Eliava Institute experience in phage research and therapy

G. Eliava Institute of Bacteriophages, Microbiology and Virology
Tbilisi, Georgia
Bacteriophages

Bacteriophages are viruses that destroy bacterial cells, pathogenic cells among them. Contrary to antibiotics, they are species-specific, having thus number of advantages to compare to traditional antibacterials.
World experience in past

- Commercial department at the Pasteur Institute (5 preps)
- German Bacteriophage Society (dried phages in tablet forms)
- German company Antipiol (Enterofagos)
- Eli Lilly Company (USA) (7 products based on phages)
- Swan-Myers of Abbot Laboratories
- Squib and sons (now belongs to Brystol Myers Squibb)
- Parke, Davis and Company (now part of Pfizer)
Short historical review

- 1916 – Tiflis Pasteur station was transformed to the Central Bacteriological Laboratory
- 1918 – George Eliava became a director of CBL
- 1923 - Inst. Bacteriology has been established by G. Eliava on the base of CBL
- 1930, 1931 - Felix d'Herelle was working at the Institute in Tbilisi
- 1937 – G. Eliava was executed
Eliava Institute, Tbilisi, Georgia

• 1918 - 1923 - Central Bacteriological laboratory, Republic of Georgia, headed by George Eliava

• 1923 - 1935 - Inst. of Bacteriology, Department of Healthcare of Georgia

• 1935 - 1937 - Inst. of Microbiology and Epidemiology

• 1938 - 1950 - Inst. of Microbiology, Epidemiology and Bacteriophages, Ministry of Health of the USSR

• 1950 - 1990 - Inst. of Vaccines and Sera, Ministry of Health of the USSR

• 1990 - 1997 - Scientific-Industrial Union “Bacteriophage”, Ministry of Industry of Georgia

• 1997 - 2005 - G. Eliava Institute of Bacteriophages, Microbiology and Virology, Georgian Academy of Sciences

• 2005 - 2011 - G. Eliava Institute of Bacteriophages, Microbiology and Virology with legal status of public law, Ministry of Science and Education

2011 – present – Ilia State University, G. Eliava Institute of Bacteriophages, Microbiology and Virology
What we were producing in past

- **Mono-phages** *(staphylococcal, streptococcal, E.coli, Pseudomonas, Dysenterial, typhoid)*
- **Poly-phages** *(Pyo, Intesti)*
- **Indicatory phages**
- **Sera to treat diphtheria, tetanus, gangrene, scarlet fever, meningococcus**
- **Sera for identification of Salmonella and Shigella**
- **Vaccines** *(anti-rabies, anthrax, brucella, smallpox, intestinal)*

*Ancient Phage preparations*
Eliava Biopreparations

Phage preparations produced at the Eliava Institute today:

- **Pyo-Phage** (5 components: *Staphylococcus*, *E. coli*, *Streptococcus*, *Pseudomonas*, *Proteus*)

- **Intesti phage** (17 components)
  - Mono-phage preparations

- **Phage mixtures**
  - (*Staphylococcal*, *E. coli*, *Streptococcal*, *Pseudomonas aeruginosa*, *Proteus*)

- **Selected phages** for individual patients
Structure of the Eliava Phage “Consortium”

- Eliava Institute
- Eliava Foundation
- Eliava BioPreparations
- Eliava Analytical Diagnostic Center
- Eliava Management Group
- Eliava Media Production
- Eliava Phage Therapy Center
What we are studying at the Eliava Institute

Phage biology
Phage selection based on virulence,
    host range, stability, complementarity for cocktails, physiology, etc.
Phage-host bacterial cell interaction
Serology and antigenic properties of phages
Physical and chemical properties of phage particles and DNA
Phage-specific proteins (i.e. “killer” proteins)
Possible fields of application of bacteriophages

- **Healthcare** - Human Therapy, Prophylaxis and Diagnostics
- **Agriculture** - Animal and Plant protection
- **Environmental Microbiology** - Monitoring
Study of phages active against *S. aureus*

- High virulence that provides substantially broad range of activity on freshly isolated clinical strains (97-98%)
- Low index of frequency of phage resistant bacterial mutants formation
- Reliable protection against bacterial restriction-modification systems (absence of GATC sequence)

*a* Electron micrograph of Sb-1 phage (magn.x200 000, bar 100nm)
*b* Structural proteins profile by SDS-PAGE
*c* Genome sequence

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Decimal numbers are used for the additional ORFs (black arrows). The four contigs of sequenced Sb-1 DNA are indicated in light blue lines on the map. Dark blue lines represent recombinant clones.
Study of phages active against *S. aureus*

*In vitro* screening:

467 MRSA from the UK collection

54 MRSA and 38 toxin-producing (not MRSA) from German Strain and Culture collection (DSMZ) against Sb-1 phage

**Results:** Staph phage revealed 98-99.5% of lytic activity against those strains
Phages against *Pseudomonas aeruginosa*

- PAT13 (head 55x55nm)
- PAT14 (head 85x85nm, tail 55x30nm)
- PAT5 (head 100x90nm, tail 160x20nm)

PFGE analyses of:
1. PAT13
2. PAT14
3. PAT5
4. Sb-1
5. Mixture of *P. aeruginosa* phages
Phages against *Acinetobacter baumanii*

**EcoR I**
1. vB_Aba G865
2. vB_Aba G866
3. vB_Aba B37
4. vB_Aba U7
5. vB_Aba U8
6. Marker

**Hind III**
1. vB_Aba G865
2. vB_Aba G866
3. vB_Aba B37
4. vB_Aba U7
5. vB_Aba U8
6. Marker

**Pvu II**
1. Marker
2. vB_Aba G865
3. vB_Aba G866
4. vB_Aba B37
5. vB_Aba U7
6. vB_Aba U8

**Sal I**
1. Marker
2. vB_Aba G865
3. vB_Aba G866
4. vB_Aba B37
5. vB_Aba U7
6. vB_Aba U8
Genotyping of clinical isolates

Study of clinical strains isolated from the Cystic Fibrosis patients in Georgia
Phages for human therapy

- **ISTC G510**, Construction of phage preparation against infection caused by *S. aureus* and *P. aeruginosa*
- **CRDF-GB-2491-TB-03**, Polyvalent bacteriophage preparation as an effective remedy against massive outbreaks of antimicrobial-resistant Salmonellosis
- **ISTC G 1369**, Microflora of inflammatory process in chronic prostatitis and urogenital tracts in men and phage therapy prospects
- **INTAS 03-51-6610**, Development of Phage-Based Therapy for Lung infections
- **ISTC G16 66**, Development of a New Phage Composite Targeted to Prevent and Treat Mixed Infections
- **STCU 5148**, Development of a novel bacterial preparation for immunotherapy of cancer
Phages for Animal Protection

- Phage preparation for treatment of bovine mastitis
- Bacteriophages against bacterial pathogens of Mediterranean cultured fish
Phages for Animal Protection

Seventh Framework Program
International Research Staff Exchange
PIRSES-GA-2009-269175

Network for the development of phage therapy in aquaculture
Phages for Plant protection

- Bacteriophages for Biocontrol of infectious diseases in potatoes, grapes, rice, cotton caused by *Erwinia spp*, *Ralstonia spp*, *Xantomonas spp.*, *Agrobacteria spp*. 
Phages for Food safety

- **GNSF**, Monitoring of salmonella distribution in poultry flacks, pig farms and in food (meat, eggs)
- **GNSF**, The prevalence of foodborne pathogens in raw milk and milk products as a potential risk for public health in Georgia
- **“Tbilisi Water” Ltd.**, Epidemiological analyses of bacterial and viral infections in Tbilisi city water
- **GRDF 08/06**, Elaboration of the standardized dairy starters for the traditional Caucasian dairy products
- **GRDF-GNSF 04/08**, Development of the standard starter(s) for the Georgian cheese type “Imeruli”
Phages Environmental Monitoring

- **STCU P486**, Application of bacteriophages against highly pathogenic bacteria (model studies)

- **STCU P448**, Study of the role of bacteriophages in the biology of sulfate-reducing bacteria

- **STCU P395**, Investigation of polymeric biofilms formed by dangerous pathogens, their formation preventing by disinfectants and bacteriophages
In the frame of DTRA funded projects

**DTRA - GG-13**

Isolation, Distribution and Biodiversity of Selected Vibrios and their Phages from the Aquatic Environment in Georgia

- Study of biodiversity and ecology of *V. cholerae* and related *Vibrio spp.*
- Identification and characterization of environmental vibrios
- Enumerate and characterize naturally occurring *Vibrio spp.* specific-bacteriophages
In the frame of DTRA funded projects

- Phage typing for identification of *Brucella spp.*
- Works for isolation of phages against *Brucella* began in 1939 at the Eliava
- TB phage (isolated in 1955) is considered by WHO as a reference for interspecies differentiation of *Brucella* spp.
- The phage reveal high lytic activity only to *B. abortus*. It adsorbs on *B. suis* but does not cause the lysis
- **GG17** – using of phages for typing the suspect *Brucella* isolated from human and animal

Electron micrograph of TB phage (Magnif. x 100 000)
In the frame of DTRA funded projects

GG17

• characterization of 45 phages from the Eliava Institute collection (Biological properties, DNA restriction, structural proteins)
• 53 suspect isolates (from NCDC and LMA) are typed by the phages
• Elaboration of new schemes for phage typing as an additional tool for diagnostic of Brucella spp.

Fig. DNA restriction and structural proteins of Brucella phages
In the frame of DTRA funded projects

**GG18** Isolation and study of bacteriophages against *Y. pestis, B. anthracis* and *F. tularensis*

• Phages against *Y. pestis*

Fig. YP/SH phage (magnif. X200000)
In the frame of DTRA funded projects

Phages against *B. anthracis*

Fig. Morphology, DNA restriction and structural proteins of *B. anthracis* phages
In the frame of DTRA funded projects

Phages against *F. tularensis*

Fig. Phage plaques polymorphism on *F. tularensis* lawn; Electron micrograph of phages active against vaccine strain of *F. tularensis*
Why Phage Therapy?

- No effective treatment except antibiotics for today
- Serious problems of antibiotic-resistance
- Phage therapy – **ecologically safe approach** (do not affect normal microflora)
- No resistance with multi-component phage preparation
- There is **no correlation** between phage- and antibiotic-resistance
- 70 years of successful experience of using phages for therapy, prophylaxis and diagnostics in FSU
- **No serious side effects** have been reported from the Eliava Institute phages, despite use in **hundreds of thousands of people** since it was introduced
Why Phage Therapy?

- Phages are available and easy to apply (different forms: tablets, in liquid, suppositories etc.)
- Compatible with the other therapy (other antibacterial remedy, vaccine, probiotics)
- Stable preparations (no cold storage and long shelf life)
- Cost effective (in comparison to antibiotics)
Clinical applications

Patient # 1. M. K., 6 years, female. The disease was confirmed by CFTR gene mutation – Δ1677. Sweat test - 126-135 mmoL/L

Initial concentration of *S. aureus* $1 \times 10^7$ CFU/mL; $8 \times 10^6$ CFU/mL of *P. aeruginosa*

Concentration of bacterial cells in sputum of the patient before and after phage application

(X) numbers indicate amount of phage treatment; a – before phage therapy; b – after phage therapy; duration between analysis corresponds 4-6 weeks
Clinical applications

Patient # 2. H. J., 25 years, male. CF was diagnosed at 1.4 years. The diagnosis was confirmed by mutation ΔF508.

Initial concentration of *S. aureus* $6 \times 10^6$ CFU/mL.; $1 \times 10^6$ CFU/mL of *P. aeruginosa*.

Concentration of bacterial cells in sputum of the patient before and after phage application

![Graph showing bacterial concentration before and after phage application](image)
ISTC support to the Eliava Institute

1. ISTC SPC 40 – Establish a computer network at the Eliava Institute
2. Support for the International Phage Meeting at the Eliava Institute (2008)
3. Renovated laboratories

Laboratory space for Microbiological Research before and after renovation
Pending project

EU Support for upgrading biosafety and biosecurity at the Eliava Institute
Thank you

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