BACTERIOPHAGES AND THEIR APPLICATIONS IN FOODS

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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://foodsecurity.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

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

By ALEXANDRA SIFFERLIN December 18, 2017

For more, visit TIME Health.
Salmonella Outbreak Number 16: This Time It Is Antibiotic Resistant And From Chicken
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.

https://www.cdc.gov/salmonella/infantis-10-18/index.html
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.


1. Superbugs
History of Bacteriophages

“...bacterial colonies...became glassy and transparent.”

Sur un microbe invisible antagoniste des bacilles dysentériques
"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 Nontyphoidal *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 Wang¹*

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2. Bacteriophages
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**
2. Bacteriophages

Phage Applications in the Agri-Food Sector

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

3. Applications of phages in foods
Foodborne Human Pathogens

Every year, 4 million Canadians are affected by foodborne 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

3. Applications of phages in foods
# Food Recall Warnings - High Risk

## Food Recall Warnings and Allergy Alerts

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

3. Applications of phages in foods
Examples of Phage Preparations for Food Safety

Non-typhoidal *Salmonella*  
*Listeria monocytogenes*  
*E. coli* O157

3. Applications of phages in foods
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/

3. Applications of phages in foods
Examples of Phage Preparations for Plant Protection

- Fire blight in apples and pears
  - *Erwinia amylovora*
  - [https://www2.gov.bc.ca/gov/content/industry/food-agriculture-seafood/animals-and-crops/plant-health/insects-and-plant-diseases](https://www2.gov.bc.ca/gov/content/industry/food-agriculture-seafood/animals-and-crops/plant-health/insects-and-plant-diseases)

- Bacterial speck in tomatoes
  - *Pseudomonas syringae pv. tomato*

3. Applications of phages in foods
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)?
Bacteriophage cocktail significantly reduces or eliminates *Listeria monocytogenes* contamination on lettuce, apples, cheese, smoked salmon and frozen foods

Meenu N. Perera, Tamar Abuladze, Manrong Li, Joelle Woolston, Alexander Sulakvelidze
4. Limitations and considerations
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 ΦJLA23, ΦKP26, ΦC119 and ΦE142 at a concentration of 10^9 PFU/mL per phage.

Phage cocktails and the future of phage therapy. - NCBI
by BK Chan - 2013 - Cited by 331 - Related articles

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
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.
Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa*

Benjamin K. Chan, Mark Sistrom, 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.

Chan et al., 2016
Will Phages Interfere with Gut Microbiota?

Negative

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

Positive

lytic Lactococcus phages

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
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

4. Limitations and considerations
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
  o soak, rinse, spray, microencapsulation

• Assessing industry & consumer perception of phage treatment
Take-Home Messages

Survival mechanisms

Bacteriophage applications

Hurdles

Agriculture and Food products

Bacterial Pathogens

Intrinsic factors & Extrinsic factors
Graduate Students

Karen Fong, PhD Candidate

Thomas Brenner, PhD Student

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Questions

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