Uses of bacteriophages

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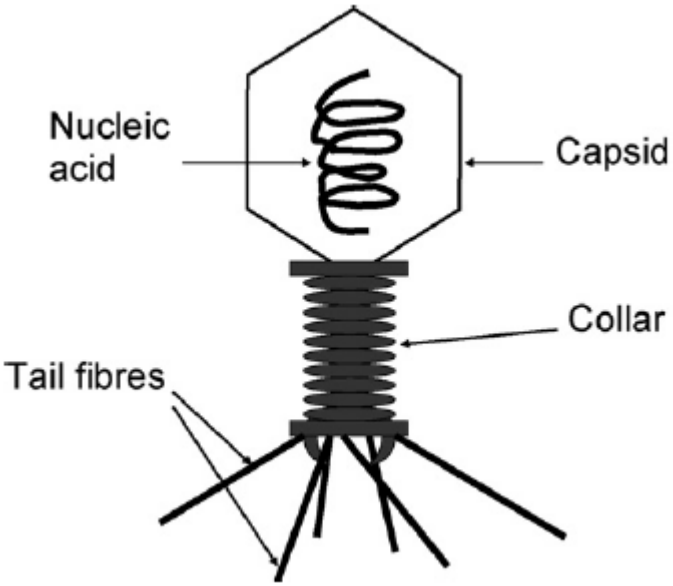
Discovery of bacteriophages

- World War I
- Viruses ”eaters of micro-organism” discovered by Felix d’Herelle
- Isolation of filterable entities able to lyse bacterial culture
- Small cleared zones on plate called “plaques”
- Treat World War I soldiers affected by *Shigella* dysentary
- Have been used clinically in Eastern Europe and the former Soviet Union since 1919
- Polish and Soviets administered phage orally, topically or systemically to treat antibiotic resistant pathogens, including *Staphylococcus, Streptococcus, Klebsiella, Escherichia, Proteus, Pseudomonas, Shigella* and *Salmonella*
- Success rate : 80-95% of phage therapy
- Not taken seriously in the West due to discovery of antibiotics
- Emergence of multi-drug-resistant pathogen, growing interest in phage therapy

Alisky *et al* 1998; Hanlon 2007; Chanishvil 2012

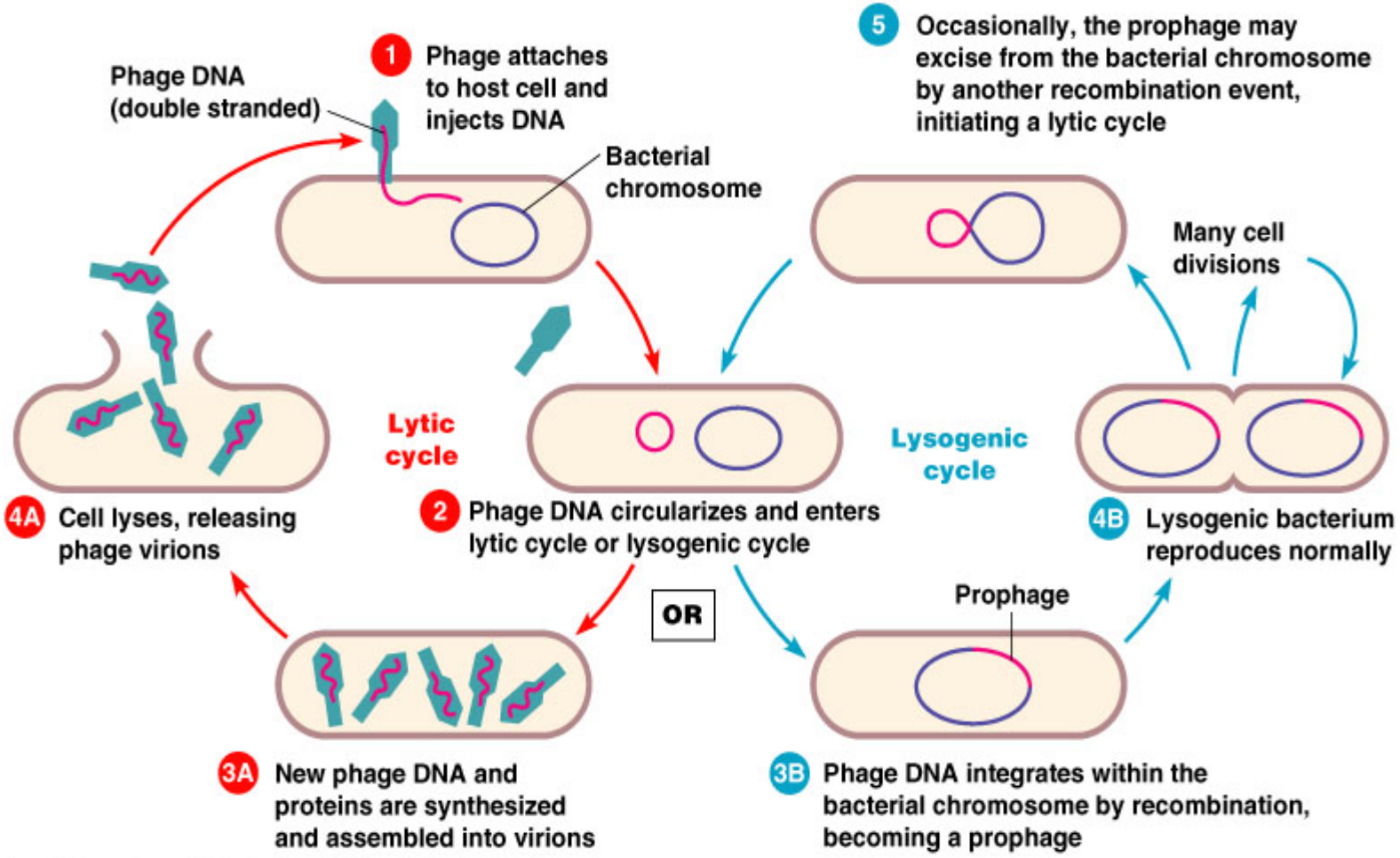
Bacteriophages(phages)

- Most abundant ‘life’ forms on Earth
- Water, soil and air
- Estimated 10^{32} bacteriophages in total on the planet
- Specifically target bacteria, not mammalian cells
- Each phage only attacks one species
- Head: Icosahedral shape capsid, RNA/DNA
- Tail: usually 6 fibers with receptors at tips, recognition of attachment sites on bacterial cell surface e.g. teichoic acid, oligosaccharide, peptidoglycan
- Two types of life cycles: lytic (virulent phage) or lysogenic (temperate phage) cycle



Brussow et al 2005; Hanlon 2007

Lytic cycle and lysogenic cycle



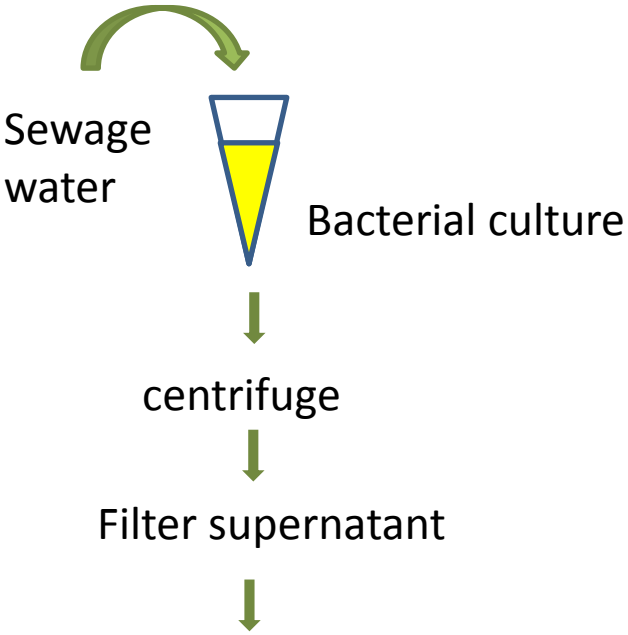
Potential use of bacteriophages in treatment of bacterial infections

- Emergency of multi-drug-resistant strains of pathogens
- New antibiotics development rate slow
- Cannot replace old drugs
- Alternative strategies for treatment of antibiotic-resistant pathogens
- Lytic phages preferred
- lysogenic phages, no host death, transfer undesirable genes (e.g. toxin genes/antibiotic resistance genes) through transduction
- Advantages over antibiotic:
  - Narrower host range
  - No side effect
  - Replicate exponentially, a self-regulating tool
  - None can replicate in eukaryotes

Kutateladze, Adamia 2010; Hodgson 2013

Isolation of bacteriophages

- Sewage water
- Bacterial culture in broth
- Inoculate the sewage water
- (specific phage binds to and replicates in host cells, lyses the bacteria eventually)
- Centrifuge to remove all cell debris
- Filter supernatant with 0.22 micron filter
- Spread O/N culture on agar plate (confluent lawn of bacteria)
- Add filtrate to center of plate
- A plaque (clear zone) will be observed where phage infect and lyze the bacteria
- phage capable of infecting the host strain has been isolated



Cerveny *et al* 2002

Phage therapy: treatment of diabetic foot infections

- Wound infections leading to foot and leg amputation: 30-50% diabetic patients
- 43% MRSA infections
- Potential use of lytic phages for treating MRSA infections in diabetic patients
- Phage MR-10 (broad host range)
- Localized foot infection caused by MRSA in diabetic mice evaluated

Chhibber *et al* 2013

Phage therapy: treatment of diabetic foot infections

- BALB/c mice injected with alloxan monohydrate
- toxic glucose analogue (150mg/kg body weight)
- Selectively damage beta-cells(insulin-producing cells) in the pancreas
- Lytic Phage MR-10
- Linezolid
- Combination therapy

Chhibber *et al* 2013

Phage therapy: treatment of diabetic foot infections

Chhibber *et al* 2013

Lesion scoring:

0- No redness, no oedema with thickness of hindpaw in the range of 0.14–0.19 mm.

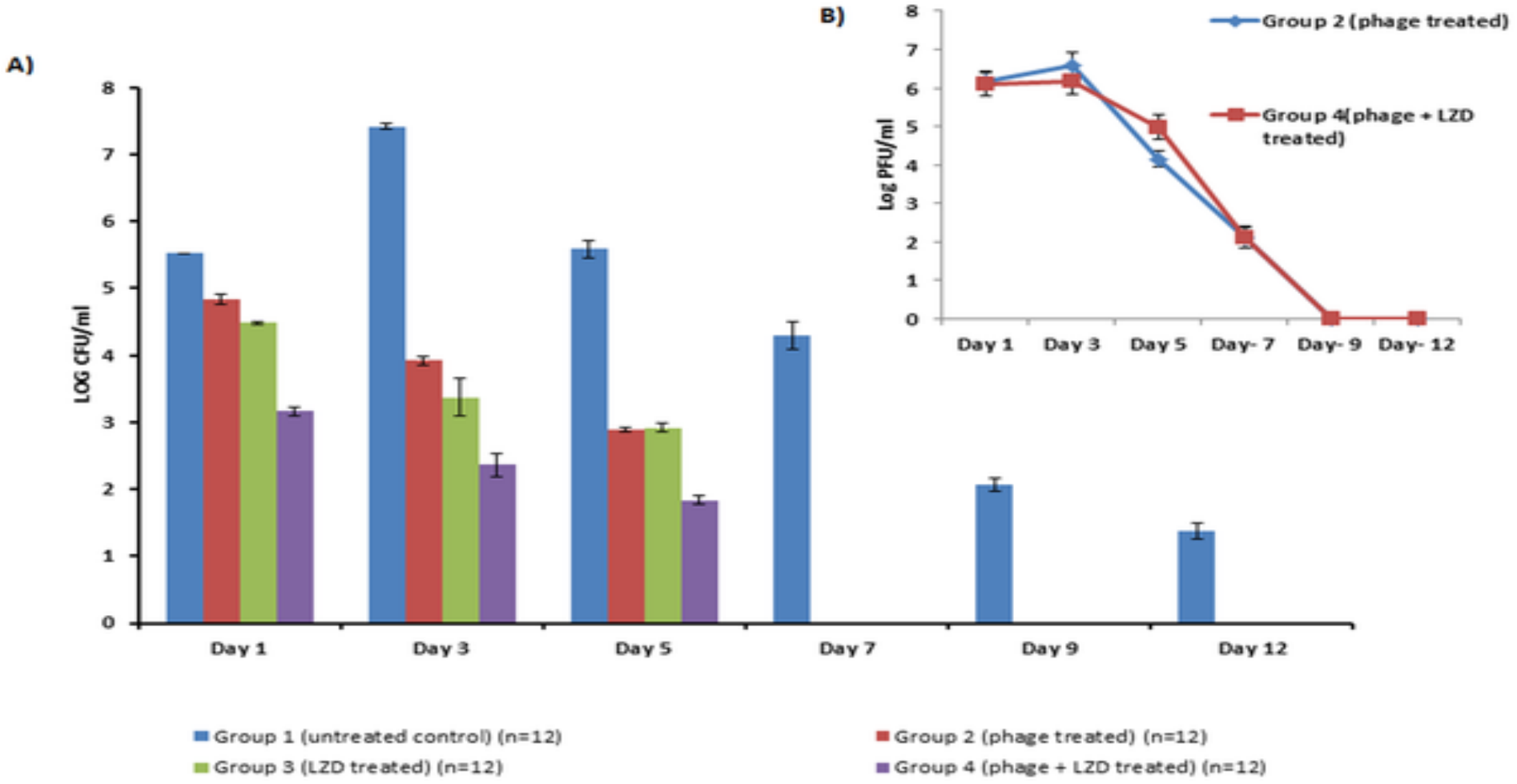
1- Slight redness with slight oedema with thickness of hindpaw in the range of 0.20–0.25 mm.

2- Visible redness and moderate oedema with thickness of hindpaw in the range of 0.26–0.30 mm.

3- Severe redness and visible oedema with thickness of hindpaw in the range of 0.31–0.36 mm.

- 4- Severe redness and pronounced visible oedema with thickness of hindpaw in the range of 0.37–0.42 mm.

Phage therapy: treatment of diabetic foot infections



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Phage therapy: treatment of diabetic foot infections

	Untreated Control		Phage MR-10 Treated		LZD treated (25 mg/kg/per oral)		Phage MR-10 + LZD treated	
Days	Lesion score	Oedema (mm)	Lesion score	Oedema (mm)	Lesion score	Oedema (mm)	Lesion score	Oedema (mm)
Day 0	0	0.16±0.01	0	0.17±0.01	0	0.17±0.02	0	0.18±0.02
Day 1	1	0.25±0.02	1	0.22±0.02	1	0.24±0.02	1	0.22±0.02
Day 3	3	0.35±0.02	1	0.28±0.01	2	0.3±0.02	2	0.25±0.02
Day 5	4	0.40±0.01	2	0.26±0.02	1	0.25±0.04	1	0.21±0.02
Day 7	3	0.32±0.02	1	0.2±0.01	0	0.18±0.01	0	0.17±0.01
Day 9	1	0.21±0.01	0	0.18±0.02	0	0.18±0.02	0	0.16±0.01
Day 12	0	0.17±0.01	0	0.17±0.01	0	0.16±0.02	0	0.17±0.01

Data is mean±S.D of a minimum of four independent values.
doi:10.1371/journal.pone.0056022.t002

Chhibber *et al* 2013

Phage therapy: treatment of diabetic foot infections

- Similar efficacy to linezolid
- Combination therapy more effective in controlling entire process of hindpaw infection in diabetic mice compared to antibiotic/phage alone
- Linezolid resistant strains emergence
- Decrease development of resistant strain
- Effective strategy for patients not respond to conventional antibiotic therapy

Phage Therapy: rescue mice with VRE bacteremia

- GI tract colonization of vancomycin-resistant *Enterococcus faecium*(VRE)
- Predispose individual to VRE bacteremia, esp. immumocompromised patients
- Clinical isolates of VRE used to induce bacterermia in mice by intraperitoneal (i.p.) injection of 10^9 CFU
- Fatal within 48 h
- *Entercoccus* phages ENB6 isolated from raw sewage at a sewage treatment plant
- Single i.p. injection of $3x10^8$ PFU of lytic phage administered 45 min after infection
- Rescue 100% of mice
- Even treatment delayed to the point where all mice are moribund
- ~50% mice rescued by a single injection of phages

Biswas *et al* 2002

Phage Therapy for infections in cancer patients

- Performed in 20 cancer patients (ages 1–66 years, 17 with solid tumors, 3 with hematological malignancies)
- All patients had concurrent bacterial infections (*Staphylococcus aureus,* 8 patients; *Pseudomonas aeruginosa*, 9; *Klebsiella pneumoniae,*6; *Klebsiella oxytoca,* 2; *Escherichia coli,* 6)
- Homogenous (12 patients) and mixed (8 patients) infections
- All patients previously treated with antibiotics without apparent response
- In all patients, bacteriophages were administered orally 3 times
- daily.
- Eight of them had localized bacteriophage treatment in addition to the oral protocol.
- No side effects of the therapy were observed
- Cure of infection achieved in all cases (cessation of suppuration, closure of wounds, eradication of pneumonia, etc.)

Weber-Dabrowska, Górski 2001

Safety of phage therapy in human

- Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy
  - T4 dose (10^3 PFU/ml), a higher phage dose (10^5 PFU/ml)
  - No adverse events related to phage application were reported
  - neither T4 phage nor T4-specific antibodies were observed in the serum of the subjects at the end of the study

- Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial
  - 42 patients with chronic venous leg ulcers, 39 patients completed the trial
  - treated for 12 weeks with either a saline control or bacteriophages targeted against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli*
  - No increase in adverse reaction associated with phage cocktail application

Bruttin, Brüssow 2005; Rhoads *et al* 2009

Commercial phage products

- JSC Biochimpharm, Biopharm L Limited
  - Republic of Georgia
  - Various phage lysates mixed for intestinal problems e.g. dysentery, salmonellosis and colitis
  - Patented and licensed liquid and tablet phage product
- EBI Food Safety
  - Netherlands
  - LISTEX P100^{TM} a cocktail of phage against *Listeria*
- Phico Therapeutics (UK), Viridax(USA)
  - Anti-MRSA phage product
  - Pre-clinical
- BioControl
  - Uk
  - Phage product for Pseudomonas infections of ear
  - Phase II clinical trial completed

Housby, Mann 2009

Enzybiotics as antimicrobials

- Bacteriophage-derived endolysins
- Released from host cell by 2 phage genome-encoded protein:
- Holins disrupt cell membrane
- Endolysins digest cell wall, peptidoglycan hydrolase
- Contain two functional domains:
- cell wall binding module(CWBM), bind to species-specific carbohydrate epitope in cell wall
- catalytic domain, cleave peptidoglycan bonding
- Lyse Gram +ve bacteria when applied exogenously
- *Staphylococcus aureus* Phage P68 *lys16* gene, cell lysis in clinical isolates of *S. aureus*
- To date, no reports of resistant strain against phage endolyins

Takáč et al 2005; Hermoso *et al* 2007; Nelson *et al* 2012; Hodgson 2013

Enzybiotics as antimicrobials

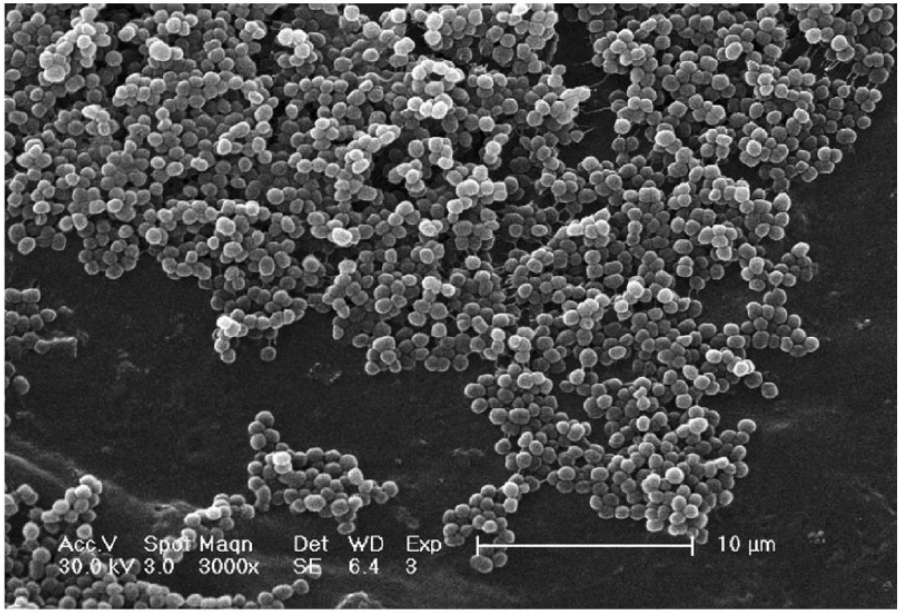
- Novel Bacteriophage lysin, broad lytic activity
- Phage lysin (PluSs2) derived from *Streptococcus suis* phage
- Activity against MRSA, VISA, *S. epidermidis, Listeria*, GBS, GES, GGS, *S. pyogenes*, *S. pneumoniae*
- Protect against mixed infection
- 128 g/ml *in vitro* reduced MRSA and *S. pyogenes* growth by 5 logs and 3 logs within 1 h respectively
- A single, 2-mg dose of PlySs2 protected 92% (22/24) of the mice in a bacteremia model of mixed MRSA and *S. pyogenes* infection
- Effective therapeutic

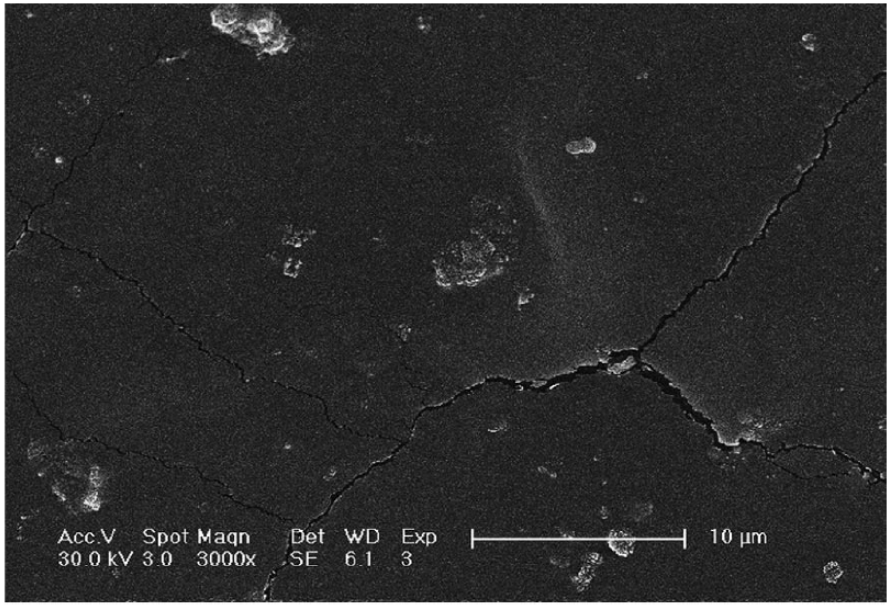
Gilmer DB *et al* 2013

Phages as biocontrol agents

- Reduce biofilm formation on indwelling catheters
- 1/3-1/2 nosocomial endocarditis cases due to infected intravascular catheters in US
- ~250,000 cases of catheter-related bloodstream infections, mortality 12-25%
- *S. aureus*, *S. epidermidis*, forming biofilm
- Current treatment: antimicrobial coating of catheters, disinfection of insertion site
- Biofilm, antimicrobial resistant strain
- A novel approach using a phage pretreatment step
- biofilm formation by *S. epidermidis* after 24 h on catheter significantly reduced in the presence of phage 456

Curtin, Donlan 2006





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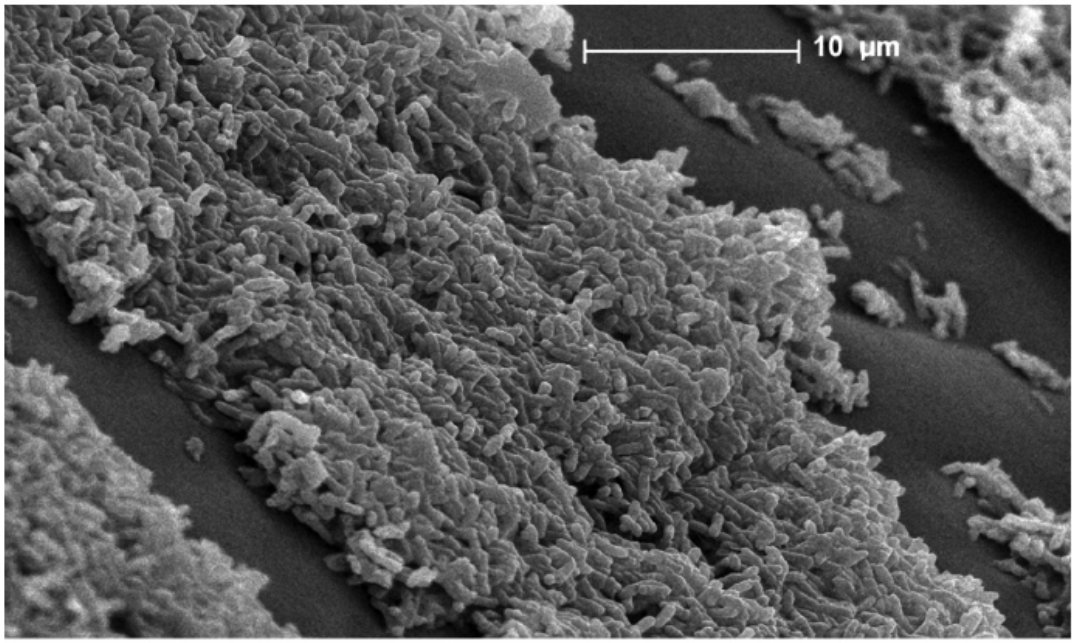
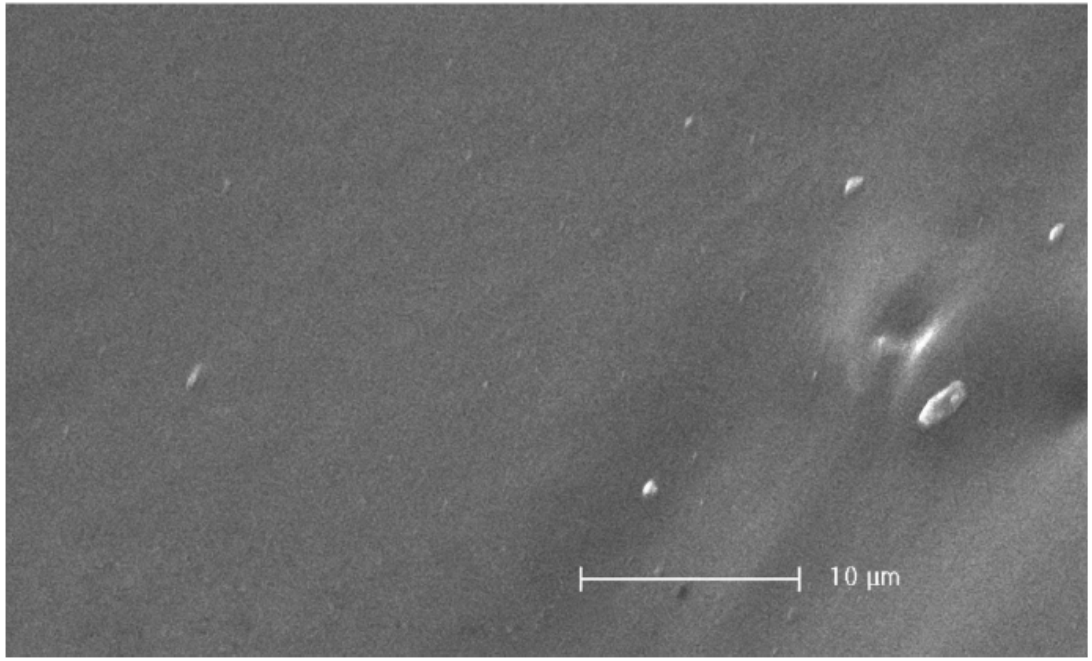


Catheter after biofilm formation by *P. aeruginosa* for 24 h



Catheter pretreated with *P. aeruginosa* phage M4 and exposed for 24 h to *P. aeruginosa*

Fu *et al* 2009

Phages as biocontrol agents

- Application of phages to control bacteria on food
- Food preservation and safety
- Food-borne illness by *Salmonella*
- Phage cocktail composed of 3 lytic phages (UAB_Phi 20, UAB_Phi78, and UAB_Phi87)
- Food (pig skin, chicken breasts and packaged lettuce) experimentally contaminated with Salmonella
- Significant bacterial reduction treated with phage cocktail

Spricigo *et al* 2013

Phages as biocontrol agents

- US Food and Drug Administration, 2006
- Approved use of phage to be added to meat and poultry products
- Mixture 6 Purified phages allowed to be used on read-to-eat (RTE) meat and poultry products
- As antimicrobial agent against *Listeria monocytogenes*
- Effective against 170 strains
- Directly consumed without additional cooking, capable multiply at low temperatures
- Spray application to RTE food surface e.g. lunch meats and hot dogs prior to packaging
- Commercially available phage preparations for food safety applications Listex™ P100 (left) and ListShield™ (right).

Peek R, Reddy KR 2006; Sulakvelidze 2013

Phages as biocontrol agents

- Phage VPp1 against *Vibrio parahaemolyticus*
- Closely associated with oysters normally consumed raw or lightly cooked

- Phage FAHc1 against *E. coli* O157 on beef

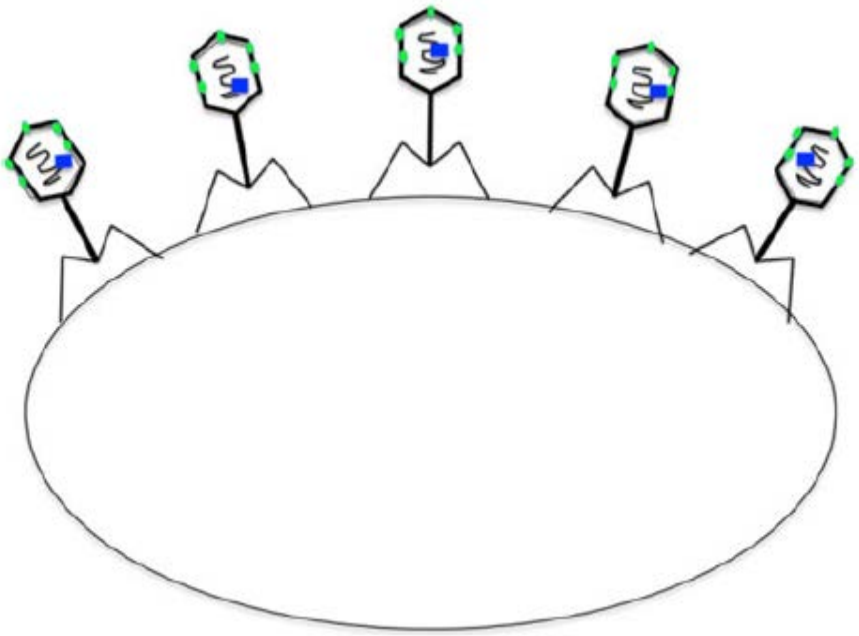
- Listex P100 to control L.monocytogenes on fresh-cut fruits and fruit juices

- *Lactococcus garvieae*, opportunistic pathogens, infectious diseases in fish
- Phage PLgY in aquaculture

Rong *et al* 2013; Hudson *et al* 2013; Oliveira *et al* 2013; Nakai, Park *et al* 2001

Genetically engineered phages as molecular tools

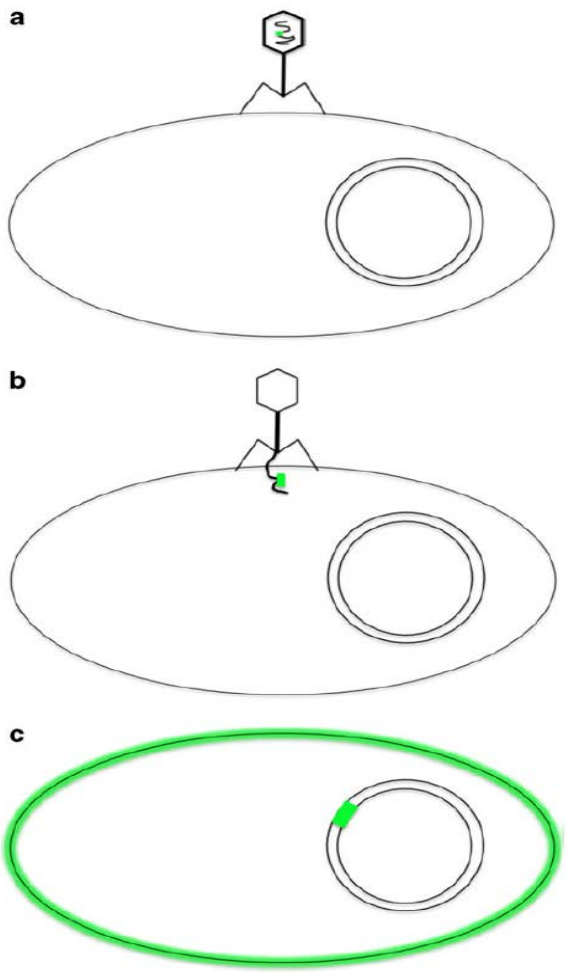
- Identification of bacterial targets
- Fluorescent labeling of phage nucleic acid or phage head or tall component
- Visual tag outline and identify bacterial cell for which the phage is host-specific



Smartt *et al* 2012

Genetically engineered phages as molecular tools

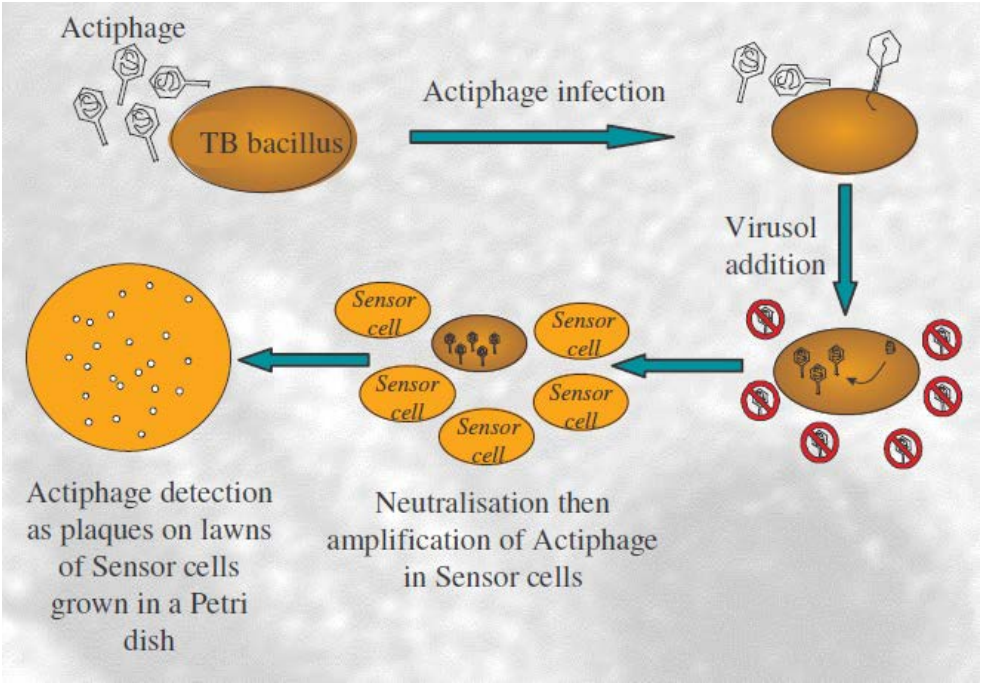
- Identification of bacterial targets
- Manipulate phage's genome to carry a reporter gene e.g. green fluorescent protein (GFP)
- Expressed when delivered to bacterial host
- Visualized by fluorescent signal



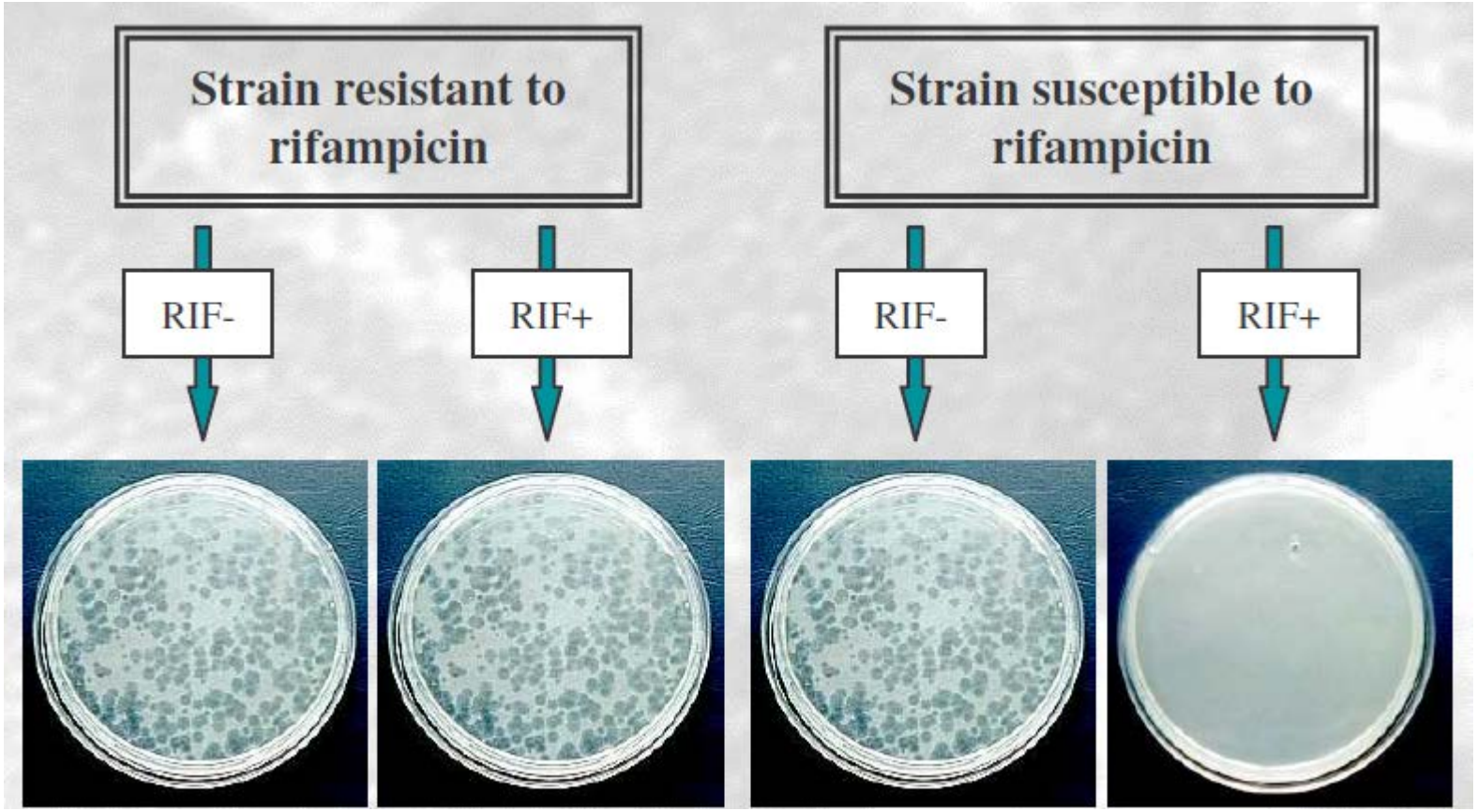
Smartt *et al* 2012

Genetically engineered phages as molecular tools

- Diagnosis of Mycobacterium tuberculosis
- Slow-growing, culturing from sputum samples requires up to 6 weeks before identification
- Commercial kit *FASTPlaque*TB™
- Sputum sample mixed with phage suspension for attachment to any MTB cells
- Unbound phages killed by virucide, derived from pomegranate extract
- Those attached not killed
- Mixed with a fast-growing non-pathogenic species of Mycobacterium which the phage also capable to infect
- Spread on agar
- Fast-grower produces a confluent lawn
- Plaques due to presence of MTB cells in sputum sample that infected prior to virucide treatment
- Plaque indicate patient suffering from TB



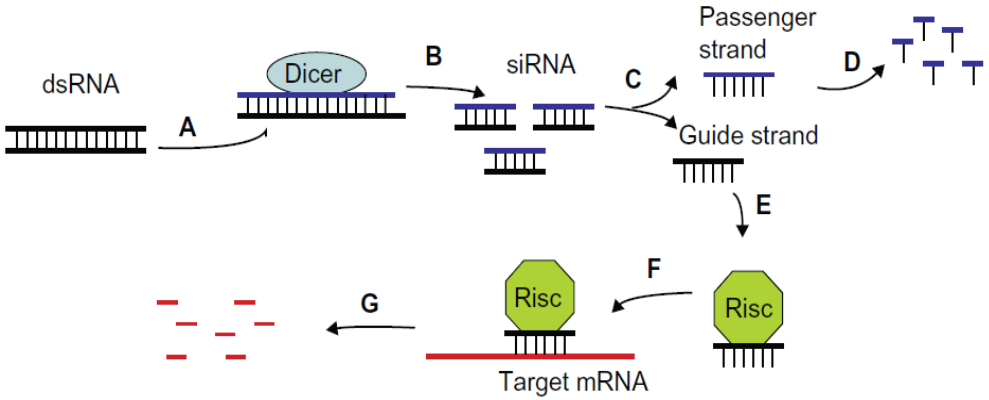
Hanlon 2013; Albert H *et al* 2004; BIOTEC Laboratories Ltd, UK



BIOTEC Laboratories Ltd, UK

Genetically engineered phages as molecular tools

- RNA interference (RNAi)
- Gene silencing
- Delivery vehicles



Chen, Xie 2012

Conclusion

- Phage therapy
  - Lytic phages against pathogens
  - Effective, safe and low cost
  - alternative strategy for multi-drug-resistant strains
- Enzybiotics as antimicrobials
  - Endolysin for Gram +ve bacterial infection
- Phages as biocontrol agents
  - Biofilm on indwelling catheters
  - Food preservation
- Phages as molecular tools
  - Bacterial identification
  - Nucleic acid delivery

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