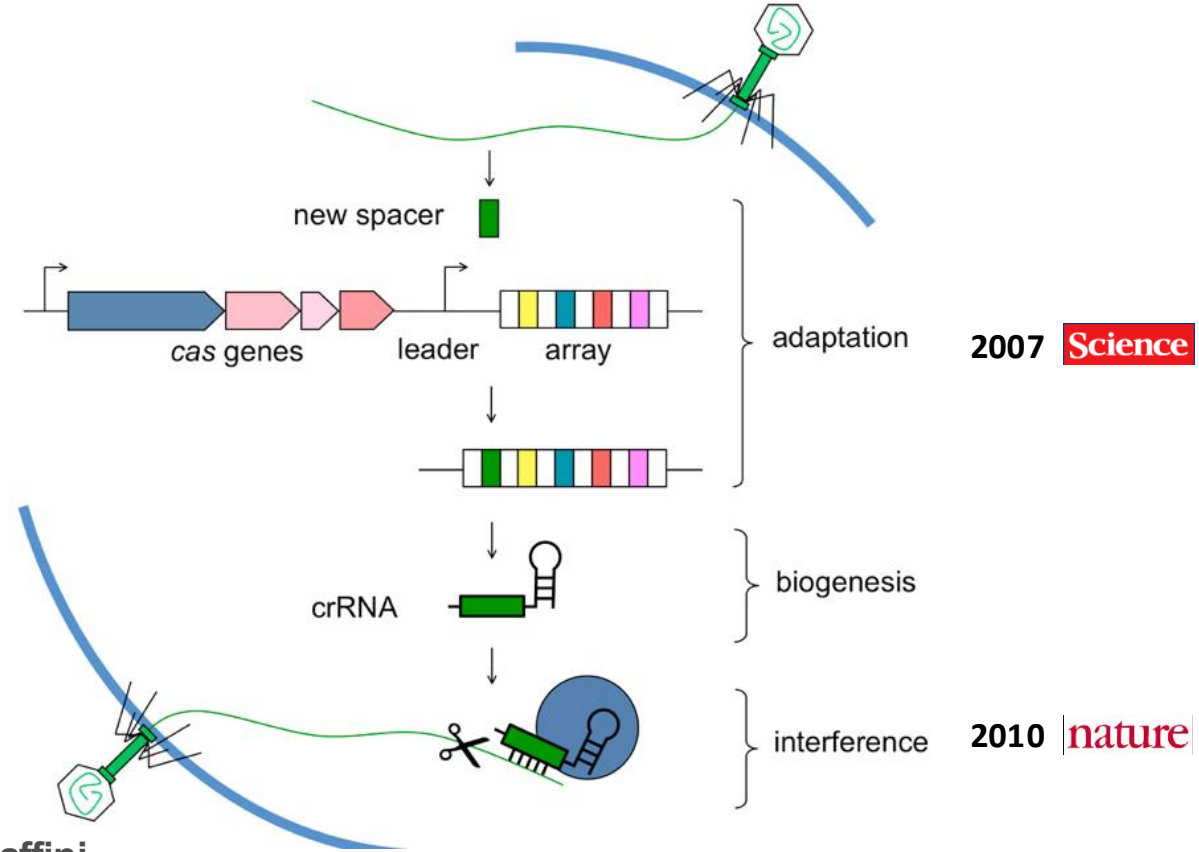
BIM#2

A

B

CRISPR-Cas in bacteria: 3 steps

Molecular details vary depending on the type and subtype

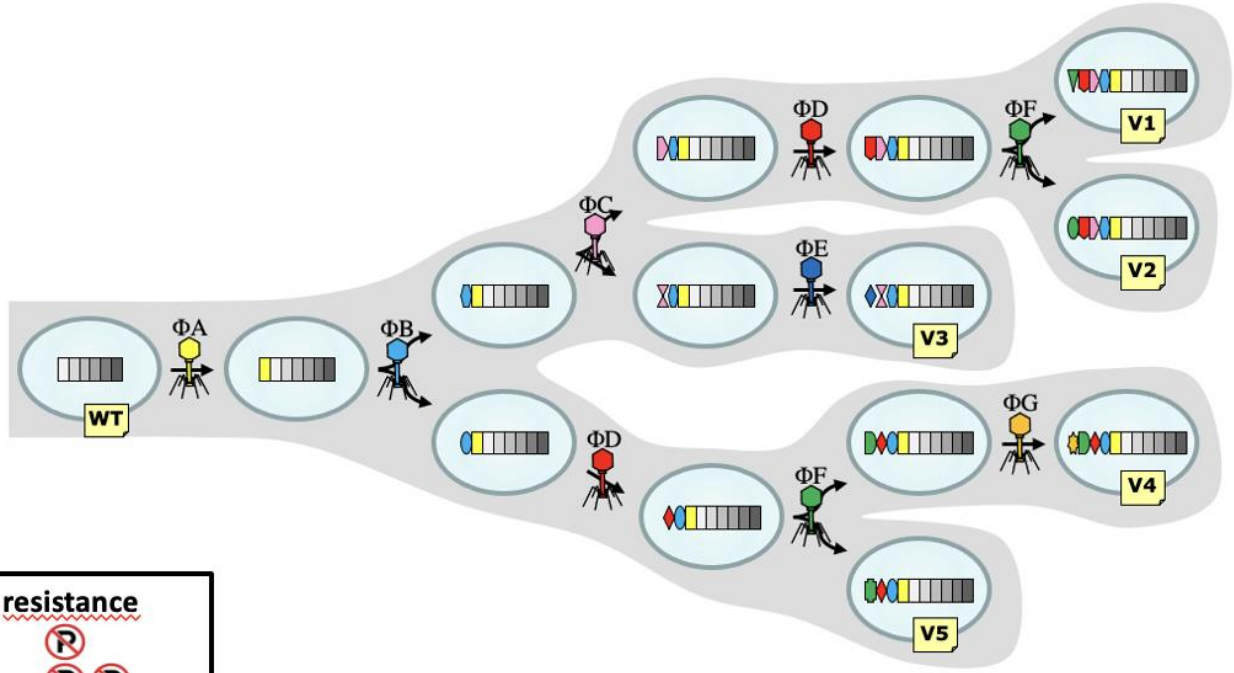


2007 Science

2010 nature

Adapted from Marraffini

- **Strains of *S. thermophilus* used by the dairy industry**
- **Naturel system providing resistance to phages**



Phage resistance

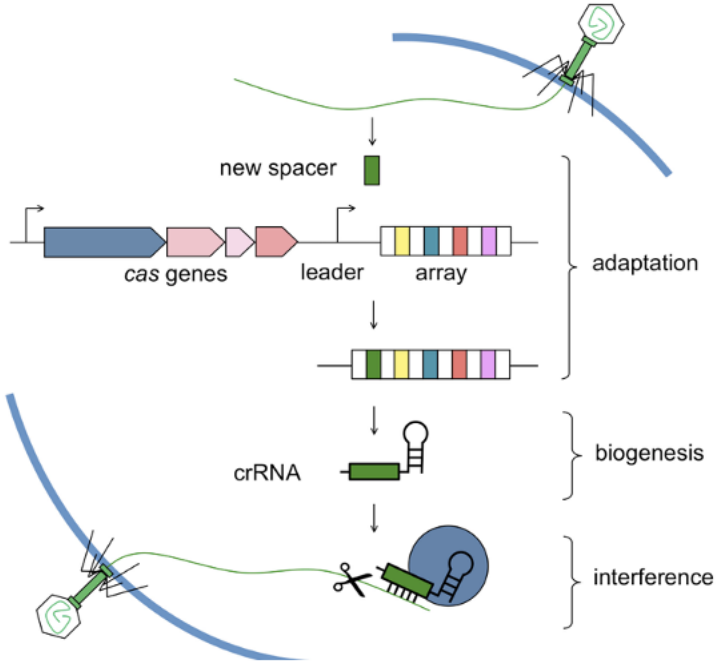
1 spacer : R

2 spacers : R R

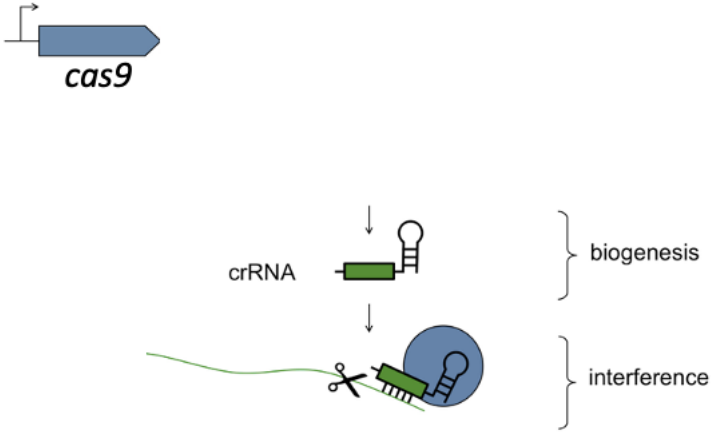
3 spacers : R R R

Adapted from Danisco

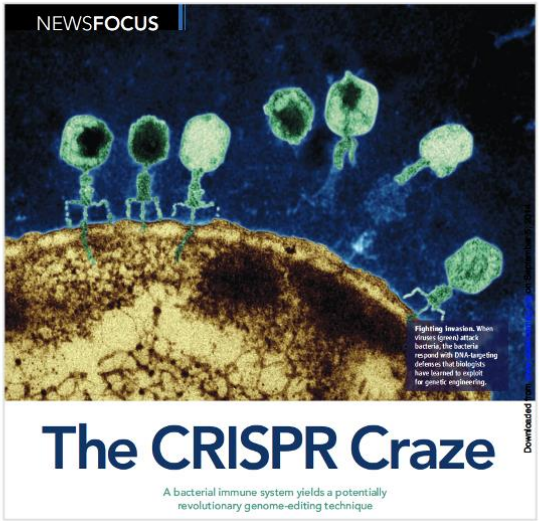
CRISPR-Cas system in bacteria



CRISPR-Cas9 Technology

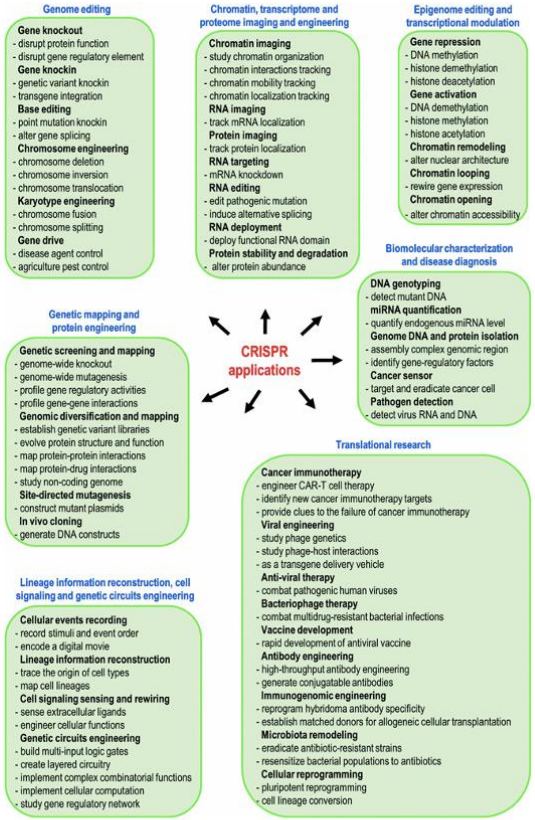


CRISPR-Cas9 *et al.*



- CRISPR-Cas12
- CRISPR-Cas13
- CRISPR-Cas14
- Base editing
- Prime editing

- Cas9 nickase
- dCas9 / CRISPRi
- CRISPRa
- epiCas9
- Multiplexing (ARNg)
- …



Lau, CRISPR J.

Victoria Gray
Sickle Cell Disease

Carlene Knight
Leber Congenital Amaurosis

KJ Muldoon
Urea Cycle Disorder

UK first to approve CRISPR treatment for diseases: what you need to know

FDA NEWS RELEASE

FDA Approves First Gene Therapies to Treat Patients with Sickle Cell Disease

CASGEVY Makes History as FDA Approves First CRISPR/Cas9 Genome Edited Therapy

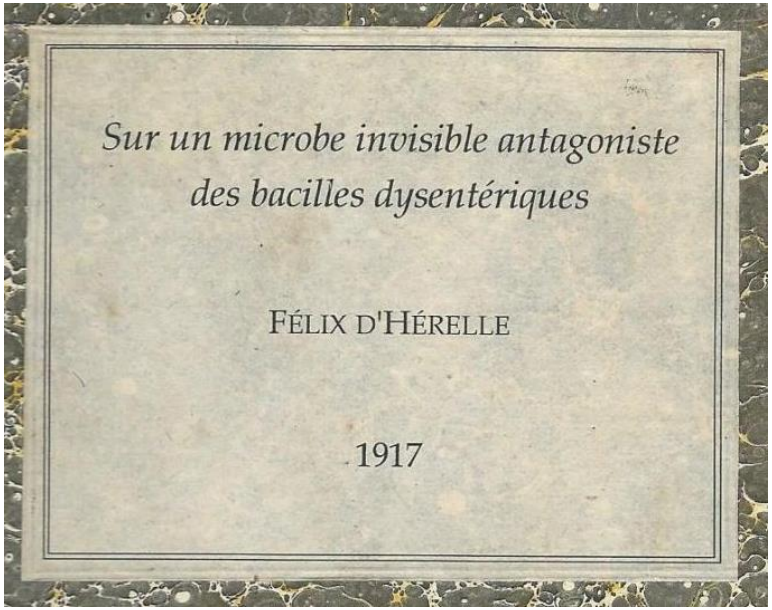
L'espoir d'une révolution médicale

Plan

- Bacteriophages
  - *Enemies*
    - Control phages
  - ***Friends***
    - **Phages as biocontrol agents**
- Conclusions & perspectives

June 2024

Félix d'Hérelle



Comptes Rendus Acad. Sci.
165, 373–375

Phages as antibacterials ?

+

- Specific
- Replicate
- Easy/cheap to produce
- Stable
- Natural
- …

-

- Specific
- Resistance
- Toxicity
- IP
- Regulatory
- …

UNIVERSITÉ LAVAL

Phage therapy : Belgium > > Canada !

Personalized bacteriophage therapy outcomes for 100 consecutive cases: a multicentre, multinational, retrospective observational study

Jean-Paul Pirnay ✉, Sarah Djebara, Griet Steurs, Johann Griselain, Christel Cochez, Steven De Soir, Tea Glonti, An Spiessens, Emily Vanden Berghe, Sabrina Green, Jeroen Wagemans, Cédric Lood, Eddie Schrevens, Nina Chanishvili, Mzia Kutateladze, Mathieu de Jode, Pieter-Jan Ceyssens, Jean-Pierre Draye, Gilbert Verbeken, Daniel De Vos, Thomas Rose, Jolien Onsea, Brieuc Van Nieuwenhuyse, Bacteriophage Therapy Providers, Bacteriophage Donors, Patrick Soentjens, Rob Lavigne & Maya Merabishvili — Show fewer authors

Nature Microbiology **9**, 1434–1453 (2024) | Cite this article

…Here we present the outcomes of a retrospective observational analysis of the first 100 consecutive cases of personalized BT of difficult-to-treat infections facilitated by a Belgian consortium in 35 hospitals, 29 cities and 12 countries during the period from 1 January 2008 to 30 April 2022….

…Clinical improvement and eradication of the targeted bacteria were reported for 77.2% and 61.3% of infections, respectively….

First patient treated with phages in Canada in 2023

ClinicalTrials.gov

Phage Therapy for the Treatment of Urinary Tract Infection

ClinicalTrials.gov Identifier: NCT05537519

Recruitment Status : Recruiting
First Posted : September 13, 2022
Last Update Posted : March 14, 2023

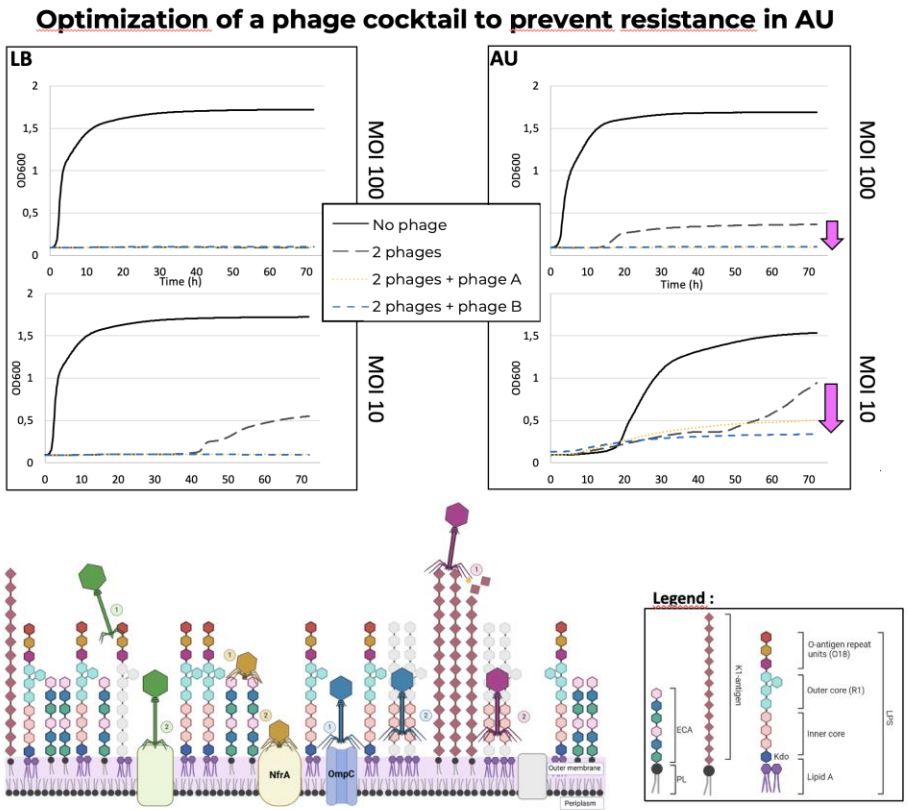
Sponsor:
Gregory German

Collaborator:
Applied Health Research Centre

Information provided by (Responsible Party):
Gregory German, Unity Health Toronto

Second patient in 2024 (Ottawa):
Periprosthetic joint infection
Staphylococcus epidermidis

Phage screening on *E. coli* strain from patient



Doré et al. unpublished

Phages in foods : biocontrol / prevention

Health Canada: Letter of no objection

Products	Target bacterial species
ListShield®	*Listeria monocytogenes*
EcoShield®	*E. coli O157:H7*
SalmFresh®	*Salmonella* spp.
ShigaShield®	*Shigella* spp.
Finalyse®	*E. coli O157:H7*
PhageGuard Lystex (Lystex P100™)	*Listeria monocytogenes*
PhageGuard S (Salmonelex™)	*Salmonella* spp.
Agriphage	*Xanthomonas campestris* *Pseudomonas syringae*

Phages and Heath Canada

Santé Canada Health Canada

Canada

Accueil > Sécurité des produits de consommation > Rapports et Publications > Pesticides et lutte antiparasitaire > Décisions et mises à jour

Sécurité des produits de consommation

Décision d'homologation RD2012-21, Bactériophage de *Clavibacter michiganensis* ssp. *michiganensis*

Agence de réglementation de la lutte antiparasitaire
Le 20 juin 2012
ISSN : 1925-0924 (version PDF)
Numéro de catalogue : H113-25/2012-21F-PDF (version PDF)

UNIVERSITÉ LAVAL

Phages in foods, plants, animals, humans

- ***Salmonella*** **(poultry, Canada-Kenya)**
- ***Staphylococcus*** **(cheese, goats, Canada-Kenya)**
- ***Acinetobacter*** **(human, Canada)**
- ***Aeromonas*** **(fish, Canada-Philippines)**
- ***Listeria*** **(packaging, Canada-Industry)**
- ***Ralstonia*** **(tomato plants, Canada-Brazil-USA)**

Phages in foods, plants, animals, humans

- ***Salmonella*** **(poultry, Canada-Kenya)**
- *Staphylococcus* (cheese, goats, Canada-Kenya)
- *Acinetobacter* (human, Canada)
- *Aeromonas* (fish, Canada-Philippines)
- *Listeria* (packaging, Canada-Industry)
- *Ralstonia* (tomato plants, Canada-Brazil-USA)

UNIVERSITÉ LAVAL

Many people involved

Université Laval

Sylvain Moineau
Damitha Gunathilake
Clément Fage
Denise Tremblay
Françoise Bourque
Nicolas Lemire
Stéphanie Loignon

International Livestock Research Institute

ILRI
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

Nicholas Svitek
Nicoline de Haan
Angela Makumi
Linda Guantai
Amos Mhone
Josiah Odaba
Zoe Campbell
Ilona Gluecks
Edwin Chelule & ILRI staff
Chicken Farmers

SyntBioLab
L'alternative aux antibiotiques

Simon Labrie
Viascheslav Liato
Rodrigue Dubar
Nicolas Lemire

Republic of Kenya
Ministry of Agriculture, Livestock, Fisheries and Irrigation

Samuel Njuguna
Jane Githinji

IDRC | CRDI
International Development Research Centre
Centre de recherches pour le développement international

UKaid
from the British people

Department of Health & Social Care

CGIAR
RESEARCH PROGRAM ON Livestock

Why chickens in Kenya ?

- 75% of families have chickens (avg. 13 / household !)
- 8% of the GDP
- 78% of poultry farmers use antibiotics (80% for treatment)
- 69% of poultry farmers use antibiotics in the last 3 months
- >60% of poultry businesses run by women
- **Could phages be used to reduce antibiotic use?**

ILRI
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE
CGIAR

Previous Studies Confirms the Feasibility of Phage Therapy Against *Salmonella* in Poultry

J. Microbiol. Biotechnol. (2013), 23(10), 1478–1483
http://dx.doi.org/10.4014/jmb.1304.04067

jmb

Therapeutic Effects of Bacteriophages Against *Salmonella gallinarum* Infection in Chickens

Sung Sik Hong[1], Jipseol Jeong[2], Jinju Lee[2], Suk Kim[2], Wongi Min[2], and Heejoon Myung[1,3]*

SCIENTIFIC REP

Microencapsulation with alginate/ for improved

Vol. 73, No. 14

Bacteriophage Therapy To Reduce *Salmonella* Colonization of Broiler Chickens

intralytix

SalmoFresh™
Phage preparation
Effective against Salmonella

Virus Genes (2018) 54:446–456
https://doi.org/10.1007/s11262-018-1549-0

Isolation and identification of *Salmonella pullorum* bacteriophage YSP2 and its use as a therapy for chicken diarrhea

Kunyuan Tie[1] · Yuyu Yuan[2] · Shiqing Yan[1] · Xi Yu[3] · Qiuyang Zhang[1] · Huihui Xu[1] · Yang Zhang[1] · Jingmin Gu[1] · Changjiang Sun[1] · Liancheng Lei[1] · Wenyu Han[1] · Xin Feng[1]

Phage cocktail SalmoFREE® reduces *Salmonella* on a commercial broiler farm

V. Clavijo,* D. Baquero,* S. Hernandez,* J. C. Farfan,* J. Arias,* A. Arévalo,† P. Donado-Godoy,† and M. Vives-Flores

ulated Bacteriophages for Enhanced Oral Phage
Salmonella spp.

Joan Colom,[a] Mary Cano-Sarabia,[b] Jennifer Otero,[a] Pilar Cortés,[a] Daniel Maspoch,[b,c] Montserrat Llagostera[a]

In 1919, d'Hérelle successfully uses phages to treat chickens infected with *Salmonella*.

Svitek *et al.* 2022, ILRI

Objectives of the project – *The roadmap toward our goal*

1

Isolate *Salmonella* from poultry farms & type strains for AMR.

2

Isolate new Salmonella phages from Kenya.

3

Test phages from the U. Laval collection for capacity to lyse Kenyan *Salmonella* strains.

4

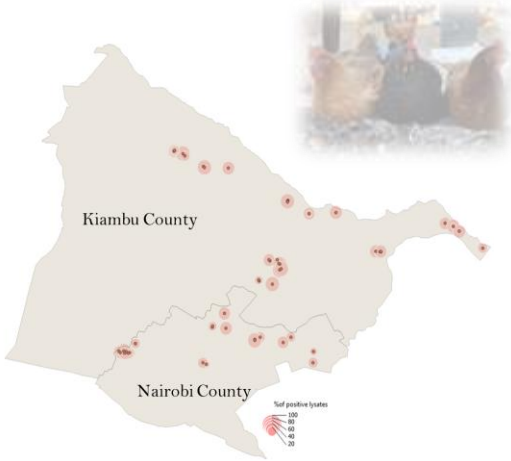
Select, produce and test phages in a *Salmonella* infection model in chicken.

5

Field trial of phages in smallholder farms.

6

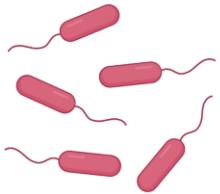
Assess the impact of phage technology & adoption by women chicken farmers.



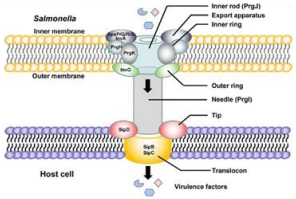
Phage Hunting in Kenya…

Over 600 samples from +70 farms/slaughterhouses in Kiambu & Nairobi!

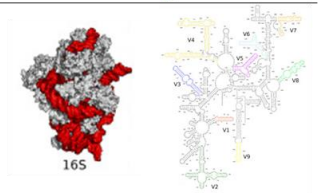
First, isolating *Salmonella*



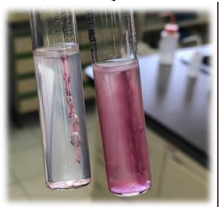
invA gene PCR



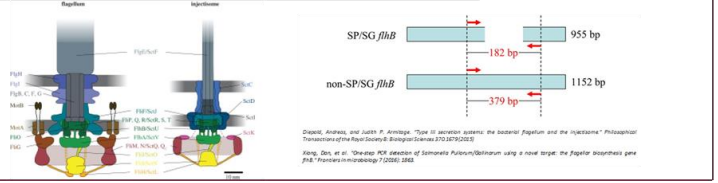
16s ribosomal RNA sequencing



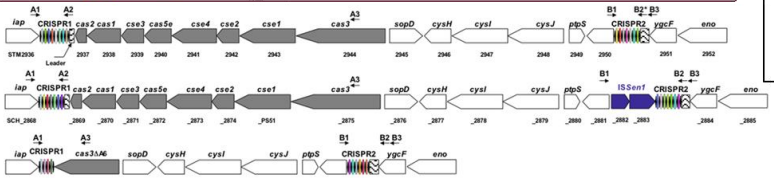
Pullorum Enteritidis



flhB gene PCR



CRISPR Locus Typing



16 confirmed strains

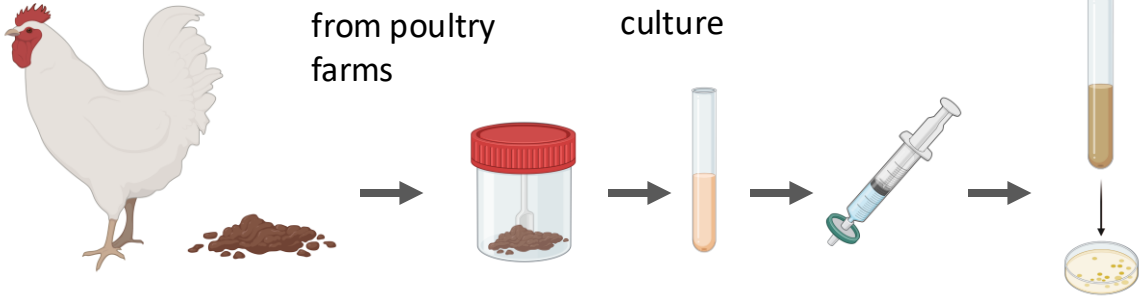
S. enterica Enteritidis # 16
S. enterica Enteritidis # 73
S. enterica Enteritidis # 177
S. enterica Enteritidis # 312
S. enterica Enteritidis # 568
S. enterica Enteritidis # 569
S. enterica Enteritidis # 572
S. enterica Kentucky # 181
S. enterica Kentucky # 182
S. enterica Kentucky # 571
S. enterica Kentucky # 172
S. enterica Heidelberg # 187
S. enterica Heidelberg # 188
S. enterica Heidelberg # 192
S. enterica Heidelberg # 194
S. enterica Crossness # 157

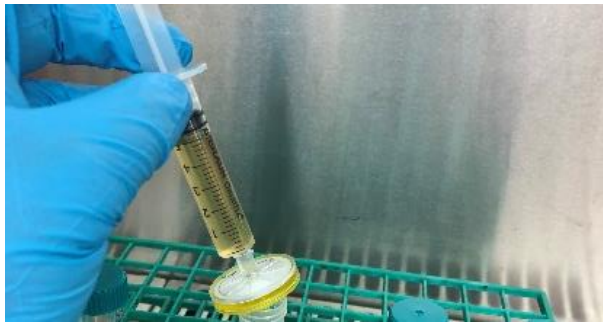
Why ?

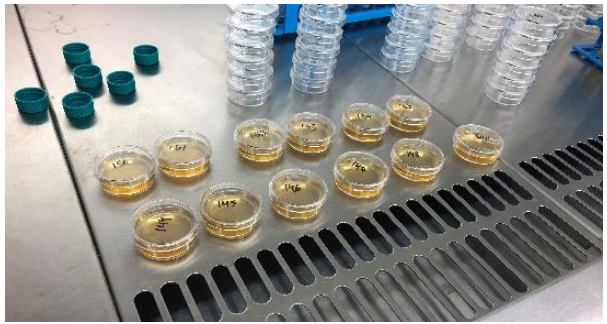
- Protocols ?
- Antibiotics ?
- Phages ?
- Usual ?

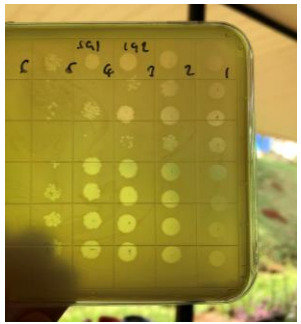
Genomes sequenced by PacBio & Illumina: under analysis

Then, isolating phages…









- # samples screened for phages: **631 (**61 chicken farms / 4 slaughterhouses)
  - 459 samples from chicken droppings
  - 114 samples from water sources
  - 58 from environmental swabs

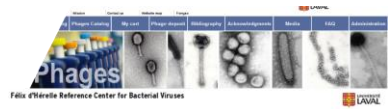
- *Salmonella*-phage + samples**: 425**
- **Plaque-purified phages: 67**

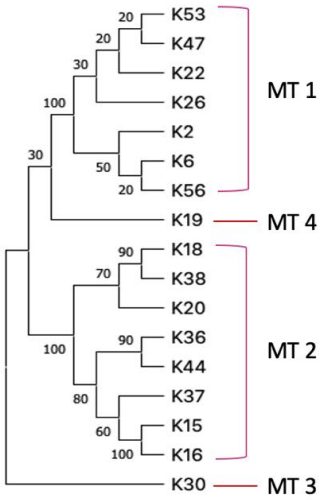
New *Salmonella* Phages!

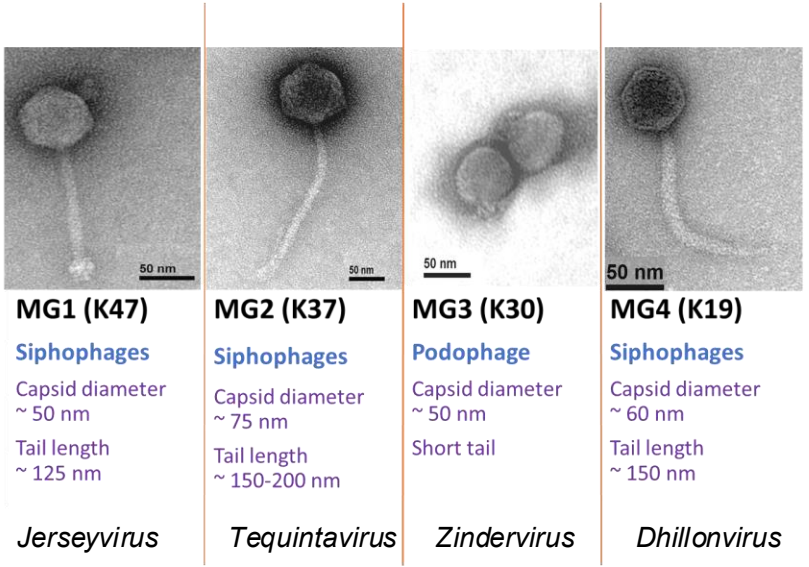
- 59 new phages (out of 67)
- 17 clusters phages
- 4 Major Groups (Genera)
- *Similar to other known phages*
- No virulence or AMR genes

+ 32

www.phage.ulaval.ca

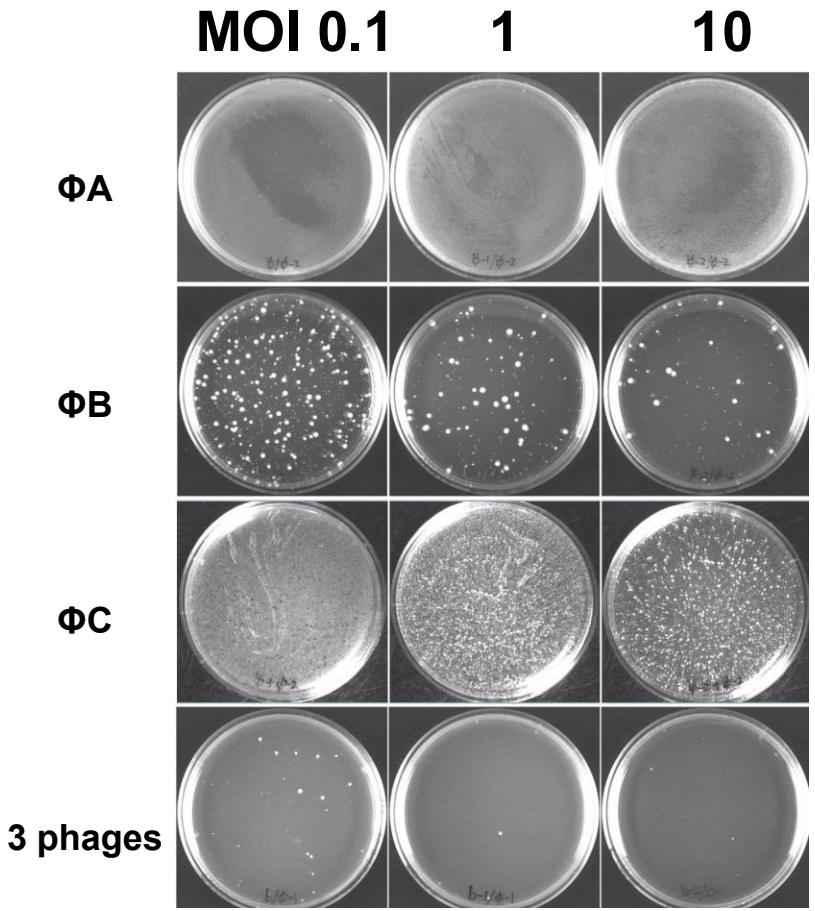






Gunathilake et al. 2024. Microbiol. Spectr.

Need to design
a phage « cocktail » to limit phage resistance



UNIVERSITÉ LAVAL

Which phages survive the harsh environment found in the chicken GIT ?

viruses

MDPI

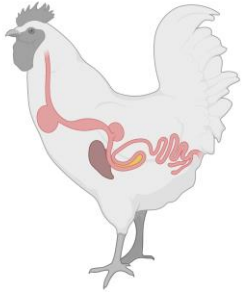
Article

Salmonella Enteritidis Bacteriophages Isolated from Kenyan Poultry Farms Demonstrate Time-Dependent Stability in Environments Mimicking the Chicken Gastrointestinal Tract

Amos Lucky Mhone [1,2], Angela Makumi [1], Josiah Odaba [1], Linda Guantai [1], K. M. Damitha Gunathilake [3,4], Stéphanie Loignon [3,4], Caroline Wangari Ngugi [2], Juliah Khayeli Akhwale [2], Sylvain Moineau [3,4,5] and Nicholas Svitek [1,*]

Phages	Total Score
ILRI_K47	46
ILRI_K1	54
ILRI_K6	55
ILRI_K3	68
ILRI_K22	68
ILRI_K9	77
ILRI_K26	82
ILRI_K11	83
ILRI_K24	84
ILRI_K14	98

Simulated Gastric and Intestinal Fluids



What about women chicken farmers and what can we learn from them?

- **24 focus groups**
- **163 respondants**

- Best value proposition is providing alternative to fowl typhoid vaccine;
- Worst value proposition is competing with affordable, widely available disinfectants like bleach;
- Drinking water administration is preferred;
- Ability to compete with antibiotics depends on cost, storage requirements, and availability.

Campbell et al., 2022, Viruses

Phages in foods, plants, animals, humans

- *Salmonella* (poultry, Canada-Kenya)
- ***Staphylococcus*** **(cheese, goats, Canada-Kenya)**
- *Acinetobacter* (human, Canada)
- *Aeromonas* (fish, Canada-Philippines)
- *Listeria* (packaging, Canada-Industry)
- *Ralstonia* (tomato plants, Canada-Brazil-USA)
- *E. coli* (several applications…)

UNIVERSITÉ LAVAL

Belgium and staphylococcal phages

- André Gratia likely isolated the first **staphylococcal** phages in the 1920s

- Gratia A. (1921) Preliminary report on a *Staphylococcus* bacteriophage. Proc. Soc. Exp. Biol. Med. 18, 192-193.

A. Gratia

J.-P. Gratia, Genetics, 2000
Photograph by G. Rulmont

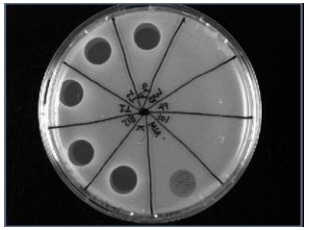
First Complete Genome Sequence of *Staphylococcus xylosus*, a Meat Starter Culture and a Host to Propagate *Staphylococcus aureus* Phages

Simon J. Labrie,[a] Lynn El Haddad,[a] Denise M. Tremblay,[a] Pier-Luc Plante,[b] Jessica Wasserscheid,[c] Jeannot Dumaresq,[d] Ken Dewar,[c] Jacques Corbeil,[b] Sylvain Moineau[a]

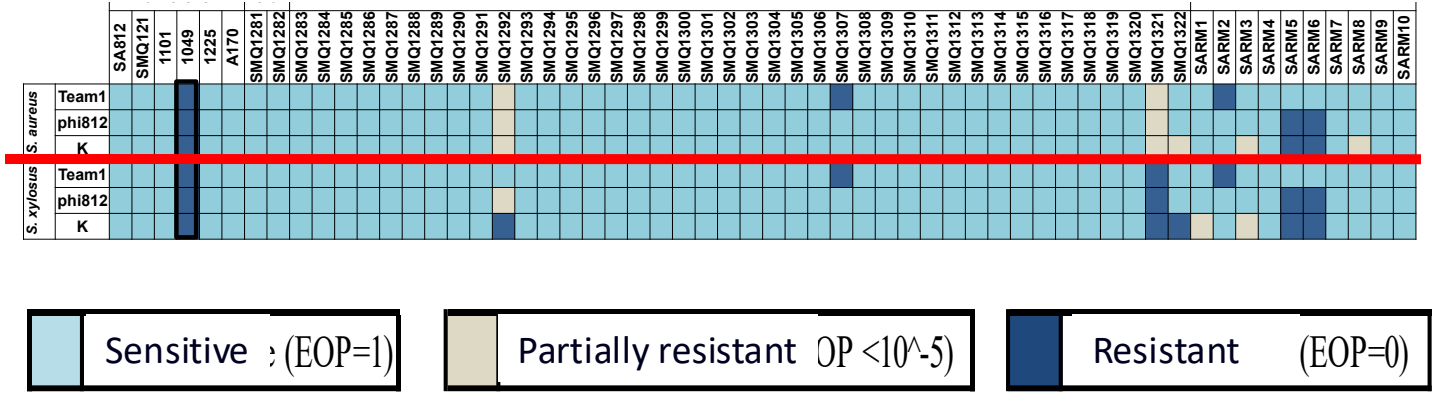
genomeAnnouncements

- **Non pathogenic staphylococci**
    - **Food grade, meat fermentation**
- **Illumina + PacBio**
    - **2,757,557 pb (1 contig)**
    - **2514 genes**
- **No known toxin (VFDB et DBETH)**
- **No functional antibiotic resistant genes (ARdb)**
- **No prophage**

UNIVERSITÉ LAVAL



Production in *aureus* or *xylosus :* No difference in efficacy

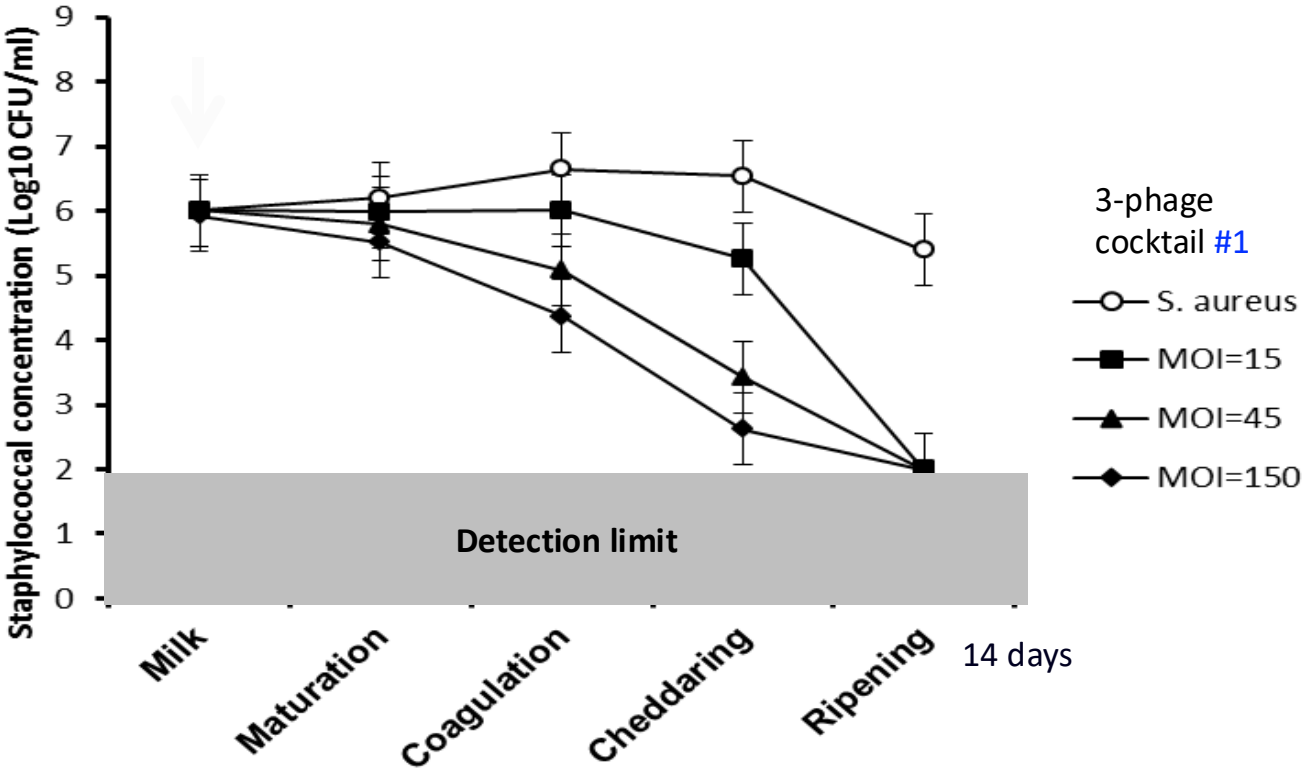


El Haddad *et al.* 2014. PLoS One

UNIVERSITÉ LAVAL

Staph phages didn't work in raw cheeses…

Staph phages did work in pasteurized cheeses



El Haddad et al., IJFM, 2016

Objectives of the project – *The roadmap toward our goal*

1

Isolate *Staphylococcal* from **goat** farms & type strains for AMR.

2

Isolate new **staphylococcal** phages from Kenya.

3

Test phages from the U. Laval collection for capacity to lyse Kenyan *Staphylococcus* strains.

4

Select, produce and test phages in a *Staphylococcus* infection model in goat.

5

Field trial of phages in smallholder farms.

6

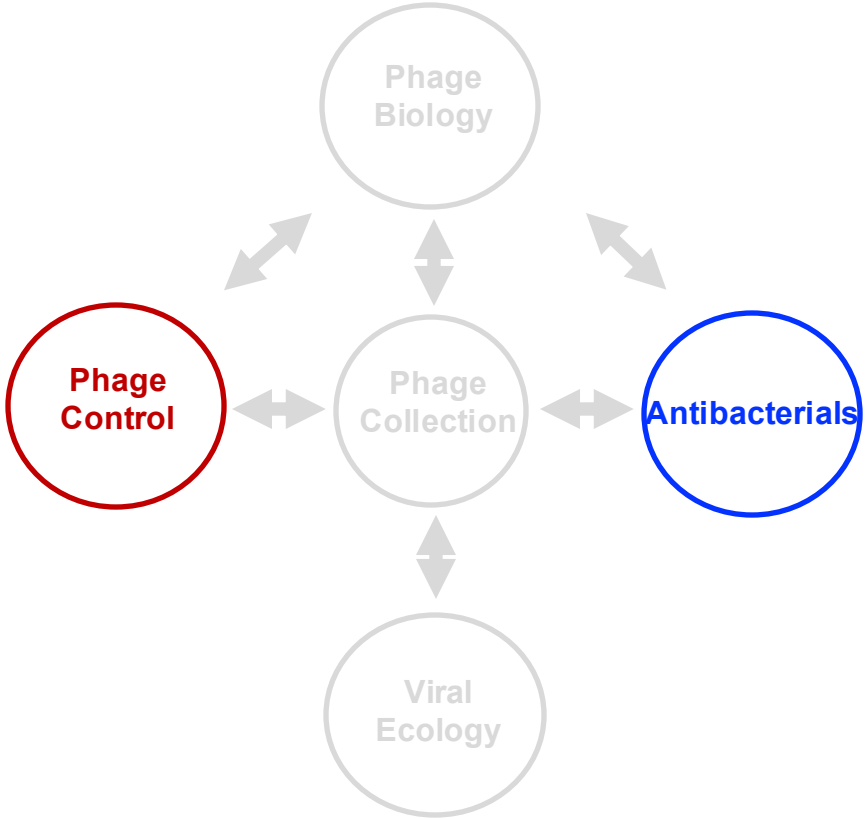
Assess the impact of phage technology & adoption by women goat farmers.

Plan

- Bacteriophages
  - *Enemies*
    - Control phages
  - *Friends*
    - Phages as biocontrol agents
- **Conclusions & perspectives**

www.moineau.bcm.ulaval.ca
www.phage.ulaval.ca

- Phages as enemies
- Know your enemies
  - Specialists
- Control strategies
- Defense systems
- Phage evolution



- Phages as friends
- Know your friends
  - Generalists
- Application strategies
- Defense systems
- Bacterial evolution

- Synergies
- Genome engineering
- Endolysins
- Etc

Labo Moineau
(www.moineau.bcm.ulaval.ca/)

CRSNG NSERC
CANADA RESEARCH CHAIRS
IDRC · CRDI
Canada
CRRC · NRC
Fonds de recherche Nature et technologies Québec
Sentinelle Nord
agropur
iff