Bacterial Viruses: the enemy of my enemy is my friend

Dr Cath Rees
School of Biosciences
Bacteriophagae (phage)

- Bacteriophage are viruses that specifically infect bacteria
- Independently discovered by two scientists:
  - Felix d’Herelle (1917) & Frederick Twort (1915)

- Both noted that these unknown agents had the ability to “eat” bacterial cells
  - Bacteriophage = “bacteria eater”
Bacteriophage hosts

- Like all viruses they have a limited Host Range
  - determines the type of cell infected
- Have evolved to bind to structures on the surface of correct host cell
- Tail structures help virus inject DNA into host
Phage infection

Bacteriophage growth

- Viruses replicate inside the host cell and produce 50+ phage per infection
- Produces enzymes to break open the host once the new viruses are made

![Graph showing bacterial growth and phage burst size](image)

**Bacterial growth**

**Phage Burst size = 100**

Nov 2013
Detecting TB cattle
The **FASTPlaqueTB Assay**

- Initially developed by UoN spin-out company for the detection of TB in human sputum samples
  - Low cost test designed for developing world markets
  - As sensitive as existing methods
  - Results gained in 48 h c.f 14 days for most rapid culture method
**FAST-Plaque**

Phage Amplification Assay

**Mycobacterial cell**

- INFECTION
  - PHAGE DESTROYED USING SELECTIVE VIRUCIDE

- NEUTRALISATION & ADDITION OF FAST GROWING CELLS TO FORM LAWN

**BACTERIOPHAGE D29**

(BROAD HOST RANGE)

**PLAQUES ON AGAR PLATE:**

GENUS IDENTIFICATION

Nov 2013
New assays: new sample preparation methods

- Method to detection and identification of Mycobacteria have been developed for:
  - Sputum
  - Milk
  - Cheese
  - Blood
New Applications: Milk Assay

- Test developed for paratuberculosis
  - Johne’s disease in cattle
  - good reproducibility and sensitivity demonstrated
New Applications: Milk Assay

- Test now being developed for Btb in raw milk
  - Specific application for artisan cheese producers
    - Raw milk used so TB not destroyed by pasteurisation
New Applications: Milk Assay

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When did the cows get TB?

• Which batches of cheese are safe?
• QA Milk test will ensure safety of product
Phage therapy
History of bacteriophage

• Development of their use as therapeutic agent performed between 1920 and 1940
  ◦ d’Herelle: cholera and other enteric diseases.
  ◦ Lieutenant-Colonel Morison: cholera and dysentery in India
    • over a million patients treated

• From 1927 onwards commercial products produced by many companies in UK, Europe and US
History of bacteriophage

- Some claims for therapeutic use included such unlikely conditions as herpes (a viral disease) or gallstones
- Research started to be published suggesting phage did not work
  - *da Costa Cruz in Brazil declared that bacteriophage therapy did not achieve effective treatment*
- **1930s and 1940s saw the discovery and development of antibiotics**
History of bacteriophage

• Therapeutic use of bacteriophages in the West being curtailed
• Clinical use continued in the countries of the former Soviet bloc

• 1980’s; Dr Betty Kutter established links with Phage therapy centre in Tbilisi, Georgia

Alfred’s story
http://blogs.evergreen.edu/phage/tbilisi-phage-therapy/alfreds-story/
Betty Kutter first met the musician Alfred Gertler at a phage conference on crutches after a catastrophic climbing accident 4 years earlier. His pulverized ankle had become badly infected, leading to osteomyelitis, multiple surgeries, IV pump of an antibiotic for a full year and other treatments. Amputation of his foot was recommended.
Alfred’s story

• Alfred saw an article in a magazine about the use of bacteriophages as antibiotics in Georgia
  • Mentioned the upcoming International Phage Biology meeting in Montreal where his parents lived
• Went along and met Betty who introduced him to the scientists she knew from Tbilisi
  • A sample of his bacteria was taken back to Georgia and the right phage identified to treat his infection
• But could not find a doctor in the US willing to administer the phage
Alfred’s story

• Betty arranged for Alfred to travel to Georgia for treatment
  • Phage were pumped into the open wounds in both sides of his ankle
  • Phage-soaked gauze applied to gradually release phage

• Within a few days, the fluid draining from his ankle was free of bacteria
  • Went home 2 weeks later armed with phage to finish the treatment
History of bacteriophage

- Wider interest in bacteriophage therapy reignited by increasing concern over antibiotic resistance
  - 1990’s successful demonstration of treatment of animals in well documented studies
  - Led to clinical trials over last 10 years
    - work being carried out to satisfy modern regulatory standards
    - **BUT** trials not all successful
      - Trial designs limited due to ethical constraints
So what is the down side?

- Bacteriophage are made up of proteins
  - Can cause an allergic response
  - Are cleared from the body by immune system
- Difficult to use for systemic infections
  - But good for skin infections

  e.g. Chronic ulcers that do not respond to antibiotics

http://polymerpharm.ge/index.php?name=Pages&op=page&pid=14
Can we overcome this?

- Delivery by medical devices

Infected wounds with $10^7$ cfu EMRSA15

Normal sutures

Bacteriophage sutures

University of Strathclyde
Treatment of gut infections

• Bacteriophage only target selected bacteria and so do not upset the body’s natural microflora
  
  • *Clostridium difficile* causes gut infections in hospital patients often following antibiotic treatment
  
  • Natural gut microbes destroyed and *C. difficile* growth gets out of control

Treatment of gut infections

Monday 21 October 2013

Discovery of virus that 'eats' bacteria that causes C. difficile could spell the end for hospital superbug

The technique represents a viable alternative to antibiotics for the treatment of bacterial infection

Pathogen control in foods
Listeria monocytogenes

- Bacterium that causes severe disease in humans
  - Very few cases each year but high number of deaths

358/603,359
Primarily affects elderly, very young, pregnant women and other immuno-compromised groups

126/389
Listeria monocytogenes

- Unusual in that it can grow at low temperatures
  - Putting food in the fridge does not stop it growing
Increase has occurred in most NHS regions and cannot be explained by seasonality, gender, underlying illness, or *L. monocytogenes* subtype.

FSA strategy 2010-2015

- The Agency aims to reduce the number of cases of listeriosis in the UK by the year 2015 through the *Listeria* Risk Management Programme.

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**LISTERIA RISK MANAGEMENT PROGRAMME (LRMP)**

<table>
<thead>
<tr>
<th>CONSUMER BEHAVIOURS / ACTIONS</th>
<th>PROCUREMENT / PROVISION OF FOOD TO THE VULNERABLE</th>
<th>INDUSTRY COMPLIANCE / ENFORCEMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AIM:</strong> To promote awareness of the risk of listeriosis and behaviours and actions that can help prevent the disease to key vulnerable groups of the UK population via those involved in advising and caring for these groups.</td>
<td><strong>AIM:</strong> To ensure that the risk of listeriosis is taken into consideration as part of public food procurement and food safety management processes in settings in which vulnerable people are cared for in the UK.</td>
<td><strong>AIM:</strong> To improve compliance of high-risk UK food industry sectors with existing legal requirements for <em>L. monocytogenes</em> in foods and ensure robust and consistent enforcement in this area.</td>
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Protecting food with phage

- Beginning to be introduced in to food and agriculture
  - Safety trials easier to perform

Now approved as a food processing aid in US, EU, Aus, Nz

http://micreosfoodssafety.com/en/listex-productdata.aspx#Uo-gU9L1ZP4
New applications emerging


...on a food product near you soon!