WWJMRD 2018; 4(5): 1-4
www.wwjmrd.com
International Journal
Peer Reviewed Journal
Refereed Journal
Indexed Journal
UGC Approved Journal
Impact Factor MJIF: 4.25
E-ISSN: 2454-6615

L. Gubeladze
Tbilisi State Medical University, G. Eliava Institute of Bacteriophages, Microbiology and Virogoly, Tbilisi, Georgia

D.Gogiashvili
G. Eliava Institute of Bacteriophages, Microbiology and Virogoly, Tbilisi, Georgia

S.Rigvava
G. Eliava Institute of Bacteriophages, Microbiology and Virogoly, Tbilisi, Georgia

M. Natidze
G.Eliava Institute of Bacteriophages, Microbiology and Virogoly, Tbilisi, Georgia

T.Kokashvili
G.Eliava Institute of Bacteriophages, Microbiology and Virogoly, Tbilisi, Georgia

G.Tsertsvadze
G.Eliava Institute of Bacteriophages, Microbiology and Virogoly, Tbilisi, Georgia

G. Kamkamidze
Tbilisi State Medical University, University of Georgia ,Tbilisi, Georgia,

M. Tediashvili
G. Eliava Institute of Bacteriophages, Microbiology and Virogoly ,University of Georgia, Tbilisi, Georgia,

Correspondence:
L. Gubeladze
Tbilisi State Medical University, G. Eliava Institute of Bacteriophages, Microbiology and Virogoly, Tbilisi, Georgia

The Isolation of Bacteriophageslytic to B. Anthracis from Environmental Sources in Georgia. 3rd Report: *B. Anthracis* –Specific Phagesisolatedin 2012 in Georgia

L. Gubeladze, D.Gogiashvili, S.Rigvava, M. Natidze, G.Tsertsvadze, G. Kamkamidze, M. Tediashvili, T.Kokashvili

Abstract
Isolation and characterization of new bacteriophages specific to *B.anthracis*is is of high importance for application in detection and identification of the pathogen, also in bio-decontamination of surfaces and devices occasionally or deliberately contaminated with *B. anthracis.* In winter 2012/2013 three new bacteriophages- BaQpo, BaQtr and BaSio were isolated on the vaccine strains of *B. anthracis* from water and soil samples obtained in 2 different regions of Georgia.The TEM study revealed theSiphoviridae type morphology for all 3 newly isolated phages,however, they differ from each other and also from the reference phages (Gamma, Fah, IM) by particular characteristics, such as head and tail size. Thephage neutralization assay done using gamma - antiphage serum showed quite close antigenic relatedness of phage BaQpo and at the less extend of BaSio phage with the gamma phage, while phage BaQtr obtained appeared to be significantly different. Restriction profiles of Ba Phage DNA's confirmed difference of phage BaQtrfrom phages Ba Qpo and BaSio.

Keywords: Anthrax, Bacillus anthracis, bacteriophages, virion morphology, phage neutralization, DNA restriction

Introduction

Anthrax is a widely spread acute zoonotic disease with high lethality, which is caused by the soil-borne, gram positive bacterium *Bacillus anthracis* [8, 17]. Anthrax has high public healthimportanceand national security implications globally. *B.anthrcis* is recognized as a biological weapon of mass destruction and although *B. anthracis* is usually is drug-sensitive bacterium, multidrug resistant strains can be deliberately engineered thus increasing the bioweapon threat [4,9].

In the Caucasus, and particularly, in Georgia Anthrax is endemic disease with certain number outbreaks every year, including human cases [10, 13, 14, and 15]. The morbidity has increased in the past 10 years with the peak in 2010-2012 concentrated within specific agricultural areas and in close proximity to urban centers [11]. Theearly diagnosis of the disease and detection of etiological agent is crucial for resolving the problems with the possible disease outbreaks and to minimize the scale of its consequences.

The control measures for spread of anthrax mainly include inactivation by physical methods such as gamma irradiation, ultraviolet light, and high pressure [3] that are not safe for human. Alternative ecologically friendly means of disinfection and disease prevention are much thought after. Bacterial viruses are naturally established controllers of bacteria in the environment and recent data strongly suggest theireffectiveness in treating bacterial diseases including those caused by antibiotic-resistant highly pathogenic microbes such as *B. anthracis*. Enrichment of existing phage collection with new bacteriophages specific to *B.anthracis* is of special importance considering their potential application for anthrax diagnosis, detection and control, including bio-decontamination [9]

The aim of presented work wasisolation of bacteriophages lytic to *B.anthrcis*from different Geographical regions of Georgia andto studies their basic characteristics.

Materials and methods.

Bacterial strains: standard vaccine strains of *B. anthracis* 34F2 (Sterne), STI, 55, I-17 ; strains of other *Bacillu*s species:*B. cereus(8 strains), B. subtilis(1), B. thuringiensis(1), B. megaterium(1)* and *B anthracoides (1)* -from the culture collection of the Eliava Institute.

Rreference bacteriophages: Gamma, IM and Fah (the phage collection of the Eliava institute).

Isolation, cloning and concentration of phages was done on the strain *B.anthracis* 34 / F2, using standard methods [1, 5, 12], as described also in our previous reports [6,7]

The virionnucleocapsidmorphology was studied by Transmission Electron Microscope (TEM).The concentrated phage suspensions were placed on carbon /formvar copper grids (EMS, USA), washed with water, negatively stained with 2% uranyl acetate and examined in the TEM 100SX (JEOL, Japan) operating at 80kV and standard instrumental magnification 50K.

The serologic relatedness was studied in the neutralization reaction using the gamma – antiphage serum (Gamma-APS), obtained earlier [6]. For each pair of phage-antiphage serum the neutralizationpercent was determined and the neutralization constant was calculated in case of high percentage of neutralization.

For the phage DNA restriction analysis DNAsworeisolatedfrompurified concentratedbacteriophage suspensions (with the titer 1-5 $x10^{11}$pfu / ml)by phenol /chloroform method [16].The isolated DNA has been dried out at room temperature and resuspended in the TAE-buffer for further procedures. Phage DNAs were digested by set of 4 restriction enzymes-SmaI, BamHI, XbaI and PstI (Promega, USA) according to the conditions provided by the manufacturer. The restriction fragmentswere separated in 0, 8% agarose gel, stained with 0, 5 mkg/mlethidium bromide and visualized in the Gel Logic System 100 (Kodak, USA).

Results and discussion

In winter 2012/2013 seven water and soil samples have been obtained in the West Georgia(3 sampling points in Qaeda district) and East Georgia (Telavi, Kumisi, Lisi, Sioni). In contrast to previous studies all samples were collected during the cold period (November- December) at the end of the rainy season. The samples were enriched with 4 standard strains of *B.anthracis*, mainly with vaccine strain *B.anthracis34f2*. The processing of 2 primary lysates obtained from enriched soil samples from Qeda and Sioni water followed by the spot test on the lawn of test cultures resulted in the lists zones, and isolation of 2 bacteriophage mixtures - BaQ and Ba Sio. The consequent cloning and concentration yielded 3 individual phages: BaQpo, BaQtr and BaSio.Some primary difference between these three bacteriophages was observed by their negative colony morphology (size and shape) that was confirmed in the studies of virion morphology.

The TEMstudy demonstrated that all the newly isolated phages belong to the Siphoviridae family with an icosahedral head and non - contractile tail [2]. However, the phages differ from each other and also from the reference phages (Gamma, Fah, IM) by particular characteristics, such as head and tail size. The virionmorphology are shown on the Fig. 1, and the parameters of virion components are presented in the table #1

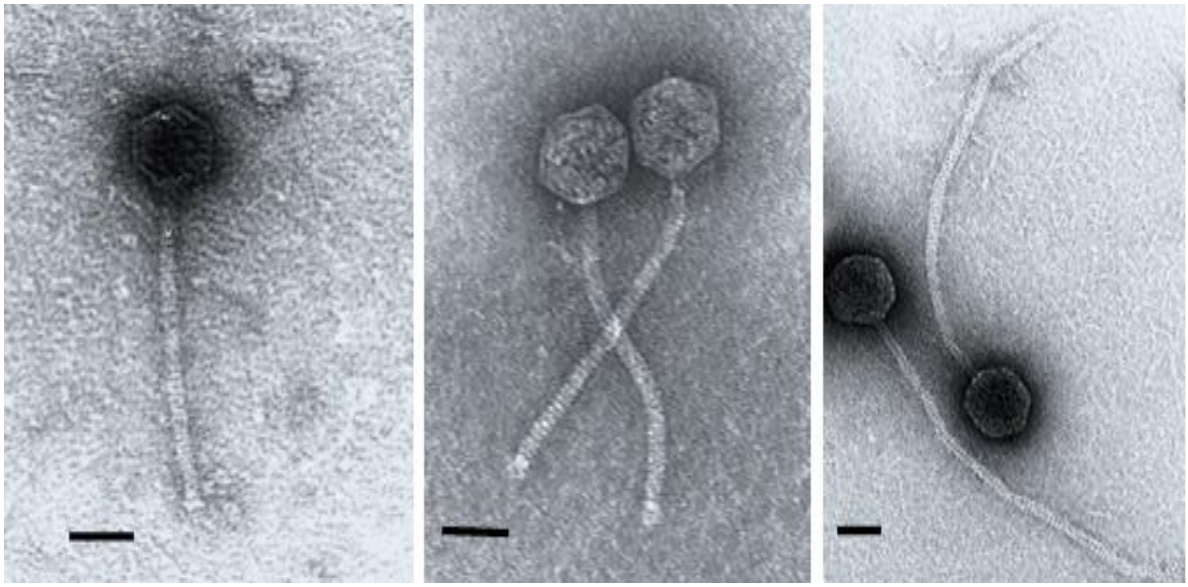

Fig. 1.Virion morphology of B.anthracis –specific phages: (a) BasSio, (b) BaQpo; (c) BaQtr; size bar indicates 50 nm

The antibacterial activity and specificity of the newly isolated Ba phages was examined against 4 vaccine strains of *B.anthracis: 34F2, STI1,#55, I 17* and other*Bacillus* species such as*B. Cereus (8 strains), B.subtilis (1), B. thuringiensis (1), B.megaterium (1)* and *B.anthracoides (1).* All three phages -BaSio, BaQpo and BaQtr demonstrated high antibacterial activity towards tested 4 strains of*B. Anthracis*, although with slightly different efficiency of plating (in the range of 0, 1- 0, 5 compared to the host strain –*B. anthracis* 34 F2). The phages showed high species -specificity not lysing majority of the tested strains of other *Bacillus* species. Only in case of someB.cereusstrains (up to 4 strains out of 8) all 3 phages produced negative plaques. It should be mentioned that reference gamma phage also lysed one of the B. *cereus* strains.

Table.1: Virion morphology parameters of phages BaSio, Ba Qtr and BaQpo

Bacteriophage	Family	Diameter of head(nm)	width of tail (nm)	length of tail (nm)
BaSio	Siphoviridae	52± 2	12±1	215± 3
BaQtr	Siphoviridae	66±3	13.5±1.5	445± 5
BaQpo	Siphoviridae	57±2	18±2	320± 4

Serological properties of phages have been studied using gamma -antiphage serum (Table#2). The studies showed that based on serological parameters of phage BaQpo (95% of neutralization, K=47.8) it can be considered as related to the gamma phage, while phage Bat originated from the same Qeda sample significantly differs from Gamma phage (22%). The Phage Ba Sio is neutralized by Gamma-APS at 78 % (the K couldn't be calculated) that indicates certain relatedness with phage Gamma.

Table.2: The results of the neutralization reaction of phages Ba Sio, BaQpo and BaQtr with Gamma- APS

	10 min	30min	10 min	30min
	%		K	
BaGamma	94%	98%	140,57	69,2
BaSio	41%	78%		--
BaQtr	7%	22%		--
BaQpo	73%	95%		47,8
BaIm	82%	91%	-	39.9
BaFah	79%	95%	-	48.04

All three Ba phage DNAs were digested with 4 restriction enzymes SmaI, XbaI, BamH1and Ps (Fig.2). Endonuclease SmaI didn't show activity towards any of tested phages DNAs. DNAof two bacteriophages - BaSioandBaQpo, related to phage Gamma, were digested by 3 endonucleases-XbaI, PStI and BamHI, producing the same numbers of restriction fragments, while DNA of the phage BaQtr was cut only by XbaI. These results indicated certain difference of phage Qtr from phages Ba Qpo and BaSio. In addition, comparison of the XbaI restriction profiles of this 3 new Ba phages with restriction profiles of Gamma, Fah and IM phages obtained using same endonucleases (electrophoregram not shown here) indicated also significant difference of phage Qtr from Ba standard phages, including phage gamma.

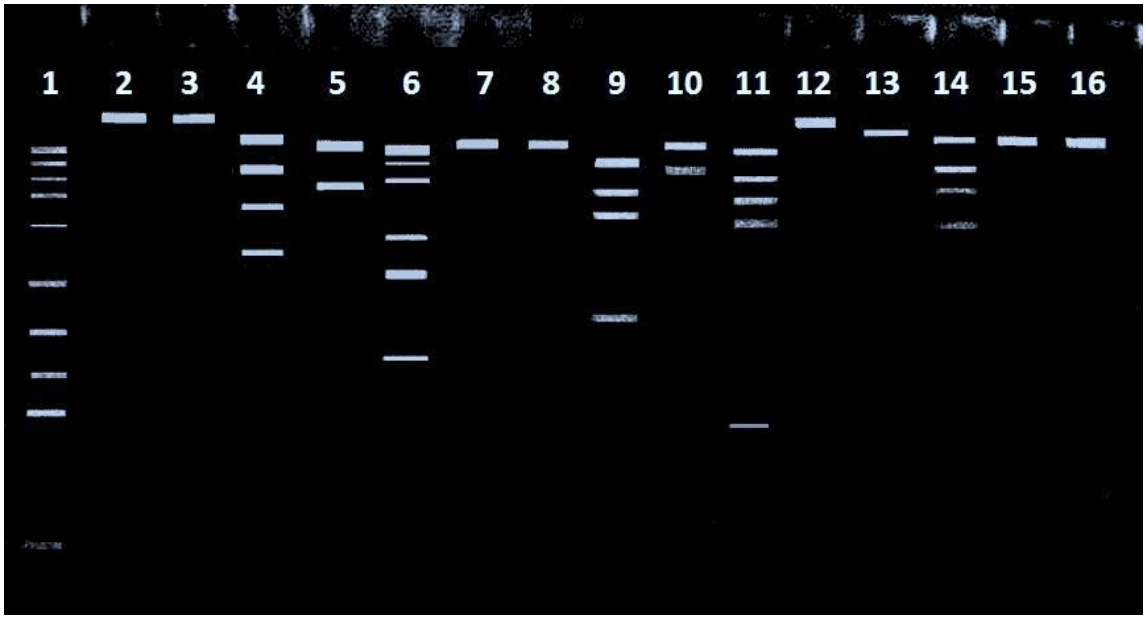

Fig.2. DNA restriction profiles of *B.anthracis* -specific phages Basio, BaQpo and BaQtr.1)Markerλ +HindIII; 2) DNA BaSio; 3)BaSio +Smal ; 4) BaSio + XbaI; 5)BaSio+ BamH1; 6) BaSio+PstI; 7) DNA BaQpo; 8) BaQpo +SmaI; 9)BaQpo+XbaI; 10)BaQpo+ BamH1; 11)BaQpo +PstI; 12) DNA BaQtr+; 13) BaQtr+SmaI; 14) BaQtr + XbaI; 15)BaQtr+BamH1; 16) BaQtr+Pst1

Conclusion

Three new bacteriophages lytic to *B. anthracis* – Ba Qtr, Ba Qpo and BaSio have been isolated from Georgian environmental sources in and characterized by basic biological properties. By vision morphology all three phages belong to Siphoviridae family, although differ by head and tail size. The BaQpo and Ba Sio show some relatednesswith each other by serological properties and restriction profiles, while phage Ba Qtr significantly differs by these characteristics. The studied Ba phages have been used in the experiments on phage-based cleaning of surfaces contaminated by *B.anthracis*.

Acknowledgment

The presented work was conducted in the frames of the Grant project STCU P486

References

1. Adams M. “Bacteriophages” 1959. “ New York, Interscience Publishers “.
2. Ackerman H.W. 2009. “Phage Classification and Characterization”. In: Bacteriophages, Methods and Protocols”. V.1. “Isolation, Characterization and interactions” (Ed. M.R.J. Clokie, A.M. Kropinski), Humana Press. Chapter13,pp. 127-140
3. Eitzen, E., J. Pavlin, T. Cieslak, G. Christopher, and R. Culpepper. 1998. Decontamination, p. 119-121. In Handbook of medical management of biological casualties, 3rd ed. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD
4. Filippov A., K.Segueev and M.Nikolich. Bacteriophages, Enviromental and therapeutic Applications”,JBioterrorism and Biodefence, v..4 No. SpecialIssue pp.S3:010 ref.150
5. Gabrilovich M. (1973). Basics of bacteriophages research. “VisheishayaSchkola”, Minsk
6. Gubeladze L, D.Gogiashvili, S.Rigvava, M. Natidze, T.Kokashvili, G.Tsertsvadze, M. Tediashvili 2017. “Comparative biological characterization of phages specific to B.anthracis isolated from environmental sources in Georgia. Communication 1. B. anthracis – specific phages isolated in Georgia in 2008-2009 “ J Experimental and Clinic Medicine, #3 pp.123-129
7. Gubeladze L, D. Gogiashvili, S.Rigvava, M.Natidze, G.Kamkamidze, G.Tsertsvadze, M.Tediashvili: 2017. “Comparative biological characterization of phages specific to B.anthracisisolated from environmental sources in Georegia. 2nd report: B anthracis specific phages isolated in Georgia in 2010-2011”. “ Black Sea" SJAR, v.37, pp.68-70
8. Jayachandran R, 2002. Anthrax:” Biology of Bacillus anthracis” Current Sience, v. 82, # 10, pp.1220-1226
9. Jonczyk-Matysiak E, B. Weber-Dąbrowska, J. Borysowski, A. Górski, 2014. “Possible Use of Bacteriophages Active against Bacillus anthracis and Other B. cereus Group Members in the Face of a Bioterrorism Threat”, Biomed Res Intl, Article ID 735413, doi:10.1155/2014/735413
10. Kukhalashvili T (2007). “Atlas of Anthrax Foci in Georgia”, Tbilisi, Georgia,
11. Kracalik I., L. Malania, N.Tsertsvadze, J. Manvelyan, L. Bakanidze, P. Imnadze, Sh. Tsanava, and J. K. Blackburn. 2014. ‘’Human Cutaneous Anthrax, Georgia 2010–2012.Emerg Infect Dis. 20(2),pp. 261–264
12. Kropinski A., A.Mazzacco, T. Waddel E Lingohr and R. Jonson: 2009. “Enuomerationof Bacteriophages by Double Agar Overlay Plaque Assay”. In: Bacteriophages, Methods and Protocols”. Volume 1. Isolation Characterization and interactions” (Ed. M.R.J. Clokie, A.M. Kropinski), Humana Press, Chapter 7. pp 69-80
13. Merabishvili M, M. Natidze, S. Rigvava, L. Brusetti, N. Raddadi, S. Borin, N. Chanishvili, M.Tediashvili, R Sharp, M.Barbeschi, P. Visca, and D.Daffonchio. 2006. “Diversity of *Bacillus anthracis* Strains in Georgia and of Vaccine Strains from the Former Soviet Union”. Applied. Environ.Microbiol, v.72, No 8, p. 5631–5636
14. National Centre of Disease Control and Public Health, Epidbulletin, 2013, v.17, issue #10
15. National Centre of Disease Control and Public Health, EpidBulletin, 2016, v.20, issue #5.
16. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular cloning, A laboratory Manual. 2 edn (Cold Spring Harbor Laboratory Press).
17. Turnbull P. C. B., R. Bőhm, O. Cosivi, et al.1998, “Guidelines for the Surveillance and Control of Anthrax in Humans and Animals, World Health Organization EMC/ZDI./98.6, 3rd edition.