

BACTERIOPHAGES AND THEIR APPLICATIONS IN FOODS

June 5th, 2019

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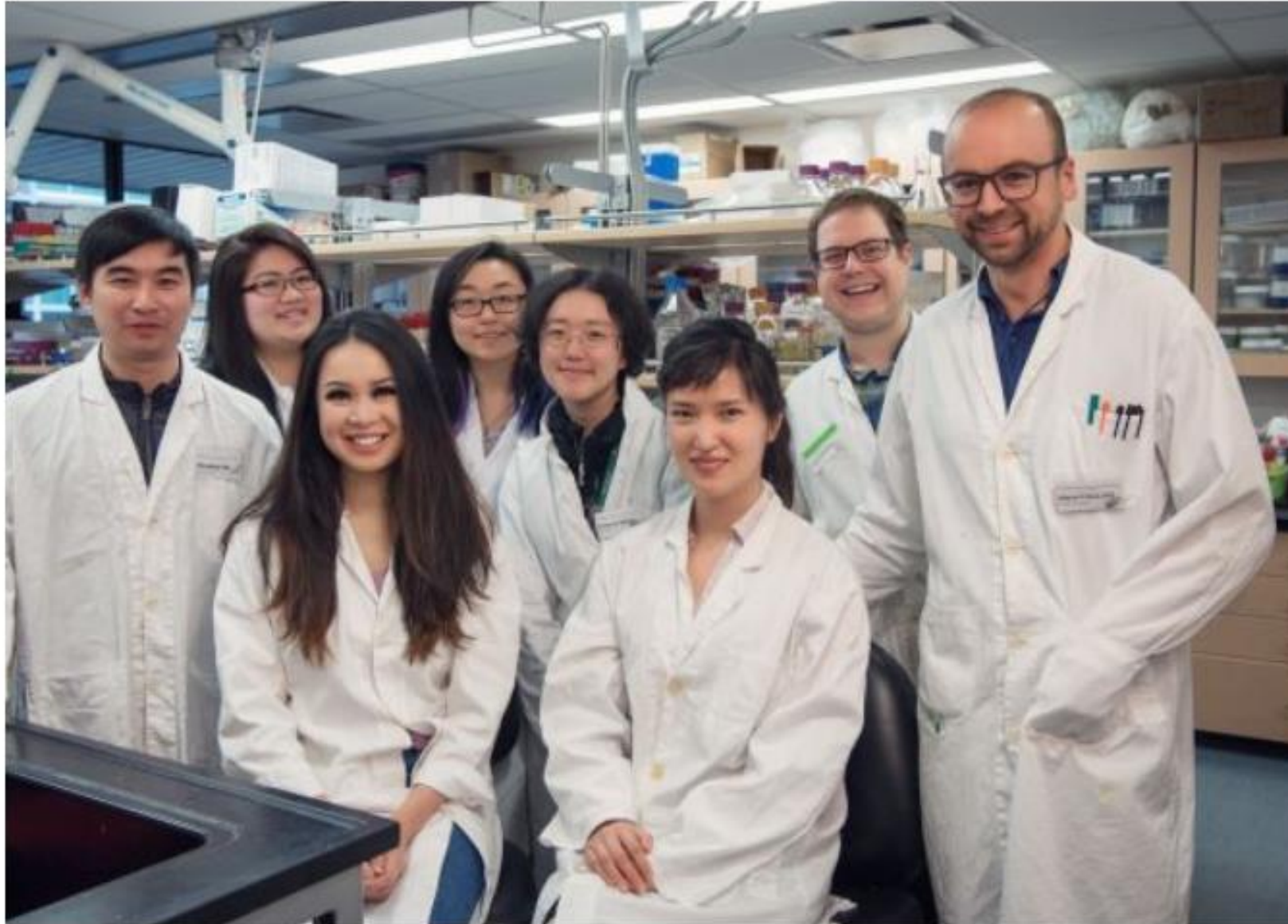


a place of mind

FACULTY OF LAND AND FOOD SYSTEMS

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The Wang Lab of Molecular Food Safety



The Wang Lab of Molecular Food Safety

We are using molecular approaches to understand the microorganisms that pose major threats to food safety, security and public health.

<http://foodsafety.landfood.ubc.ca/>

Today, We'll Discuss About:

- Superbugs
- Bacteriophages (phages)
- Applications of phages in foods
- Limitations and considerations
- What's the next for phage applications in foods?
- Take-home messages

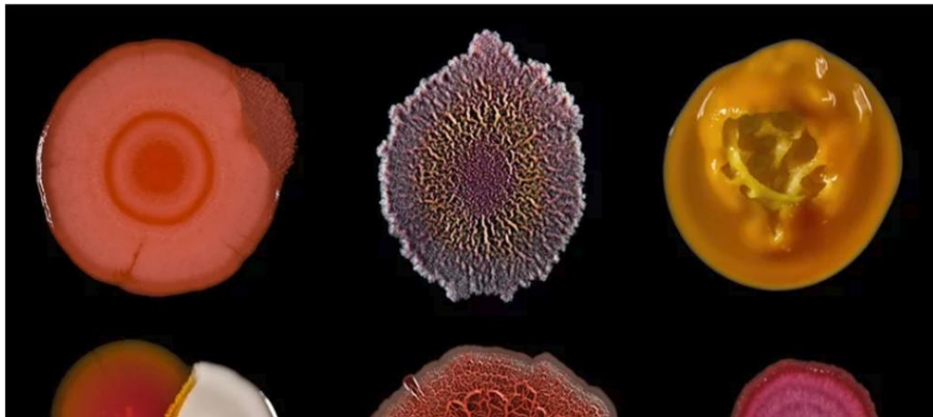
Superbugs in the Media

TIME

Superbugs Are Nearly Impossible to Fight. This Last-Resort Medica...



Superbugs Are Nearly Impossible to Fight. This Last-Resort Medical Treatment Offers Hope



By **ALEXANDRA SIFFERLIN** December 18, 2017



TIME
Health

For more, visit *TIME Health*.

Hospital NewsTM
CANADA'S HEALTH CARE NEWS AND BEST PRACTICES

Salmonella Outbreak Number 16: This Time It Is Antibiotic Resistant And From Chicken



Bruce Y. Lee Contributor ⓘ

Healthcare



CDC and public health and regulatory officials in several states investigated a multistate outbreak of multidrug-resistant [Salmonella](#) infections linked to raw chicken products. The U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) monitored the outbreak.

Final Outbreak Information



- As of February 21, 2019 this investigation is over.
- A total of 129 people infected with the outbreak strain of *Salmonella* Infantis were reported from 32 states.
 - Twenty-five people were hospitalized.
 - One death was reported from New York.

At A Glance

- [Reported Cases](#): 129
- [States](#): 32
- Hospitalizations: 25
- Deaths: 1

...predicted resistance to some or all of the following antibiotics: ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, fosfomycin, gentamicin, hygromycin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole.

What are Superbugs?

"Superbugs" is a term used to describe strains of bacteria that are resistant to the majority of antibiotics commonly used today.

<https://www.mayoclinic.org>

Antibiotic Resistance is a naturally occurring phenomenon



Bacteria adapt to the drugs that are designed to kill them and change to ensure their survival.

<https://gohighbrow.com/discovery-of-penicillin-1928/>, <https://www.mayoclinic.org>

History of Bacteriophages



THE LANCET,]

MR. F. W. TWORT: ULTRA-MICROSCOPIC VIRUSES.

[Dec. 4, 1915 1241

AN INVESTIGATION ON THE NATURE OF ULTRA-MICROSCOPIC VIRUSES.¹

BY F. W. TWORT, L.R.C.P. LOND., M.R.C.S.
(From the Laboratories of the Brown Institution, London.)

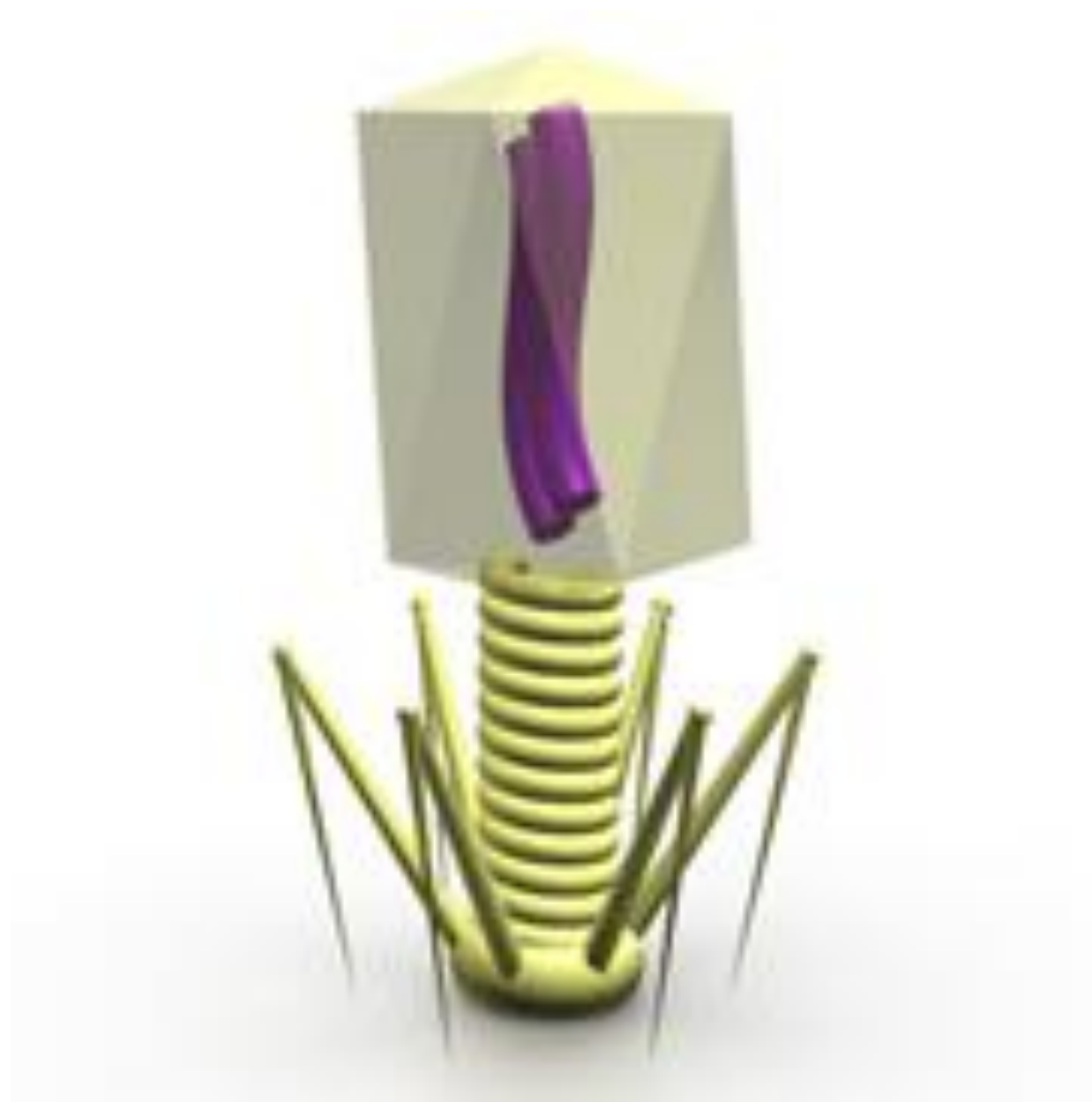
*"...bacterial colonies...became glassy and
transparent."*



*Sur un microbe invisible antagoniste des bacilles
dysentériques*

F d'Herelle - CR Acad. Sci. Paris, 1917; 165:373-5.

**"bacteriophage" means
"bacteria eater"**

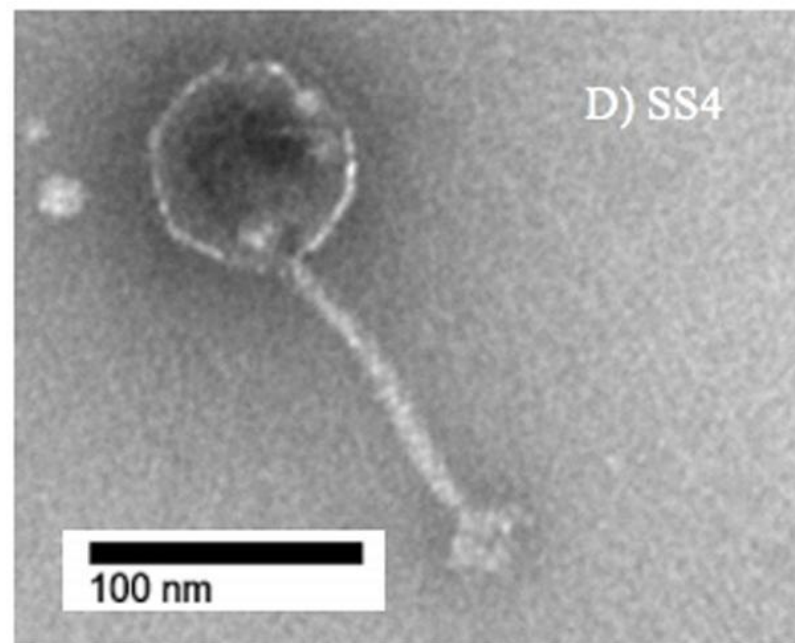
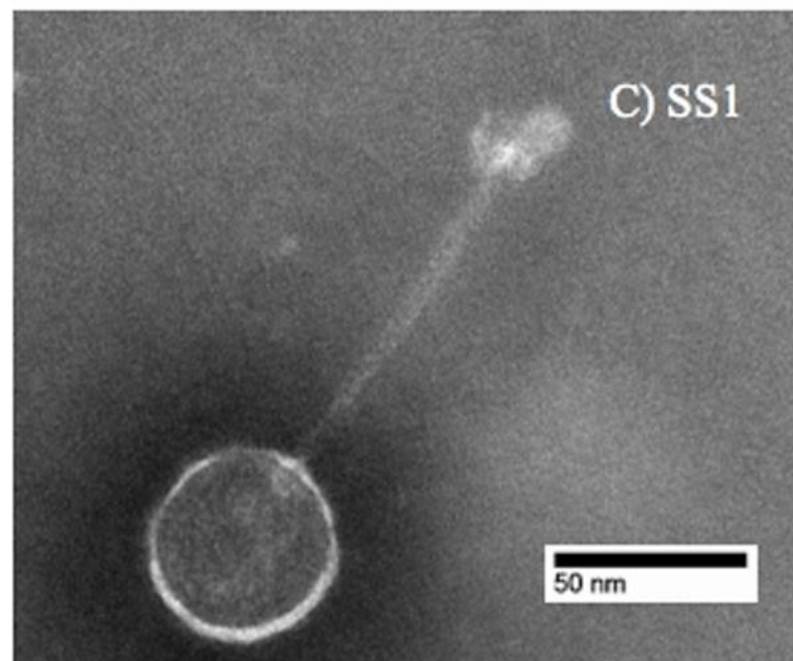
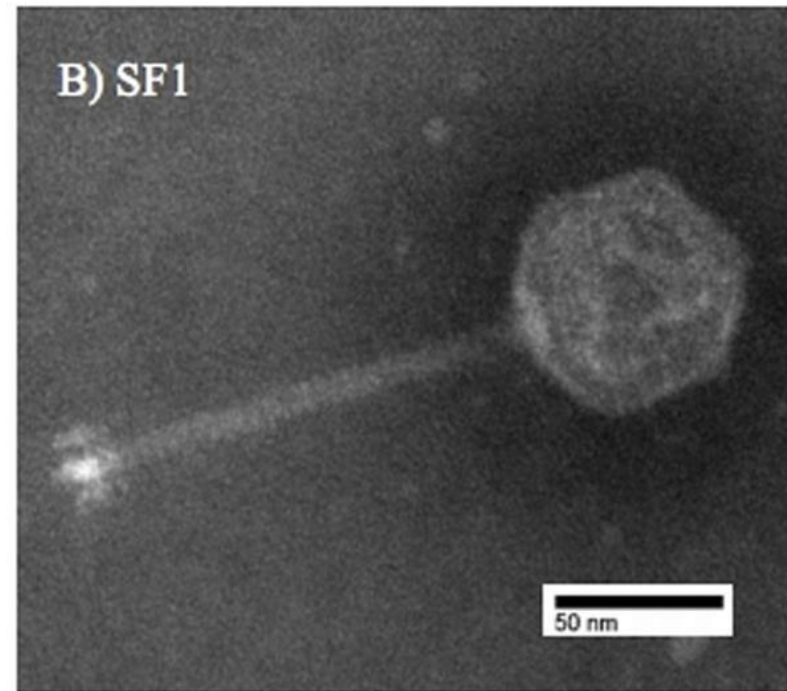
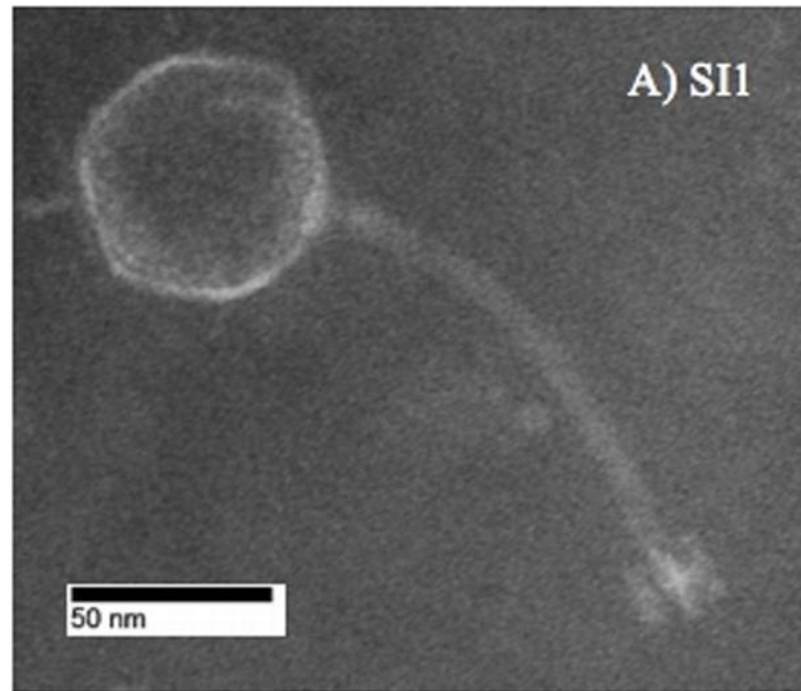


<https://www.nature.com/scitable/definition/bacteriophage-phage-293>

Characteristics of Phages

- Predators of bacteria
- Most abundant replicating biological entity on Earth
- Tiny
 - 0.2-0.4 microns (5 – 10X smaller than bacteria)
- Cause lysis (**killing**) of hosts - lytic phages only
 - Very specific to bacterial hosts
 - Harmless to humans, animals & plants

How do phages look like under the microscope?



Fong et al., 2017. Frontiers in Microbiology

How are phages obtained?

Characterization of Four Novel Bacteriophages Isolated from British Columbia for Control of Non-typhoidal *Salmonella in Vitro* and on Sprouting Alfalfa Seeds

 Karen Fong¹,  Brett LaBossiere¹,  Andrea I. M. Switt²,  Pascal Delaquis³,  Lawrence Goodridge⁴,  Roger C. Levesque⁵,  Michelle D. Danyluk⁶ and  Siyun Wang^{1*}

¹Food, Nutrition, and Health, University of British Columbia, Vancouver, BC, Canada

²Escuela de Medicina Veterinaria, Facultad de Ecología y Recursos Naturales, Universidad Andres Bello, Santiago, Chile

³Agriculture and Agri-Food Canada, Summerland, BC, Canada

Isolation of a *Salmonella* phage



Ditch water from
Abbotsford, BC

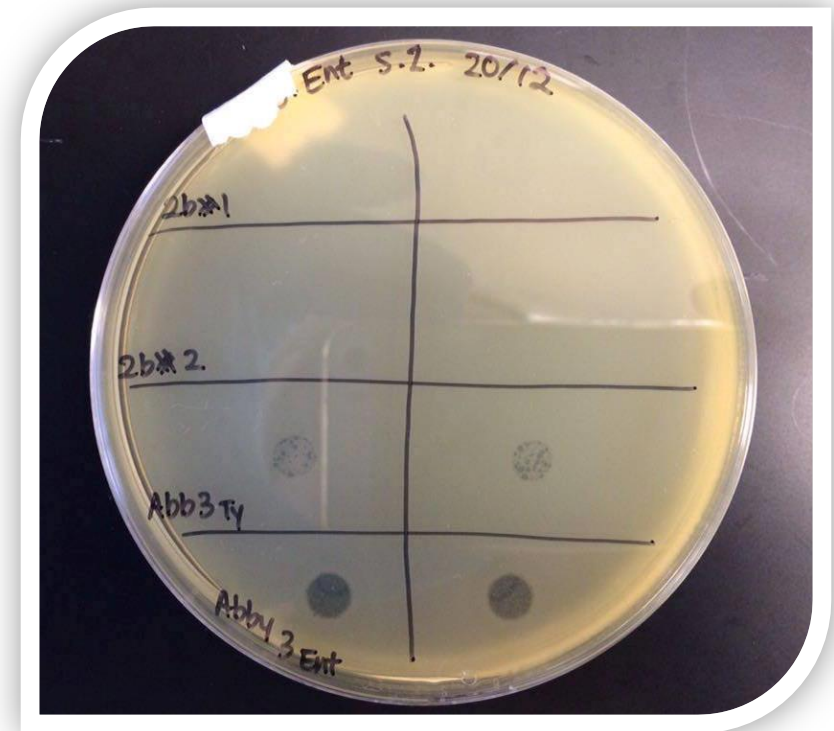


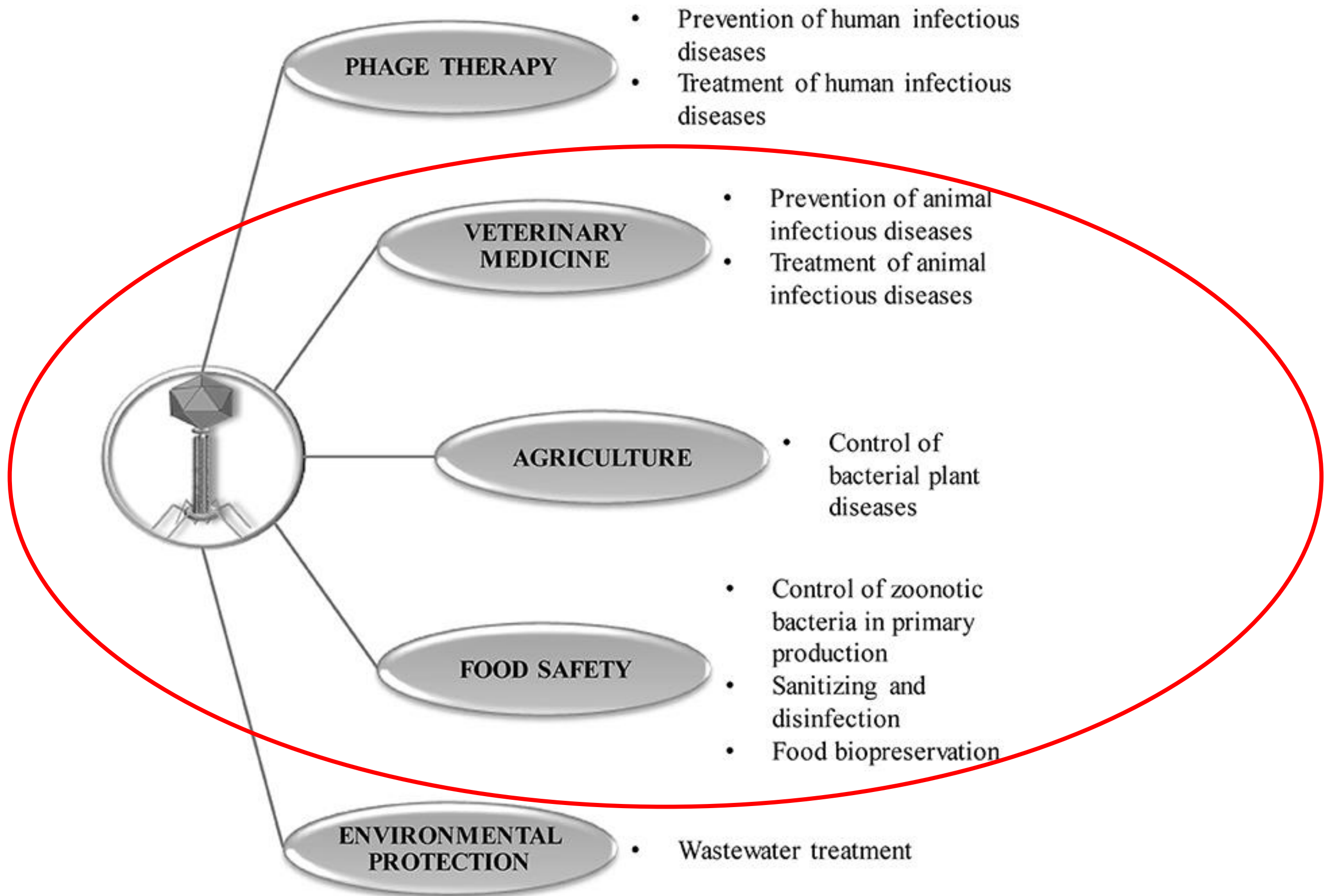
Characterization of *Salmonella* phage SI1



In vitro testing – done before they are tested on foods

- Host range
- Kills *S. Enteritidis* & *S. Typhimurium*?
 - causing highest proportions of *Salmonella* outbreaks worldwide
- Environmentally stable?
 - pH
 - temperatures
- Produces ~83 phage progeny in 25 mins





Fernández et al., 2018. Application of Bacteriophages in the Agro-Food Sector: A Long Way Toward Approval. Front. Cell Infect. Microbiol.

2. Bacteriophages

Phage Applications in the Agri-Food Sector

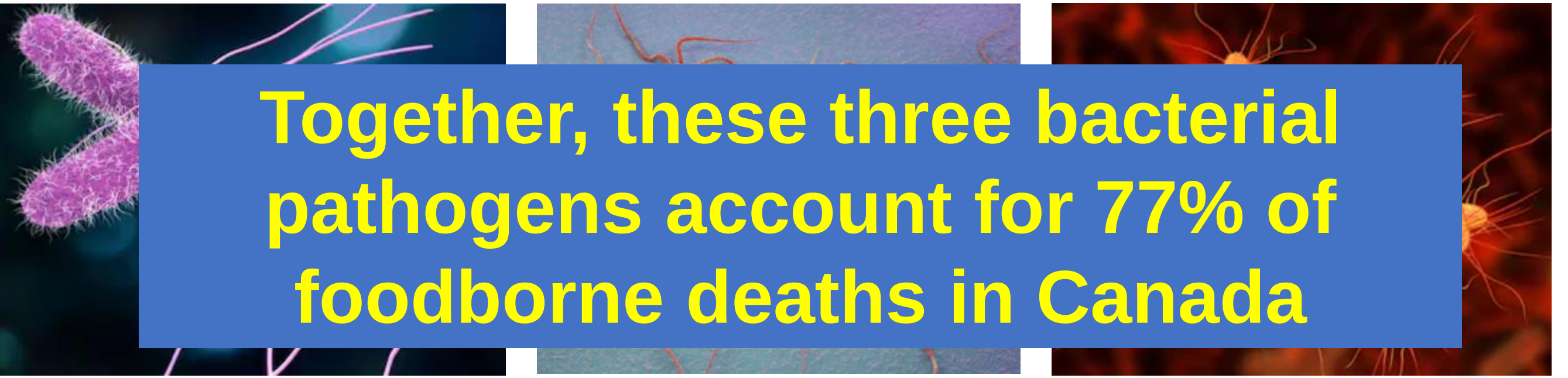
- Food Safety
 - Foodborne human pathogens
- Veterinary Medicine
 - Animal pathogens
- Plant Protection
 - Plant pathogens



Foodborne Human Pathogens

Every year, 4 million Canadians are affected by food-borne illnesses

- 11,600 hospitalizations
- 238 deaths



Together, these three bacterial pathogens account for 77% of foodborne deaths in Canada

**Non-typhoidal
*Salmonella***

No. 1 cause of hospitalizations by bacterial pathogens

Listeria monocytogenes

No. 1 cause of foodborne deaths

***E. coli* O157**

Cause kidney failures



Food Recall Warnings - High Risk

Food Recall Warnings and Allergy Alerts

Posted	Recall	Class	Distribution
2019-04-26	Food Recall Warning - Certain Celebrate brand frozen profiteroles and eclairs recalled due to <u>Salmonella</u>	Class 1	Alberta, British Columbia, Manitoba, New Brunswick, Nova Scotia, Ontario, Possibly National, Quebec, Saskatchewan
2019-04-26	Food Recall Warning - Ecoideas brand Organic Skinned Tigernuts recalled due to <u>Salmonella</u>	Class 2	Ontario, Possibly National, Quebec
2019-04-24	Updated Food Recall Warning - GPM brand Pea Shoots recalled due to <u>Listeria monocytogenes</u>	Class 1	Alberta, British Columbia, Possibly National, Saskatchewan
2019-04-19	Food Recall Warning - GPM brand Pea Shoots recalled due to <u>Listeria monocytogenes</u>	Class 1	Alberta, British Columbia
2019-04-18	Food Recall Warning - Kirkland Signature brand Harvest Burger - Veggie Burgers recalled due to possible presence of pieces of metal	Class 2	British Columbia, Ontario, Possibly National, Quebec
2019-04-14	Food Recall Warning - Feeding Change brand Young Thai Coconut Meat recalled due to <u>Salmonella</u>	Class 2	British Columbia, Ontario, Possibly National

Phages as Food Antimicrobials

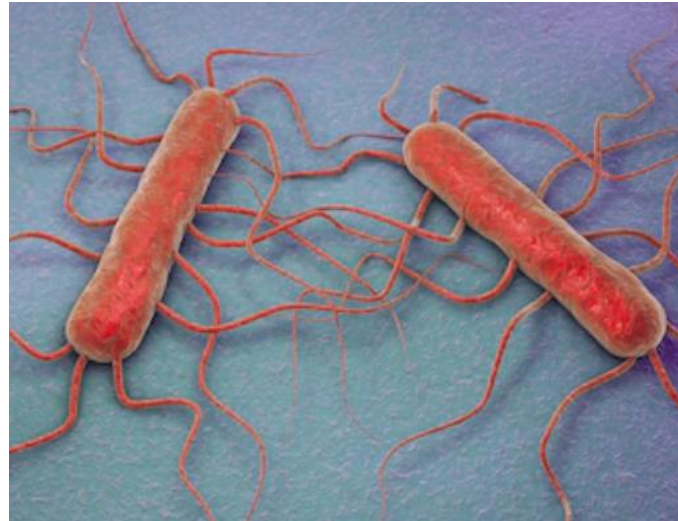
They offer several desirable attributes:

1. "Green" technology (organic, clean label, non-GM, Kosher)
2. Kill ONLY the specific target bacteria
usually do not cross genus or species barriers
3. Safe for human consumption
4. Self-replicating and self-limiting
5. Ubiquitously distributed in nature

Examples of Phage Preparations for Food Safety



Non-typhoidal *Salmonella*



Listeria monocytogenes



***E. coli* O157**



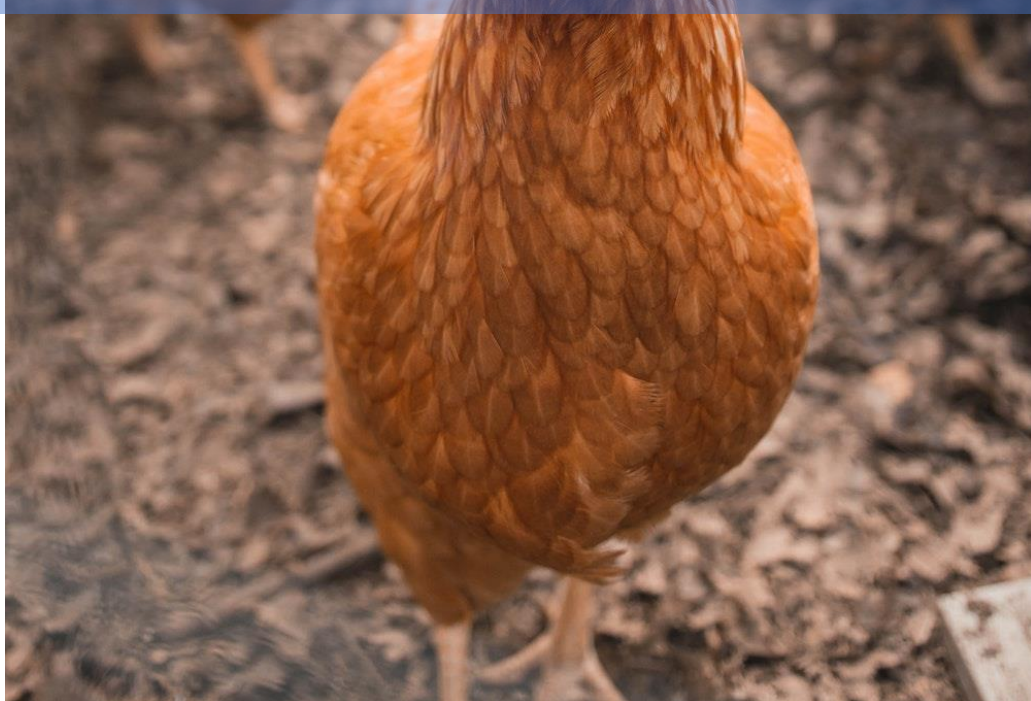
Considerations for Selecting Phage Preparations for Food Safety

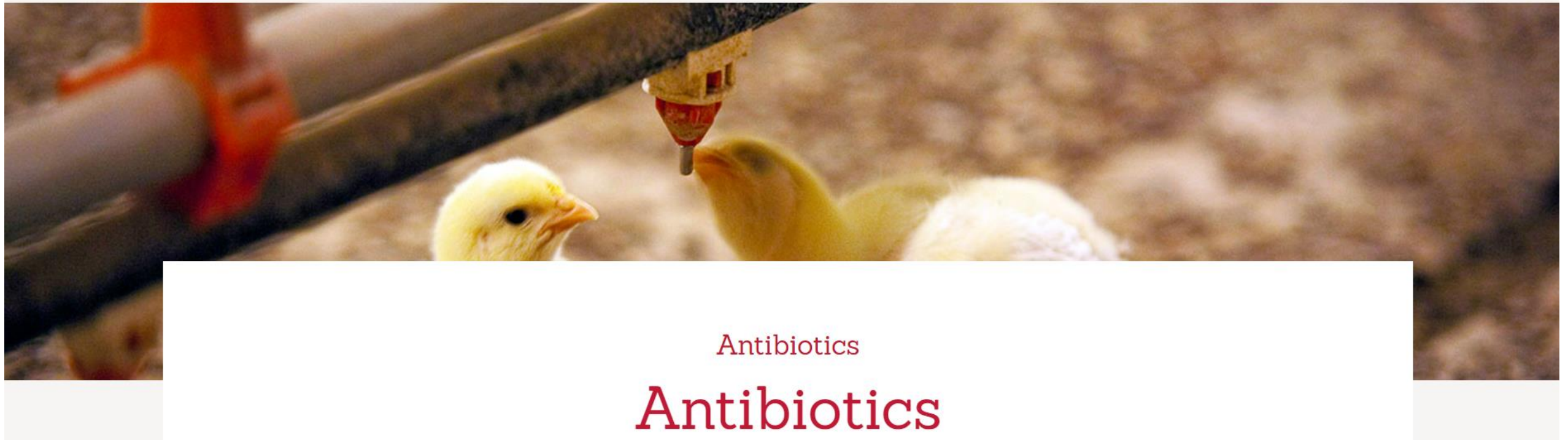
- Approval status? (by Health Canada?)
- Which strains do they target to?
 - E.g., *Salmonella* has more than 2,600 serotypes
- Which foods were used in the validation studies?
 - Any peer-reviewed publications?
- How effective this preparation is under a certain food processing/storage condition?

Examples of Phage Preparations for Veterinary Medicine



Salmonella, E. coli, Staphylococcus aureus...





...The biggest step in this strategy to date was the elimination of Category I antibiotics throughout the chicken sector. Category I antibiotics are those considered most important to human health, and as of May 15, 2014, their preventive use was no longer permitted in the Canadian chicken sector.

In May 2017, Chicken Farmers of Canada announced that the chicken sector would be eliminating the preventative use of Category II antibiotics by the end of 2018 and that a goal had been set to eliminate the preventive use of Category III antibiotics by the end of 2020.

<https://www.chickenfarmers.ca/antibiotics/>

Examples of Phage Preparations for Plant Protection



Fire blight in apples and pears
Erwinia amylovora

<https://www2.gov.bc.ca/gov/content/industry/agriculture-seafood/animals-and-crops/plant-health/insects-and-plant-diseases>



Bacterial speck in tomatoes
Pseudomonas syringae pv. *tomato*

<http://blogs.cornell.edu/livegpath/gallery/tomato/bacterial-speck-of-tomato/>

Limitations and Considerations

- Many phages are found to be effective in the lab, but will they perform on a farm, in a food processing facility or in foods?
- Bacteria can develop resistance to the treatment of the same phage.
- Will phages negatively affect human health (gut microbiota)?





Food Microbiology

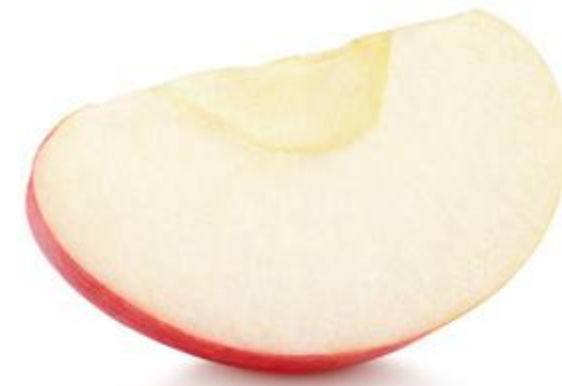
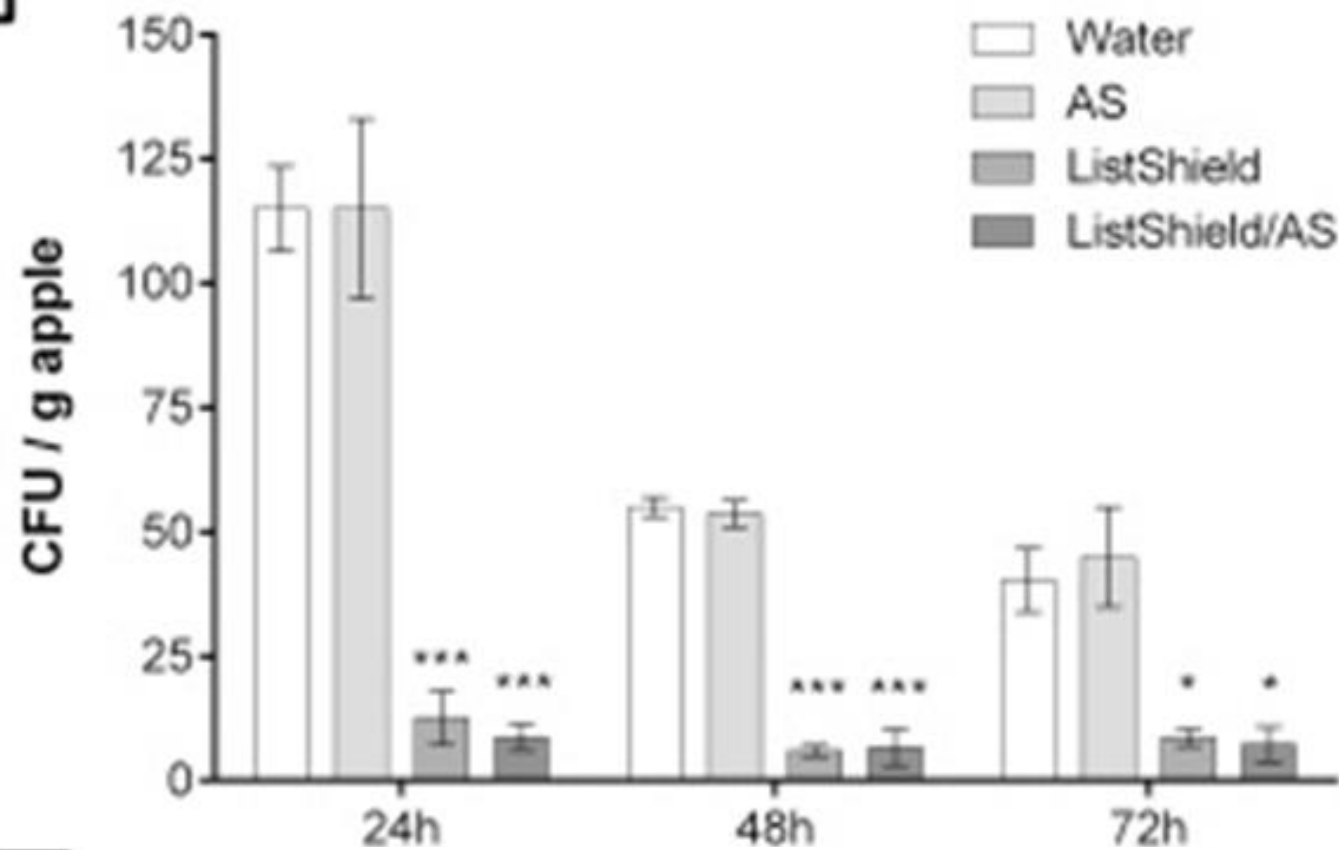
Volume 52, December 2015, Pages 42-48



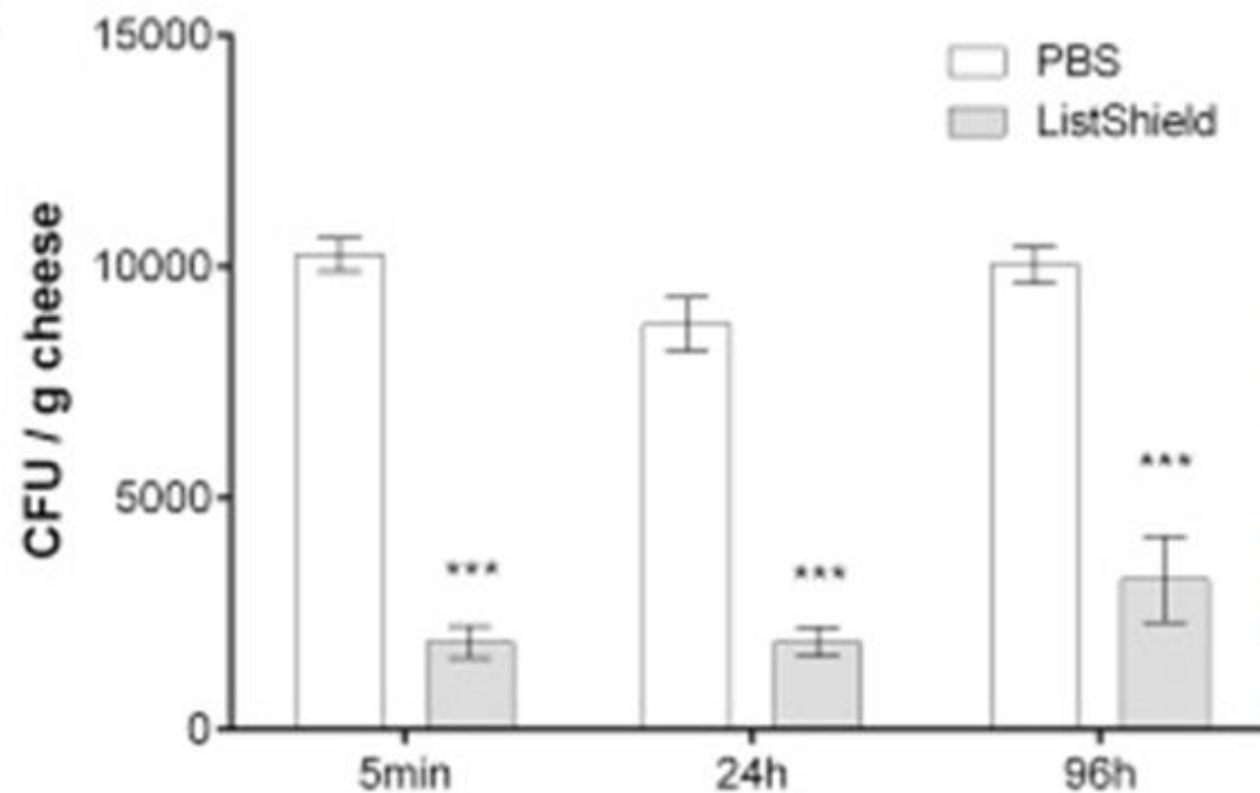
Bacteriophage cocktail significantly reduces or eliminates *Listeria monocytogenes* contamination on lettuce, apples, cheese, smoked salmon and frozen foods

Use of a bacterio

Meenu N. Perera , Tamar Abuladze, Manrong Li, Joelle Woolston , Alexander Sulakvelidze

A

82% reduction (0.8 log)

B

68% reduction (0.5 log)



Pasteurization – 5 log reduction



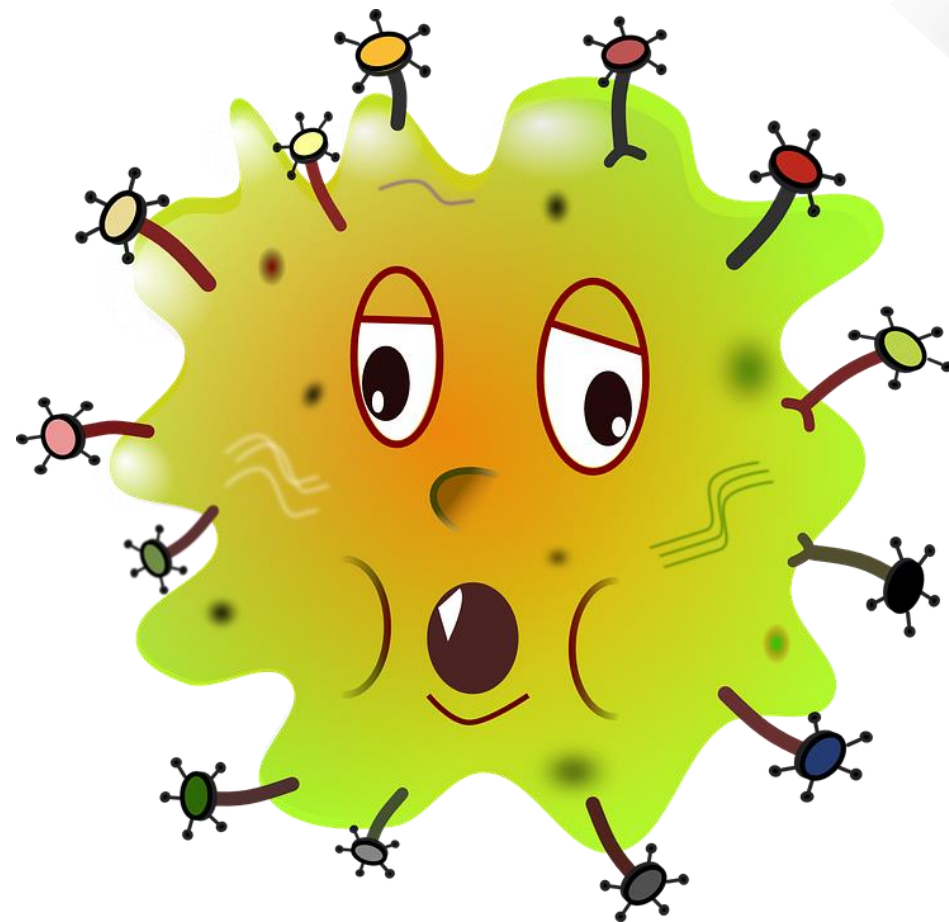
These foods differ in moisture content, acidity, nutrient content, native microbiota, storage conditions, and many others!





Optimal method of application: soaking, rinsing, spraying, microencapsulation...??

Bacteria do develop resistance to phage treatments.



“Now, here, you see, it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!”

- Lewis Carroll



The Red Queen hypothesis

(Van Valen, 1973):

“In a prey-predator relationship, changes (e.g. running faster) on the one side may lead to near extinction of the other side. The only way the second side can maintain its fitness is by counter-adaptation (running even faster).”

Bacteriophage **cocktail** for biocontrol of Escherichia coli O157:H7 - PLOS

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0195023> ▼

by K Ramirez - 2018 - Cited by 3 - Related articles

May 15, 2018 - The free **phages cocktail** was prepared with the phages Φ JLA23, Φ KP26, Φ C119 and Φ E142 at a concentration of 10^9 PFU/mL per phage.

Phage **cocktails** and the future of phage therapy. - NCBI

<https://www.ncbi.nlm.nih.gov/pubmed/23701332> ▼

by BK Chan - 2013 - Cited by 331 - Related articles

Future Microbiol. 2013 Jun;8(6):769-83. doi: 10.2217/fmb.13.47. **Phage cocktails** and the future of phage therapy. Chan BK(1), Abedon ST, Loc-Carrillo C.

Design of a Broad-Range Bacteriophage **Cocktail** That ... - NCBI - NIH

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5971607/>

by F Forti - 2018 - Cited by 11 - Related articles

May 25, 2018 - With the aim to assemble a **phage cocktail** that displays a broad host range and genetic diversity, we selected 6 virulent phages, PYO2, DEV, ...

INTRODUCTION · RESULTS · DISCUSSION · MATERIALS AND METHODS

Phage **cocktails** and the future of phage therapy - Future Medicine

<https://www.futuremedicine.com/doi/pdf/10.2217/fmb.13.47>

by BK Chan - 2013 - Cited by 333 - Related articles

resistance • bacteriophage. • diversity of bacterial pathogen • **phage cocktail**. • polyphage evie w. For reprint orders, please contact: reprints@futuremedicine.



Article | [OPEN](#) | Published: 26 May 2016

Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa*

Benjamin K. Chan, Mark Sstrom, John E. Wertz, Kaitlyn E. Kortright, Deepak Narayan & Paul E. Turner 

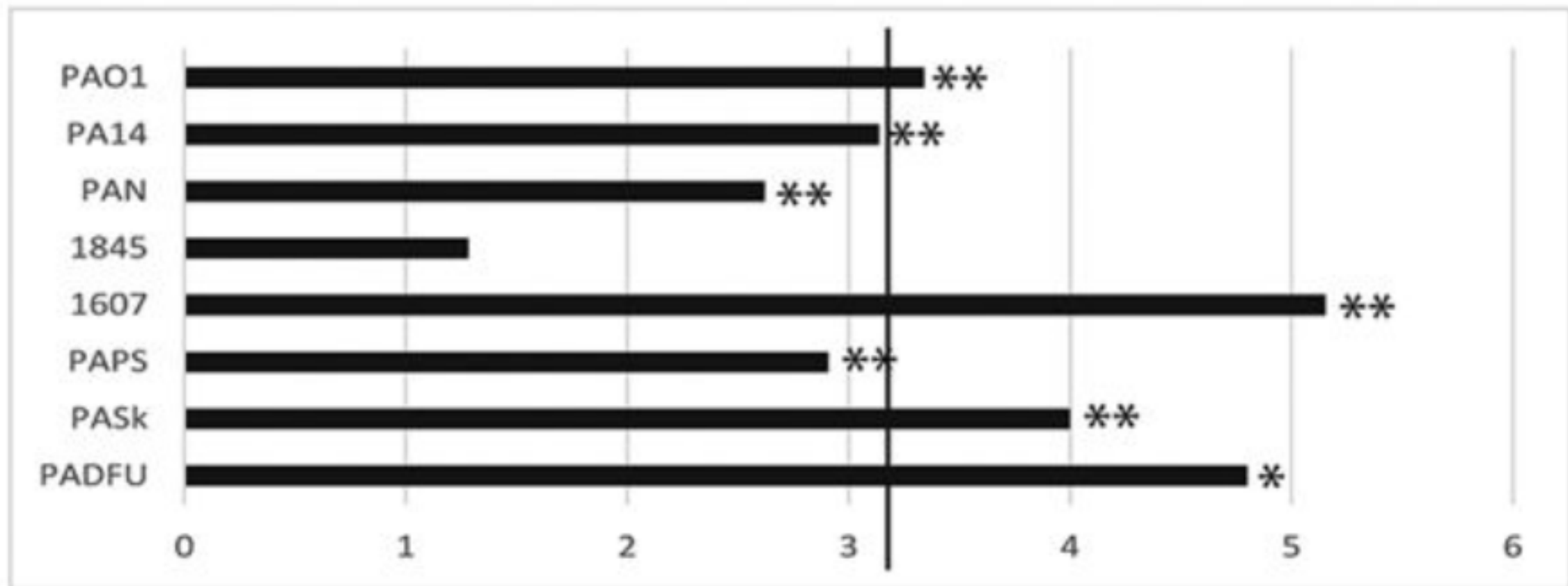
Scientific Reports **6**, Article number: 26717 (2016) | [Download Citation](#) 

Abstract

Increasing prevalence and severity of multi-drug-resistant (MDR) bacterial infections has necessitated novel antibacterial strategies. Ideally, new approaches would target bacterial pathogens while exerting selection for reduced pathogenesis when these bacteria inevitably evolve resistance to therapeutic intervention. As an example

Improved Antibiotic Efficacy

Tetracycline



Selection for phage resistance causes a trade-off resulting in enhanced sensitivity to 4 drugs drawn from different antibiotic classes.

Will Phages Interfere with Gut Microbiota?

PUBLIC RELEASE: 10-JUN-2018

Negative

Bacteriophages: Are they an overlooked driver of Parkinson's disease?

AMERICAN SOCIETY FOR MICROBIOLOGY

lytic *Lactococcus* phages



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Bacteriophages Synergize with the Gut Microbial Community To Combat *Salmonella*

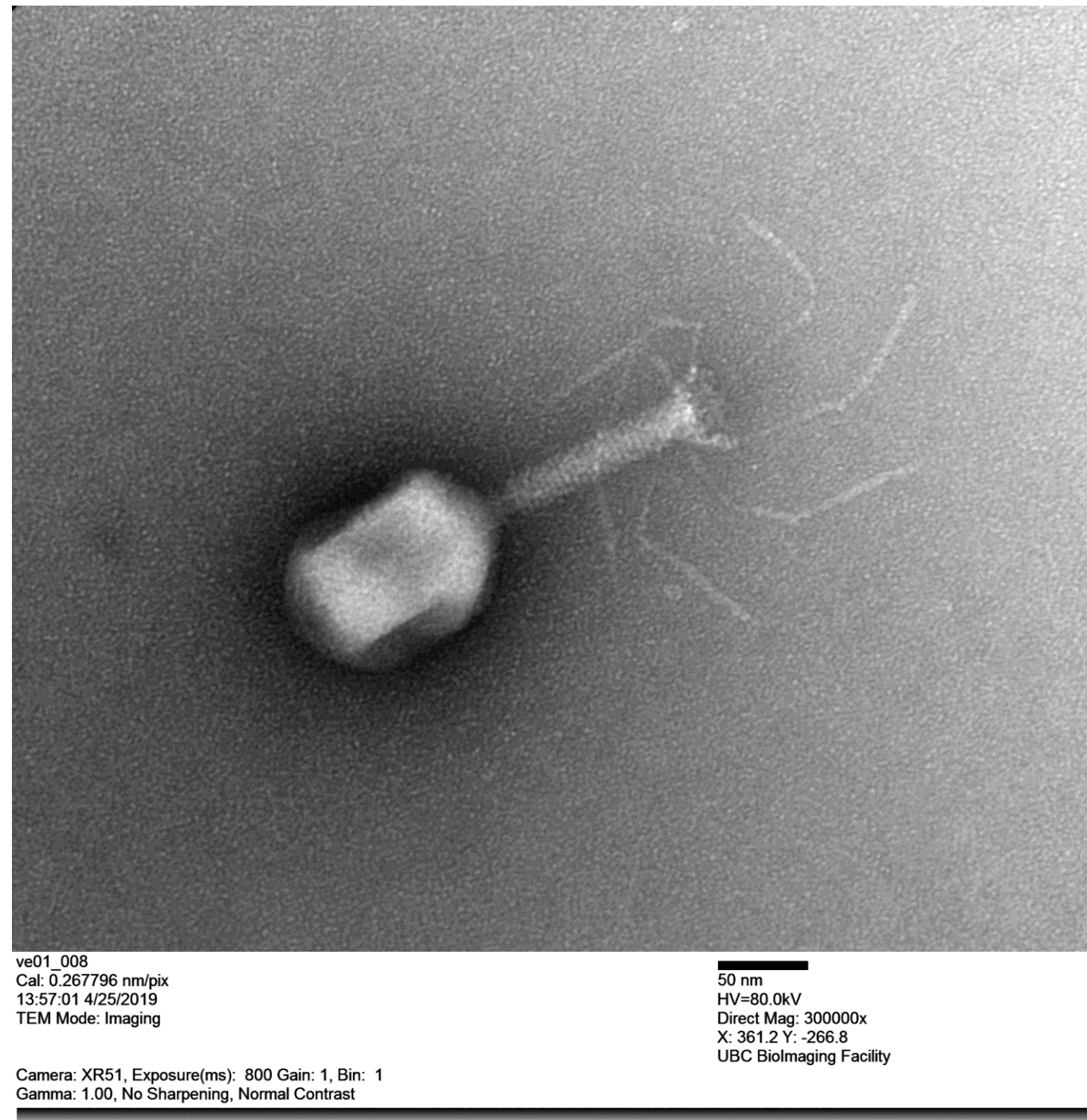
Yue O. O. Hu, Luisa W. Hugerth, Carina Bengtsson, Arlisa Alisjahbana, Maike Seifert, Anaga Kamal, Åsa Sjöling, Tore Midtvedt, Elisabeth Norin, Juan Du, Lars Engstrand

Katrine L. Whiteson, Editor

DOI: 10.1128/mSystems.00119-18

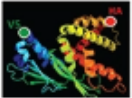


4. Limitations and considerations



This *E. coli* O157 phage is stable at pH>4,
but dies off when the pH is below 4

Ma et al., unpublished



1,244

Views

0

CrossRef citations
to date

54

Altmetric

Listen

Brief Report

A bacteriophage cocktail targeting *Escherichia coli* reduces *E. coli* in simulated gut conditions, while preserving a non-targeted representative commensal normal microbiota

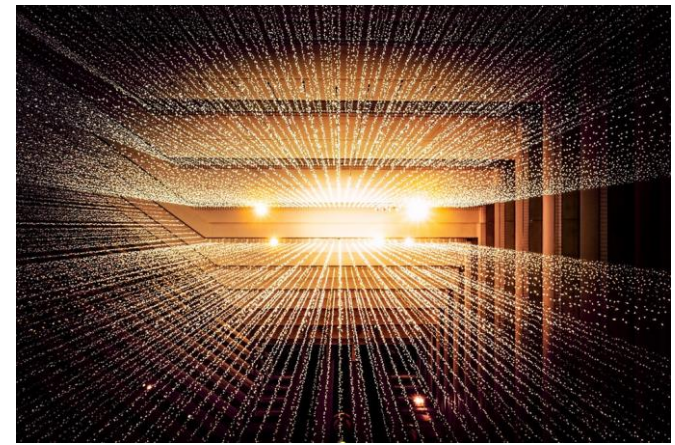
Tomasz Cieplak   , Nitzan Soffer, Alexander Sulakvelidze & Dennis Sandris Nielsen

Pages 391-399 | Received 15 Sep 2017, Accepted 26 Feb 2018, Accepted author version posted online: 08 Mar 2018, Published online: 24 Aug 2018

Considerations for Phage Preparations in Agri-foods

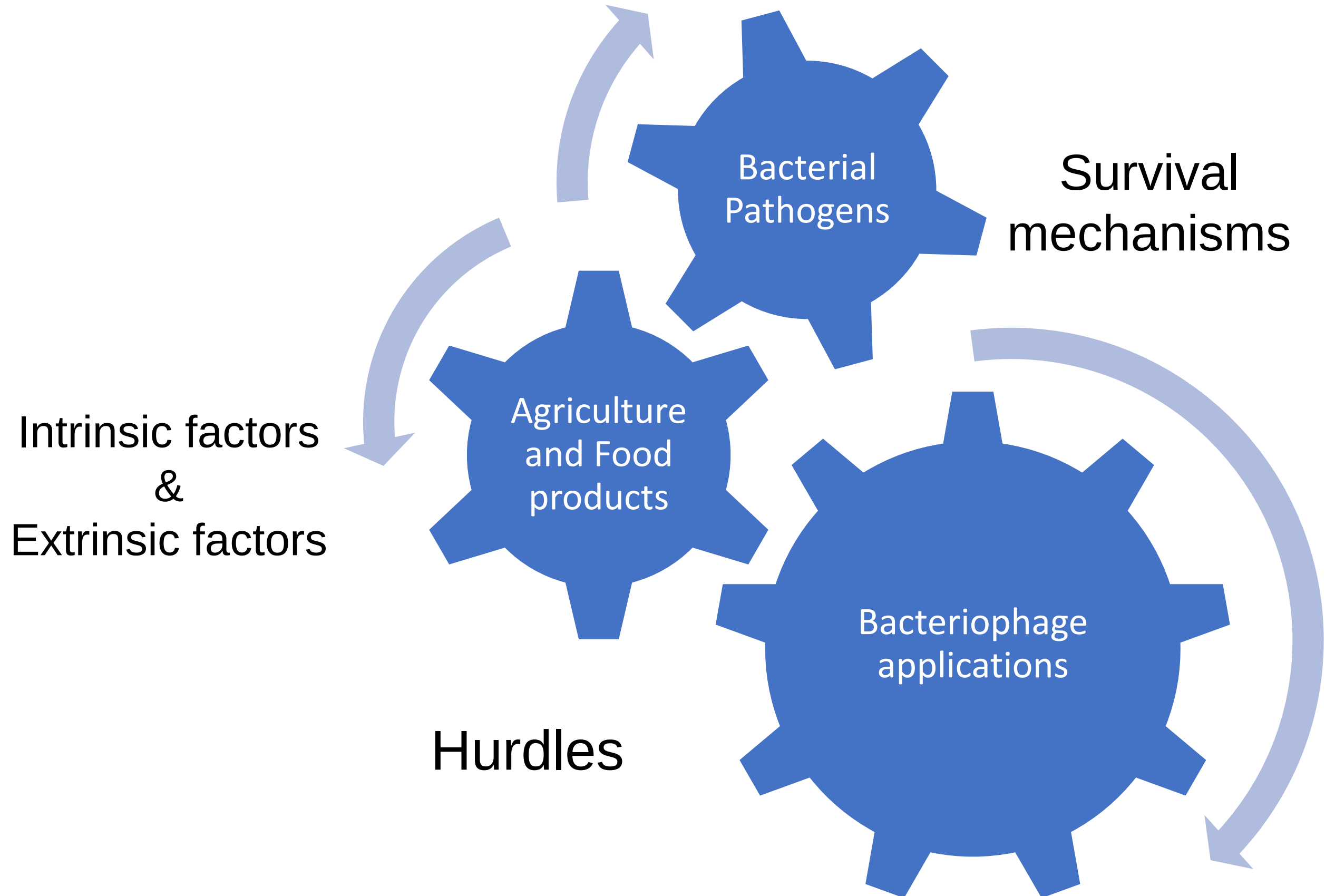
- Effective against the target bacterial pathogen
- Stable under the environmental factors relevant to their applications
 - Intrinsic (e.g., pH, water activity, nutrient content, biological composition/structure of foods)
 - Extrinsic (e.g., UV exposure, temperature, relative humidity, native microbiota)
- Do not cause harm to the gut microbiota
- Easy to apply
- Cost effective

What's Next for Phage Biocontrol in Agriculture and Food Products?



- Validating the effects on different bacterial serotypes/strains under a variety of relevant conditions
- Evaluating effects of hurdle treatments
- Evaluating optimal method of application
 - soak, rinse, spray, microencapsulation
- Assessing industry & consumer perception of phage treatment

Take-Home Messages



Graduate Students



Karen Fong, PhD Candidate



Thomas Brenner, PhD Student



Catherine Wong, PhD Student



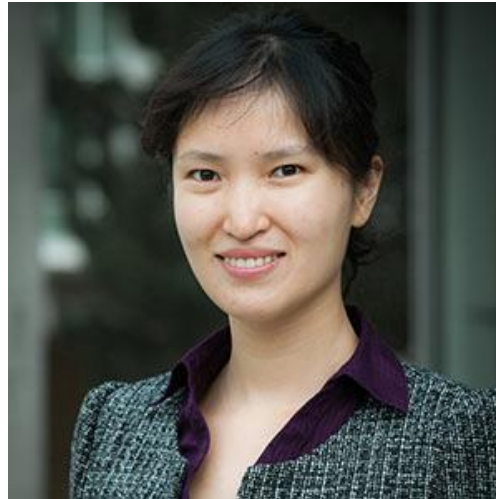
Yvonne Ma, MSc student

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Questions



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