

*Bacteriophages - beneficial microbes at
their best*

World Congress on Beneficial Microbes: Food, Pharma, Aqua &
Beverages Industry

Valencia, Spain

Bacteriophages - beneficial microbes at their best

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Alexander Sulakvelidze, Intralytix, USA:

Bacteriophages - new class of beneficial microbes and their practical applications

Joelle Woolston, Intralytix, USA:

Bacteriophage-based biocontrol for gently modifying food microflora and improving food safety

Lawrence Goodridge, McGill University, Canada:

Reprogramming the gut: Bacteriophages and their roles in modifying the gastrointestinal microflora

Andrey Aleshkin, Gabrichevsky Moscow Research Institute for Epidemiology and Microbiology, Russia:

Phagebiotics in treatment and prophylaxis of foodborne infections

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Bacteriophages - new class of beneficial microbes and their practical applications

Alexander Sulakvelidze, Ph.D.
Intralytix, Inc.

Beneficial Microbes: Food, Pharma, Aqua & Beverages Industry
August 25- 27, 2015 Valencia, Spain



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SAFETY BY NATURE™



Bacteriophages / Phages

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Viruses that attack bacteria

Estimated 3.5 billion years old

Discovered by Frederick Twort (1915) and Felix d'Herelle (1917)

D'Herelle coined the name *bacteriophage* (bacteria-eater)

Most ubiquitous organisms in nature

Highly specific for bacterial host

Play a key role in maintaining balanced bacterial populations in all ecosystems



The Most Ubiquitous Organisms on Earth

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- 10 Phage per microbe
- In the U.S.A., approx. 3×10^9 coliphages shed per person per day
- 1 mL of non-polluted water contains 2×10^8 PFU of phages
- Total number of phages on Earth is estimated to be $1 \times 10^{30} - 1 \times 10^{32}$
(100 000 000 000 000 000 000 000 000 000 000 phages)
- Phages have been found in commercial sera (e.g., $> 10^3$ PFU/ml in calf serum)
- Phages have been found in human vaccines (e.g., $> 10^2$ PFU/ml in poliovirus vaccine)
- Phages are common in human mouth, where they are harbored in dental plaque and saliva

All things compared...

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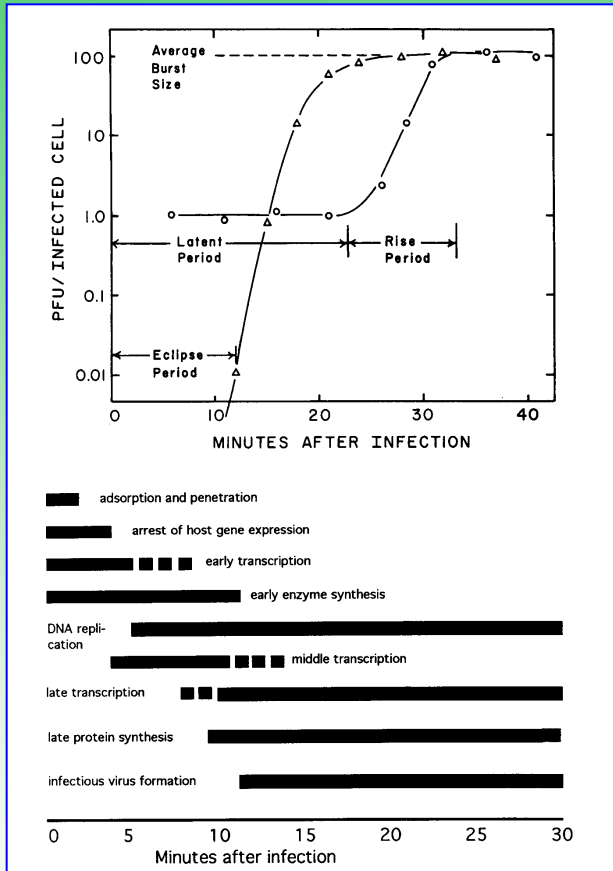


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Timing of Lysis

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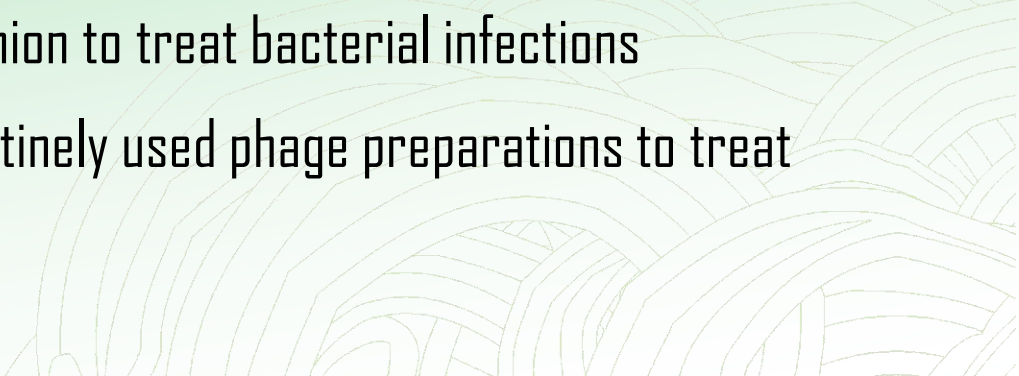


Source: Karam JD et al (ed.), Molecular Biology of Bacteriophage T4, ASM Press, Washington, DC



History of Therapeutic Use

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- First therapeutic application in 1919
 - First published report on therapeutic use in 1921
 - In 1930-1940s, Eli Lilly and Co. manufactured several therapeutic phage products, including preparations against Staphylococci and Streptococci
 - From the 1920s to the current day, phage products have been utilized in Eastern Europe and the former Soviet Union to treat bacterial infections
 - Russian and German Armies routinely used phage preparations to treat wounded soldiers
- 

Clinical Applications

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Phages have been administered to humans:

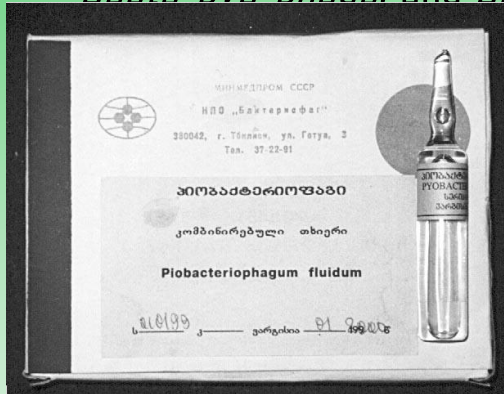
- (i) orally, in tablet or liquid formulations (10^5 - 10^{11} PFU/dose),
- (ii) rectally,
- (iii) locally (skin, eye, ear, nasal mucosa, etc.), in tampons, rinses and creams,
- (iv) as aerosols or intrapleural injections, and
- (v) intravenously

There have been no reports of serious complications associated with the use of phages. In the United States, phage phi X174 has been used to monitor immune function in patients

Some of the commercial phage products

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Laboratoire du Bactériophage: *Bacté-coli-phage, Bacté-rhino-phage, Bacté-intesti-phage, Bacté-ovo-phage, and Bacté-staphy-phage*



ate, Neiso-lysate,

luese



Eliava Institute: *IntestiPhage, PyoPhage*

EBI Food Safety (now Microeos): *Listex*

Intralytix, Inc: *ListShield, EcoShield, SalmoFresh*

Potential Applications

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Food safety

“Green” industrial and household cleaners / bio-pesticides

Probiotic products / phage preparations for fine-tuning GI microbiome

Cosmetics and dental care (including probiotic preparations)

Pet food & food supplements

Hospital decontamination & biofilm reduction

Plant infections

Animal health products (bacterins & drugs)

Drugs / human therapeutics (e.g., wound infections)

Diagnostic uses

Phage enzymes



Phages – platform technology for fine-tuning microflora

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- Phages present a novel and unique platform technology for fine-tuning gut, nasal, skin, etc. microflora
- Series of potential products can be developed based on that platform
- These novel products can address diseases of both infectious (e.g., shigellosis) and non-infectious etiology (e.g., obesity)
- Flexibility to update the cocktails

Are phages probiotics?

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Latin preposition *pro* ("for") and the Greek adjective *βιωτικός* (biotic), from the noun *βίος* (bios, "life")

Old Testament (Genesis 18:8): Abraham owed his longevity to the consumption of sour milk.

Lilly and Stilwell (1965): "Substances secreted by one microorganism that stimulate another microorganism."

Parker (1974): "Organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance"

Fuller (1989): "Live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance"

WHO (2001): "Live microorganisms which when administered in adequate amounts confer a health benefit on the host."

NCCAM, NIH "Probiotics are live microorganisms (in most cases, bacteria) that are similar to beneficial microorganisms found in the human gut."

Phages as probiotics

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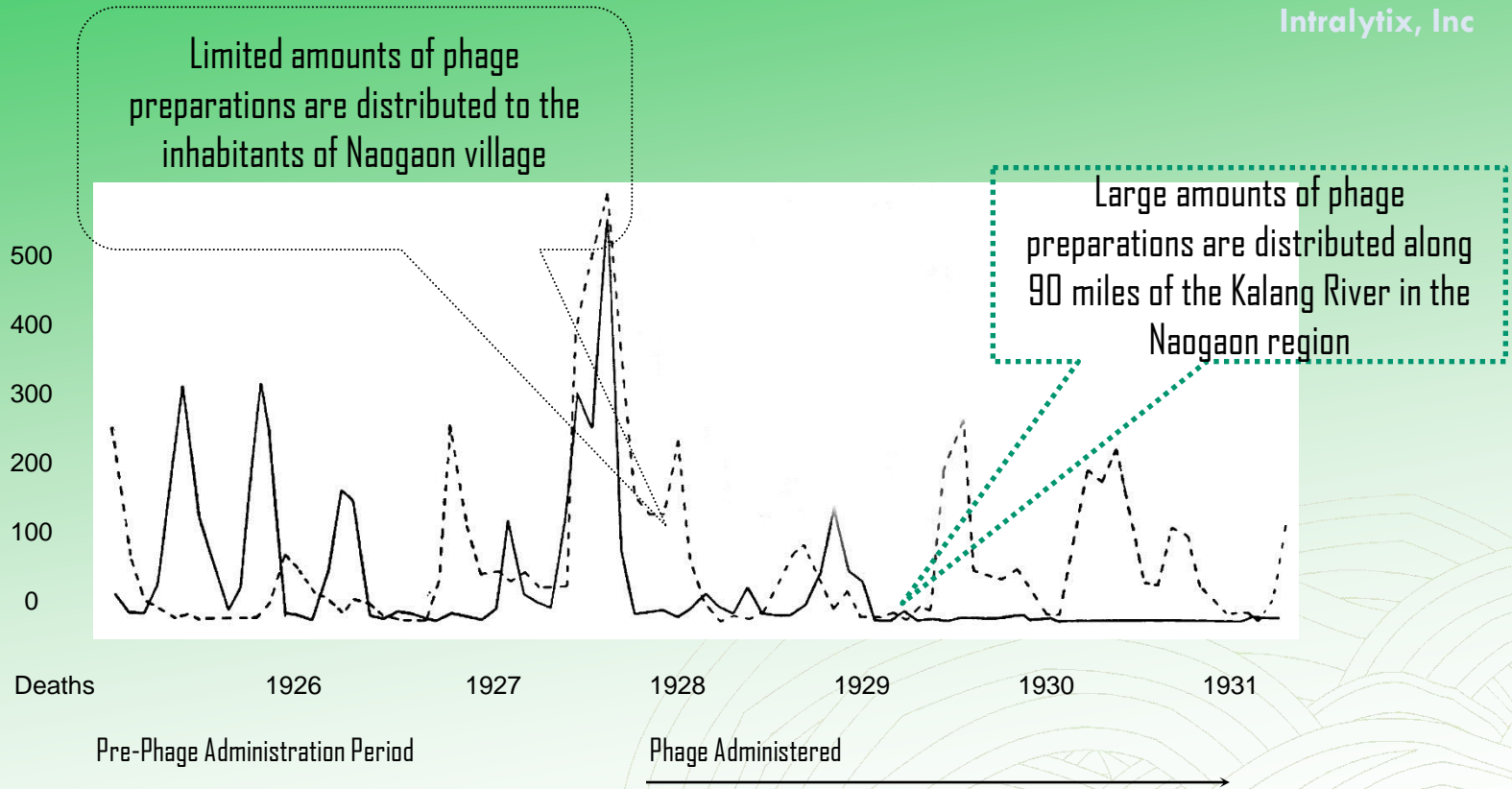
The approach of using phages prophylactically is similar to that used for bacteria-based probiotic preparations, which are administered over a period of time and act by favorably conditioning the gut's microflora.

The key difference between the approach commonly used for bacteria-based probiotics and our current approach for phage-based probiotic preparations is that:

- the former introduces nonpathogenic bacteria into the gastrointestinal (GI) tract in order to interfere with the ability of pathogenic bacteria to colonize the GI tract and cross the intestinal mucosa
- whereas, the latter will target specific pathogenic bacteria in the GI tract.

Phages against cholera: the "Cholera Study"

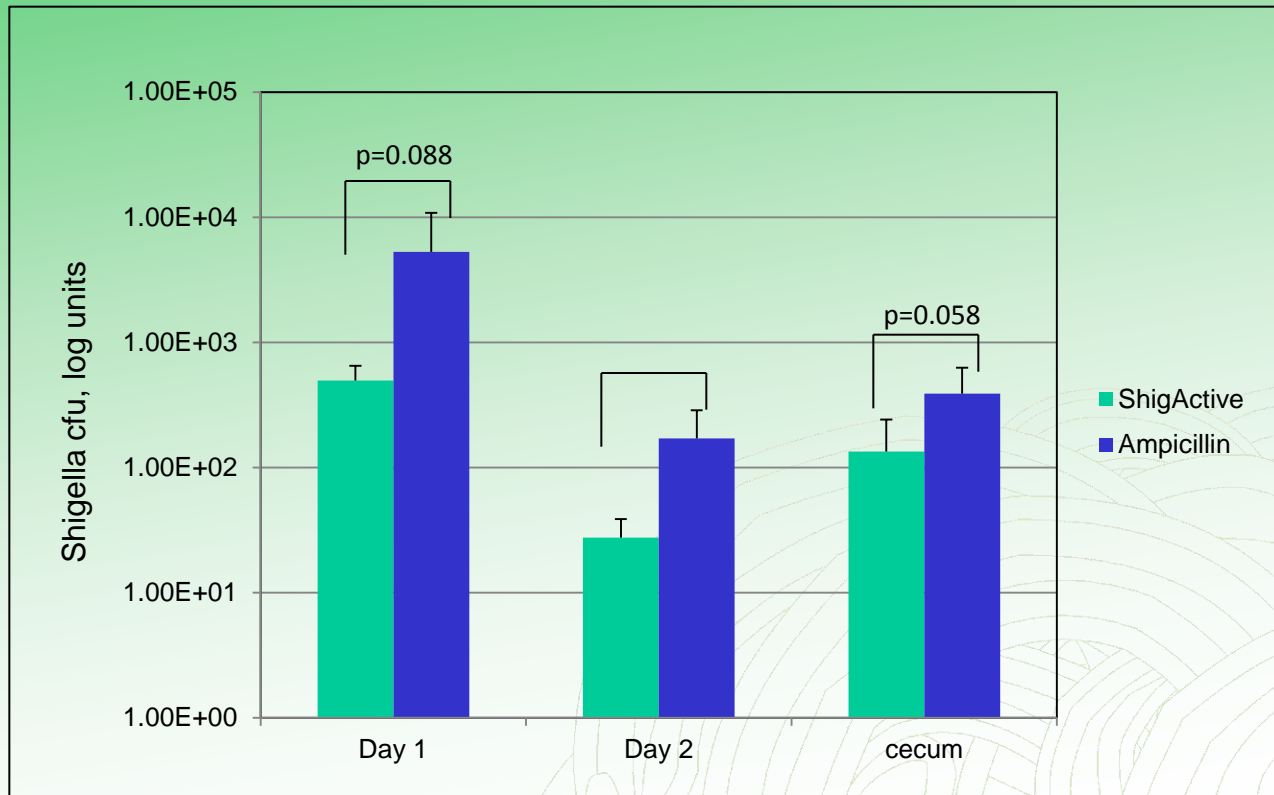
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Summers, W. C. Felix d'Herelle and the origins of molecular biology. Yale University Press, 1999.

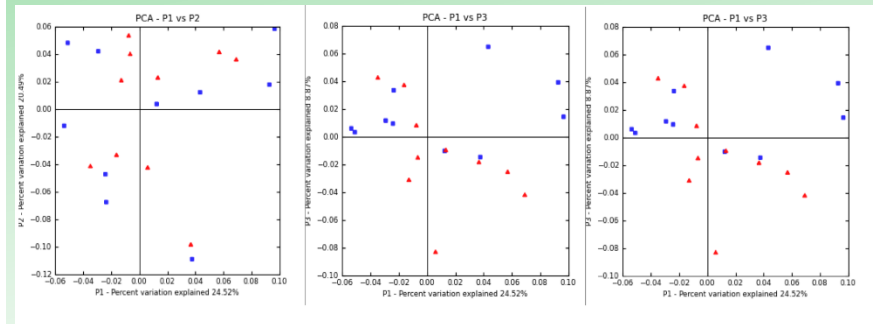
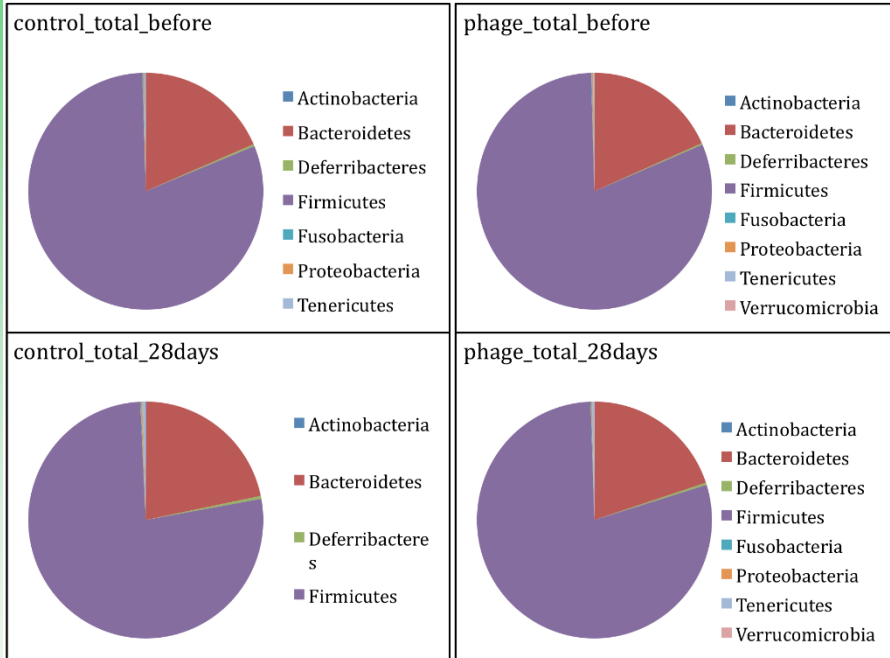
Shigella counts between ampicillin-treated and ShigActive-treated mice

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No differences in the proportions of the sequences on the phylum level in control and ShigActive-treated groups

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Blue squares - controls
Red triangles - phage-treated animals

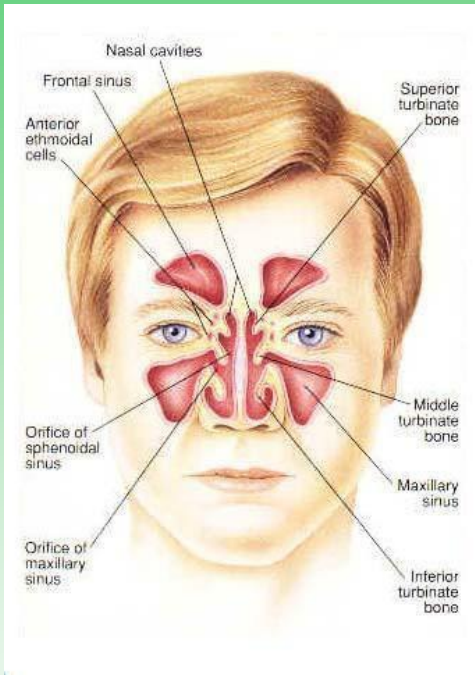
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Nosocomial infections



Phages for maintaining healthy nasal microflora

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- ❖ Healthcare-associated infections (HAIs) are a major cause of human morbidity and mortality.
- ❖ Ca. 1 out of every 20 hospitalized patients in the USA contracts a HAI, and the total medical cost to hospitals ranges from \$35.7 billion to \$45 billion/year
- ❖ Ca. 92 million persons in the USA have nasopharyngeal colonization with *S. aureus* (ca. 2.3 million are MRSA)
- ❖ *Broad spectrum antimicrobials (e.g., Mupirocin) alter the natural homeostatic balance of the local microbial community and may lead to an increase in HAIs caused by non-S. aureus (e.g., Gram-negative) bacteria*

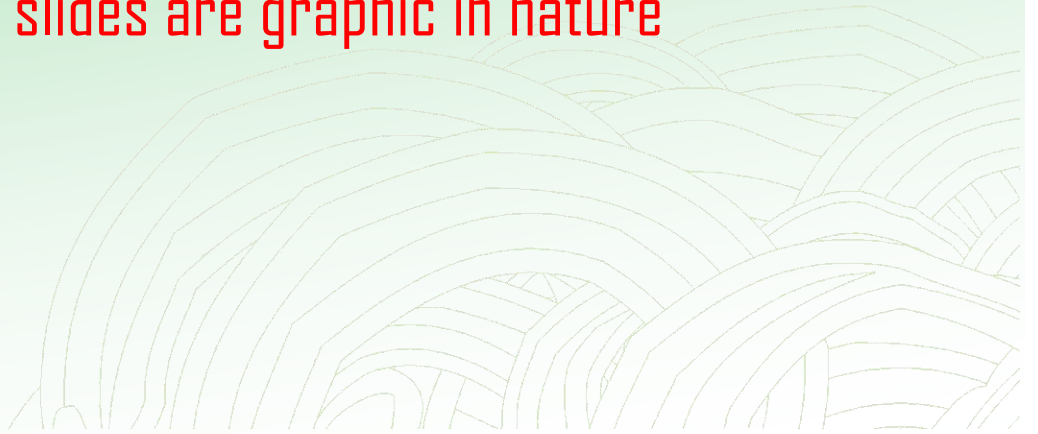
Image from: www.tonybaino.com

Phages and Wound Infections

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(Including Treating Combat-Related Injuries)

WARNING: Some slides are graphic in nature



Pictures of combat-related injuries are courtesy of Dr. Guram Gvasalia

PhagoBioDerm: 1st clinical trial

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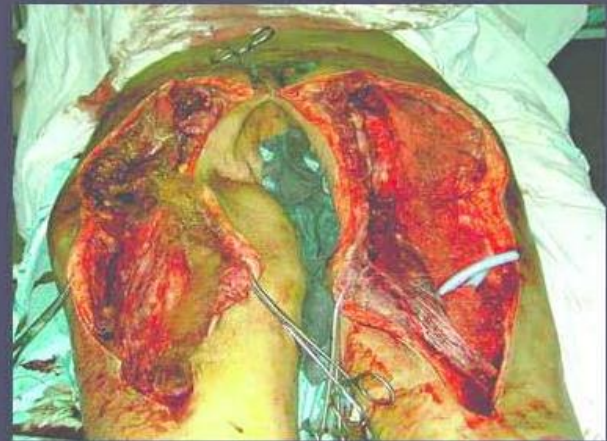


The use of PhagoBioDerm for wound healing in an 80-year-old female patient. The pictures show (from left to right) the initial lesion, application of PhagoBioDerm (day zero), and wound healing on days 10, 30, and 90, respectively

Source: Int J Dermatol. 2002 Jul;41(7):453-8.

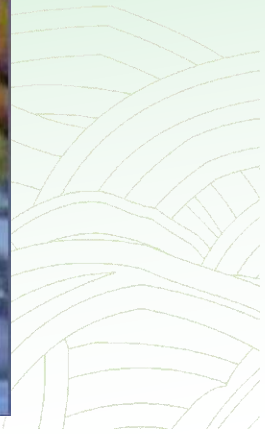
Extensive Wounds Before Treatment With Bacteriophages

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Phages Administered As Aqueous Solutions, Using Ultrasonic Dispenser

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Phages Administered as Biodegradable Films (Left) or Powder (Right)

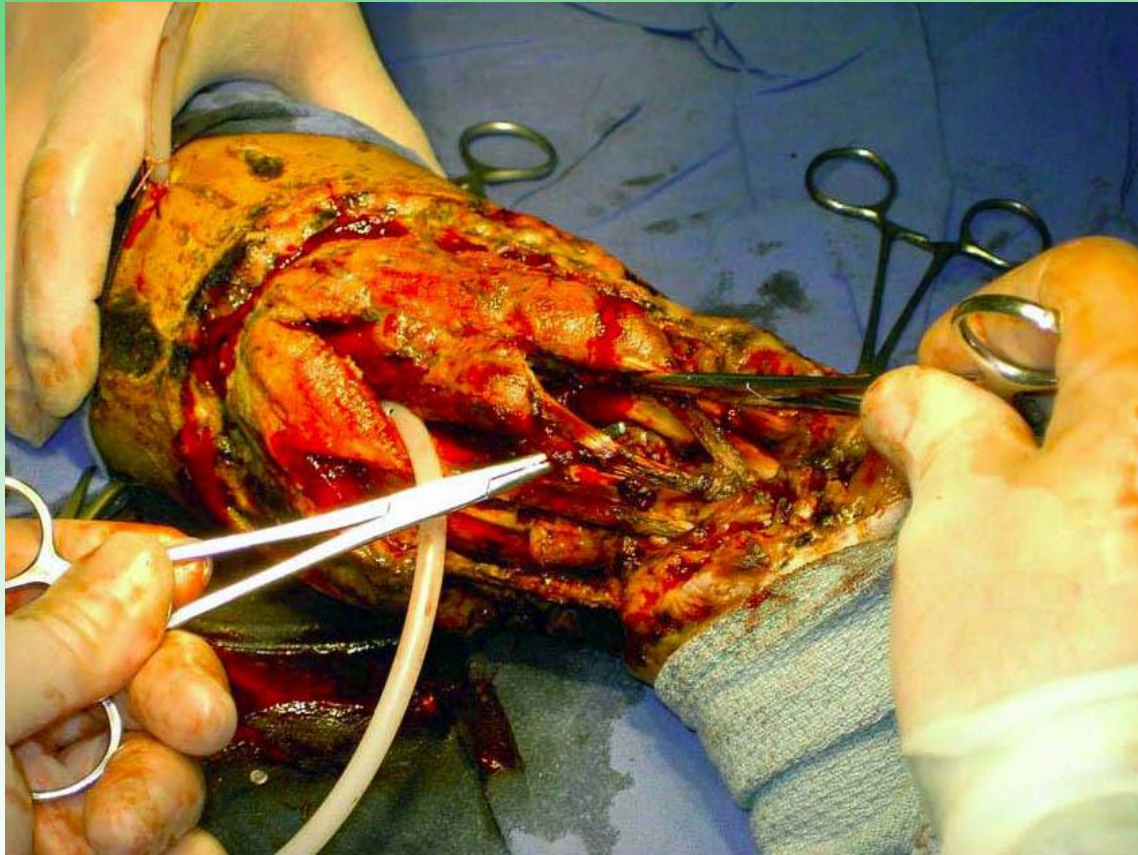
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Courtesy of Dr. Guram Gvasalia
Tbilisi, Georgia

Injury Due to Mine Explosion. 10th day. Treatment with Phage Ongoing.

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Courtesy of Dr. Guram Gvasalia
Tbilisi, Georgia

Injury Due to Mine Explosion. 1.5 months. Treatment with Phage Ongoing.



Courtesy of Dr. Guram Gvasalia
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Injury Due to Mine Explosion. 2 months.

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Courtesy of Dr. Guram Gvasalia
Tbilisi, Georgia

Numerous other “probiotic” applications

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Food Safety

Bacteriophage-based biocontrol for gently modifying food microflora and improving food safety

Veterinary / animal health

Reprogramming the gut: Bacteriophages and their roles in modifying the gastrointestinal microflora

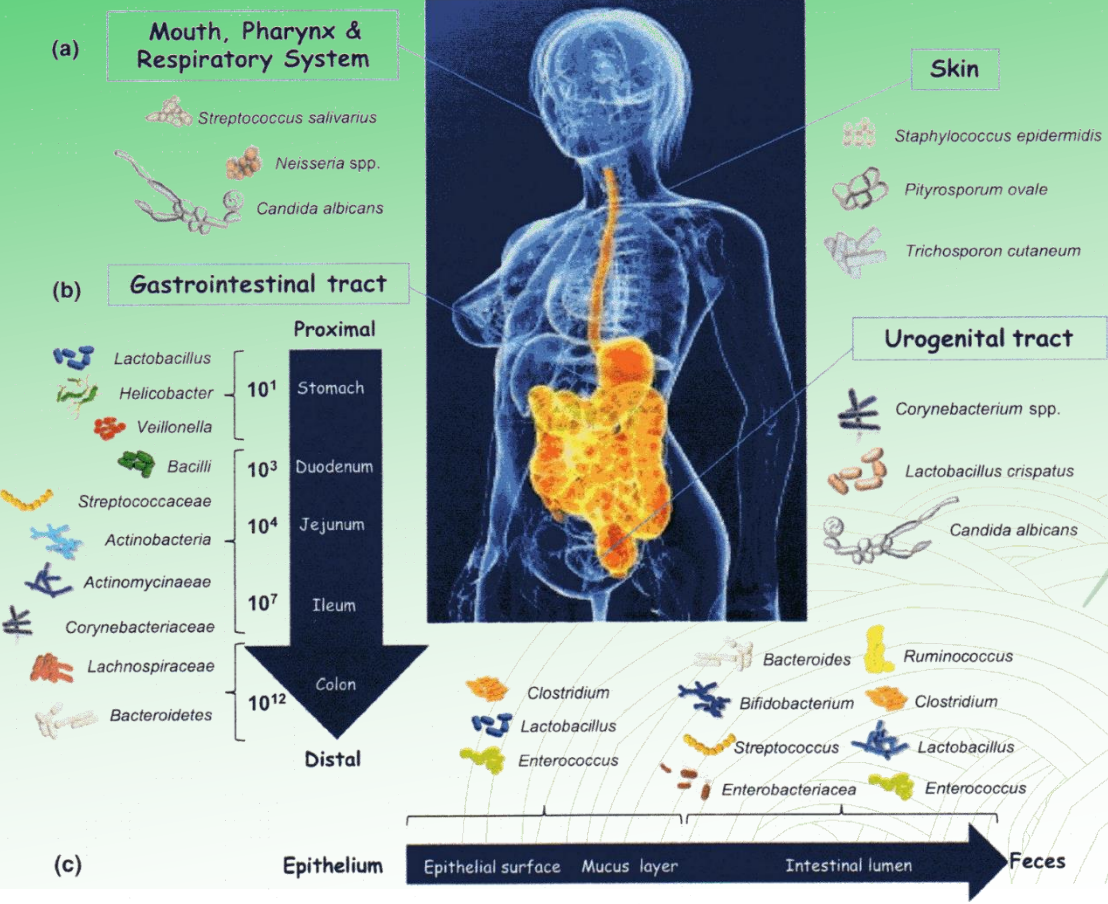
Improving human health

Phagebiotics in treatment and prophylaxis of foodborne infections



Microbial Population in the Human Body

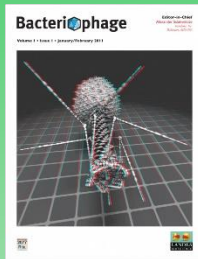
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From: S. M. Bakhtiar et al, FEMS Microbiol Lett, 2013, 1-8.

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