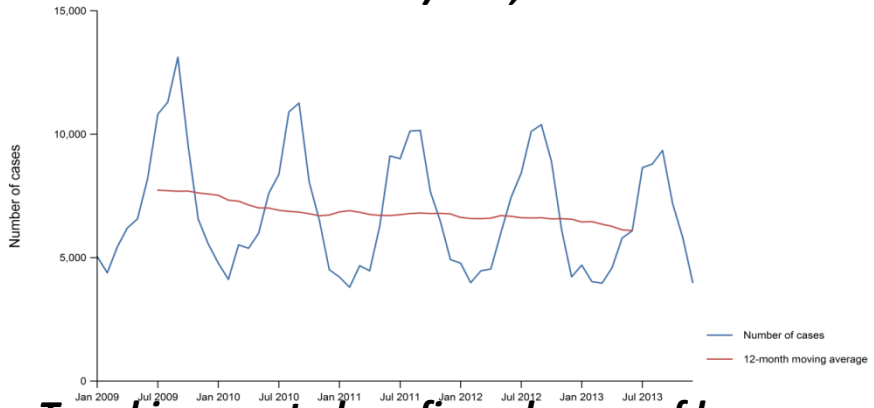
Phagebiotics in treatment and prophylaxis of foodborne infections

Andrey Aleshkin

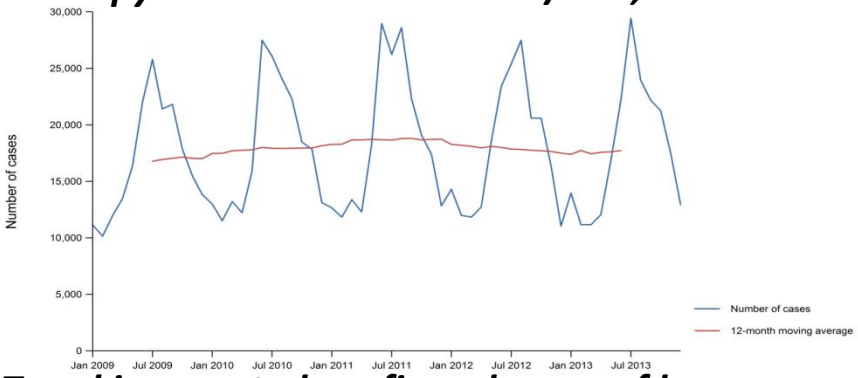
Bphage LLC, Moscow, Russia

Gabrichevsky Moscow Research Institute for Epidemiology and Microbiology, Russia

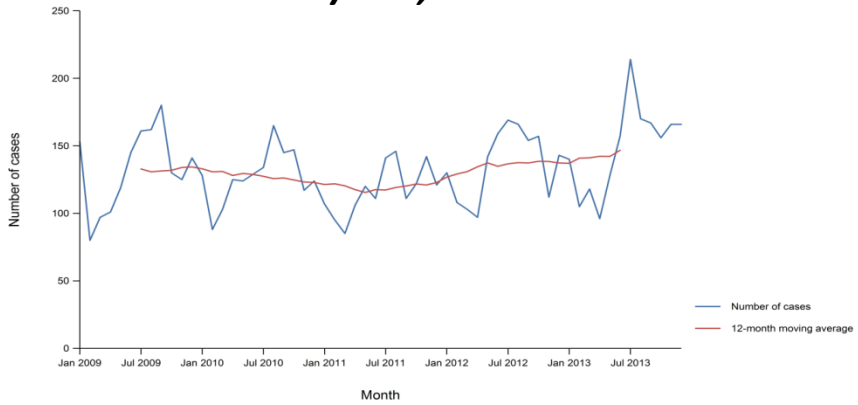
Trend in reported confirmed cases of human salmonellosis in the EU/EEA, 2009-2013



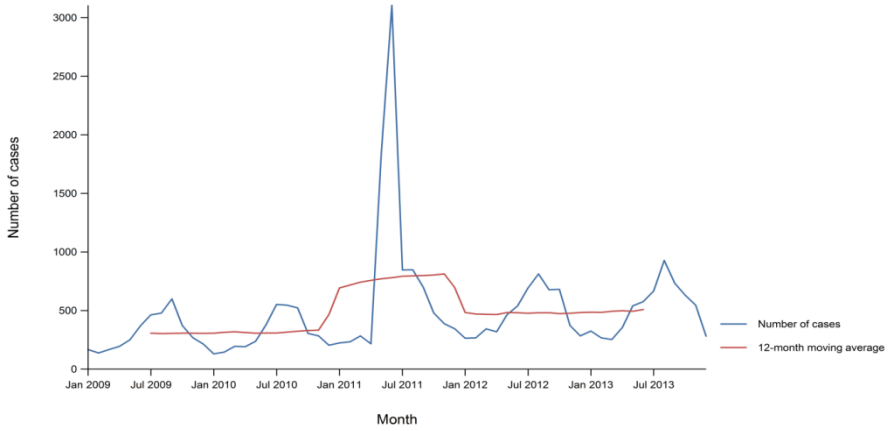
Trend in reported confirmed cases of human campylobacteriosis in the EU/EEA, 2009-2013



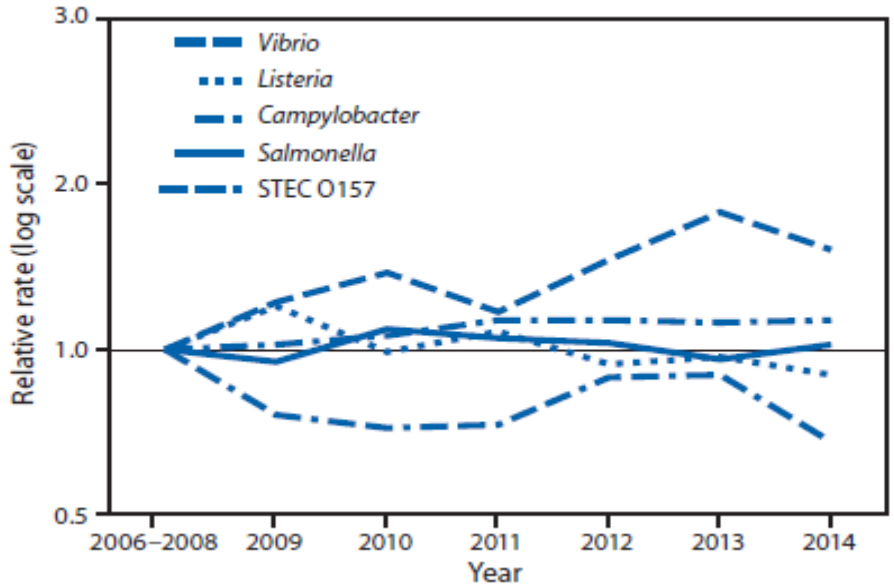
Trend in reported confirmed cases of human listeriosis in the EU/EEA, 2009-2013



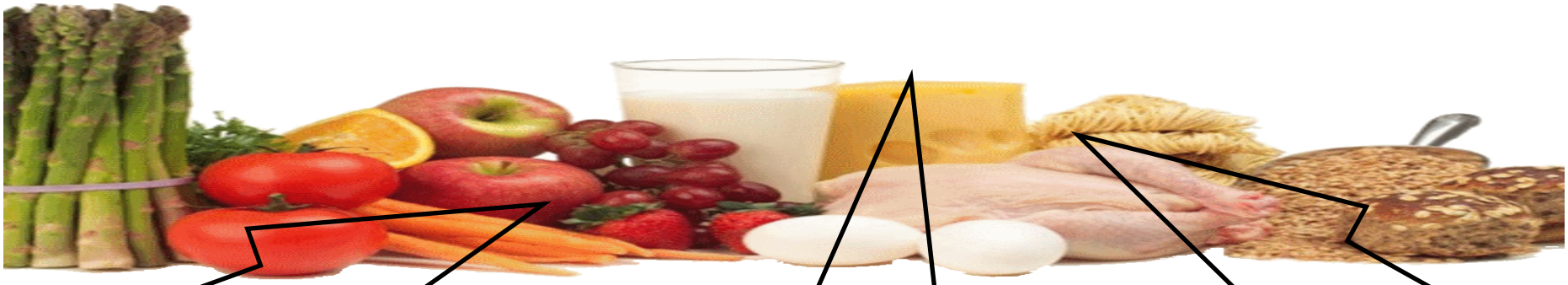
Trend in reported confirmed cases of human VTEC infections in the EU/EEA, 2009-2013



Trend in reported confirmed cases of salmonellosis, campylobacteriosis, listeriosis, VTEC and Vibrio infections in the US, 2006-2014



A NEW SEGMENT OF FOOD SUPPLEMENTS BASED ON BACTERIOPHAGES



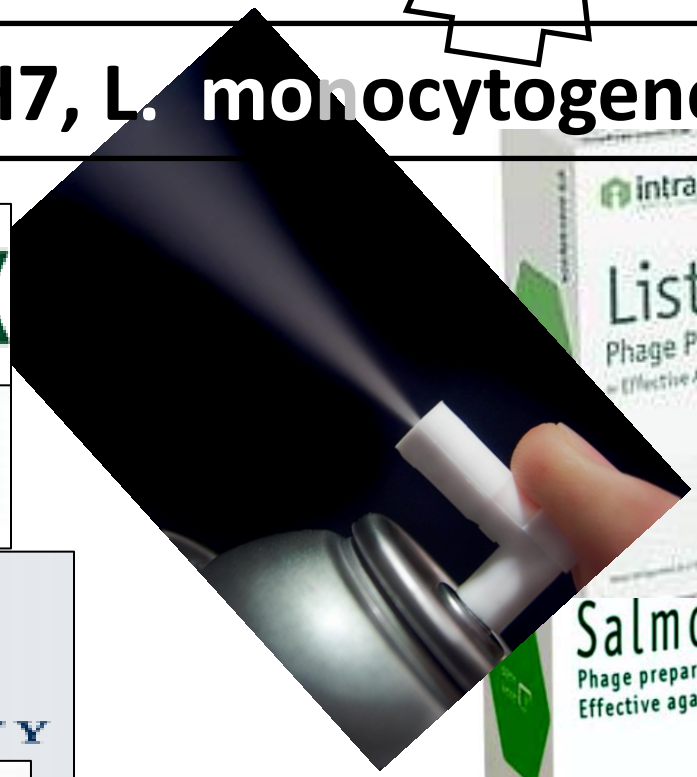
E. coli O157: H7, L. monocytogenes, S. enterica…

intralytix
SAFETY BY NATURE

GangaGen

EBI FOOD SAFETY

OMNILYTICS™
THE PHAGE COMPANY





The centenary history of phage treatment and prophylaxis of foodborne infections

- **Phase 1. The 1900s through the 1940s.**

Using phage prophylaxis and treatment in cases of foodborne infections during armed conflicts and large-scale outbreaks

- **Phase 2. The 1950s through the 1960s.**

With the emergence of antibiotics commercial production of medicinal phages ceased and they were considered as most convenient objects of virologic and genetic research.

- **Phase 3. The 1970s through the 1990s.**

Human trials of bacteriophage was further actively developed in the USSR and Poland, despite the skeptical attitudes of the Western scientists.

- **Phase 4. 2000 and beyond.**

Growing number of antibiotic resistant strains actualized a second birth of bacteriophage.

Invisible microbes antagonistic to the dysentery bacillus





An invisible microbe that is antagonistic to the dysentery bacillus

1917 • F. d'Herelle

d'Herelle, F. 1917. Sur un microbe invisible antagoniste des bacilles dysentériques. *Comptes rendus Acad. Sciences*, Vol. 165, pages 373-375.

From the feces of several patients convalescing from infection with the dysentery bacillus, as well as from the urine of another patient, I have isolated an invisible microbe endowed with an antagonistic property against the bacillus of Shiga. This discovery is particularly easy to make in cases of ordinary enteritis following upon dysentery infections. In convalescent cases in which enteritis does not occur, the antagonistic microbe disappears very soon after the disappearance of the pathogenic bacillus. In spite of a number of examinations, I have never found this antagonistic microbe in the feces of dysentery cases during the period of infection, nor in normal subjects.

It is very simple to isolate the anti-Shiga microbe. A tube of broth is seeded with four to five drops of feces, and the tube is placed in the incubator for 18 hours at 37°, and the contents then filtered through a Chamberland candle filter, L$_3$. When a small quantity of an active filtrate is added to a broth culture of the Shiga bacillus, or

d'Herelle, F. 1917. Sur un microbe invisible antagoniste des bacilles dysentériques. *Comptes rendus Acad. Sciences*, Vol. 165, pages 373–375.

The history of bacteriophage studies prior to their official discovery by F. d'Hellere

Original title	English title
Frankland P. Ueber das Verhalten des Typhusbacillus und des Bacillus coli communis im Trinkwasser. Zeitschrift für Hygiene und Infektionskrankheiten 1895; 19:393–407	On the behavior of the typhoid bacillus and the common Bacillus coli in drinking water
Hankin ME. L'action bactéricide des eaux de la Jumma et du Gange sur le vibrion du choléra. Annales de l'Inst Pasteur 1896; 10:511–23	The bactericidal action of waters of Jumna and Ganges on the cholera microbe
Hankin ME. Les microbes des rivières de l'Inde. Annales de l'Inst Pasteur 1896; 10:175–6	The microbes of the rivers of India
Gamaleya NF, 1898. Бактериолизны–ферменты, разрушающіе бактерій. Russ Arch Pathol Clin Med Bacteriol 6:607–13	Bacteriolysins-ferments destroying bacteria[1]
Klein A. Die physiologische Bakteriologie des Darmkanals. Archiv für Hygiene 1902; 45:117–76	The physiological bacteriology of the intestinal canal
Krencker E. Über Baktericidie von Bakterienfiltraten. Inaug-Diss, Straßburg 1903	On the bactericidal activity of bacterial filtrates
Lode A. Experimentelle Untersuchungen über Bakterienantagonismus. I. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten 1903; 33:196–208	Experimental studies on bacterial antagonists. I
Eijkman C. Ueber thermolabile Stoffwechselprodukte als Ursache der natürlichen Wachstumshemmung der Mikroorganismen. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten 1904; 37:436–49	On the thermolabile metabolites as a cause of the natural growth inhibition of microorganisms
Conradi H, Kurpjuweit O. Ueber spontane Wachstumshemmung der Bakterien infolge Selbstvergiftung. I. Muenchener Medizinische Wochenschrift 1905; 52:1761–4	On the spontaneous growth inhibition of bacteria due to self poisoning. I
Kantorowicz A. Bakterien-Antifermente und Bakteriolyse. Munchener Medizinische Wochenschrift 1909; 56:897–900	Bacterial fermentation inhibitors and bacterial lysis
de Waele H. Protéolase et antiprotéolase dans les cultures microbiennes. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten 1909; 50:40–4	Proteolytic enzymes and enzyme inhibitors in microbe cultures
Twort FW. An investigation on the nature of ultra-microscopic viruses. Lancet 1915; 2:1241–3	An investigation on the nature of ultra-microscopic viruses

First clinical application of the dysentery bacteriophage prepared by F. d'Hellere August 1, 1919, Paris



Victor-Henri Hutinel
1849-1933

Hôpital Necker – Enfants Malades supreetas

Assistance Publique – Hôpitaux de Paris





Georgy Grigoryevich Eliava
1892-1937

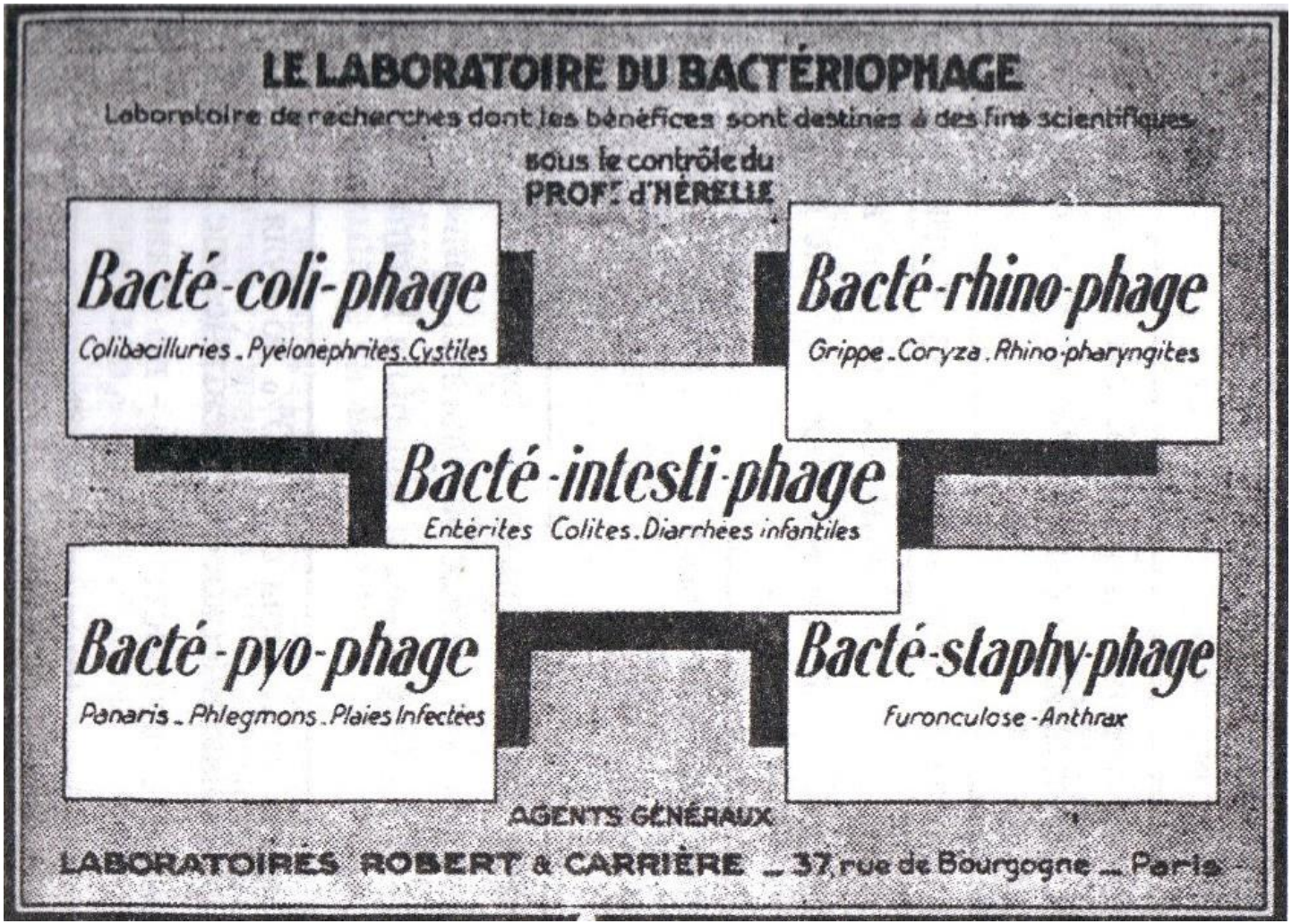


Institute of Bacteriology founded by G. Eliava in Tbilisi in 1923 (later Tbilisi Research Institute of Vaccines and Sera)



Félix d'Herelle
1873-1949

Manufacturing center «Laboratoire du Bactériophage» founded by Félix d'Hérelle and Theodore Mazure in France in 1931



Bacteriophage producers outside of Russia in 1920s-1930s

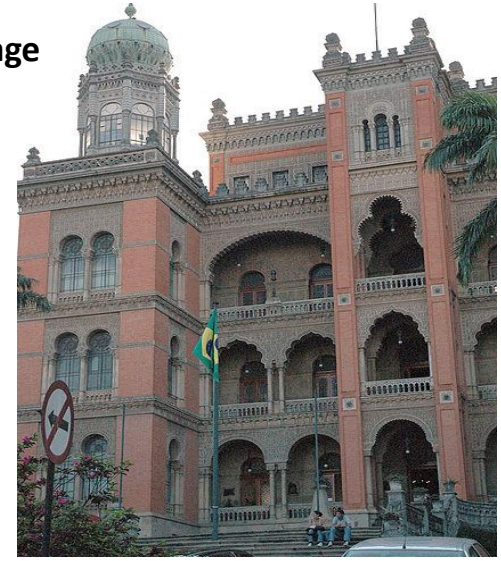
E.R. Squibb&Sons, USA
Staphylococcal bacteriophage



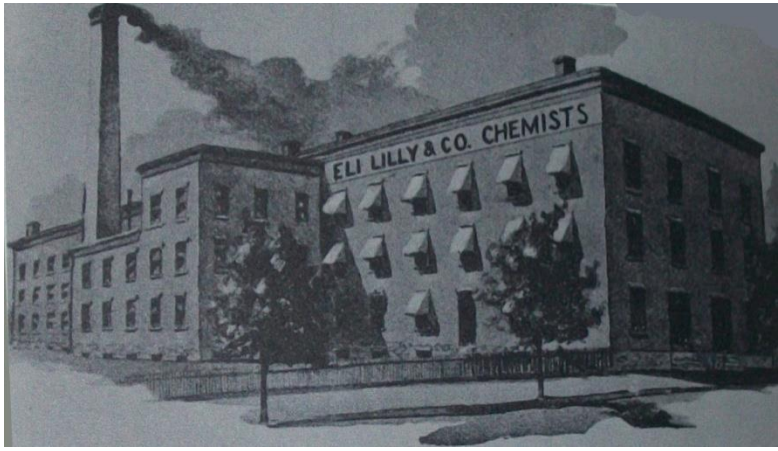
Swan-Myers (Abbot Laboratories), USA
Staphylococcal bacteriophage



Oswaldo Cruz Foundation, Brazil
Dysentery bacteriophage



Eli Lilly & Co. , USA
Colo-lysate, Ento-lysate, Staphylo-lysate…



Bacteriophage producers in the USSR in 1930s-1940s

Krestovnikova V.A. (1947) Phage therapy and phage prophylaxis and their development through the work of Soviet researchers

- Mechnikov Research Institute of Vaccines and Sera, Moscow
- Leningrad Research Institute of Vaccines and Sera
- Tbilisi Research Institute of Vaccines and Sera
- Mechnikov Research Institute of Vaccines and Sera, Ufa
- Perm Research Institute of Vaccines and Sera
- Gorky Research Institute of Vaccines and Sera
- Ukrainian Research Institute of Epidemiology and Microbiology
- Mechnikov Research Institute of Vaccines and Sera, Kharkiv
- Khabarovsk Research Institute of Epidemiology and Microbiology
- Tashkent Research Institute of Vaccines and Sera
- Tomsk Research Institute of Vaccines and Sera
- Kazakhstan Research Institute of Epidemiology and Microbiology

Application of bacteriophages for treatment and prophylaxis of dysentery (USSR, 1930s-1940s)

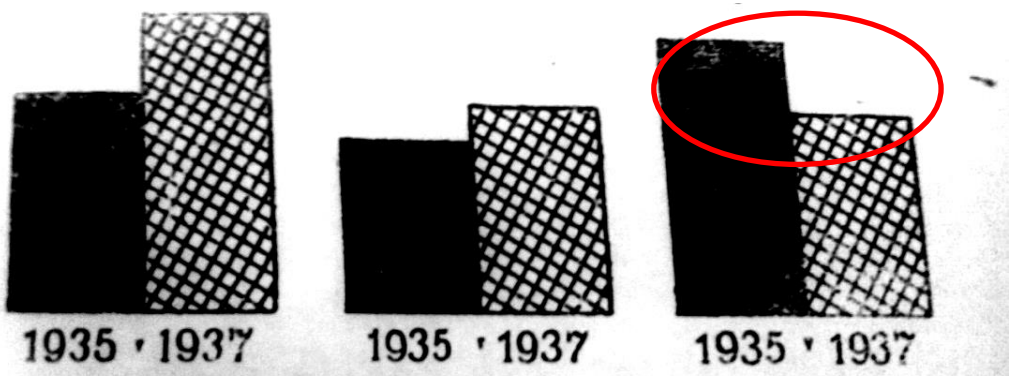
Strategy for oral administration of phage preparations

- Kaplan A.S. (1941) Distribution and maintenance of a polyvalent dysentery bacteriophage in the body of white mice after a single feeding
- Karpov S.P., Yavorskaya B.M. (1949) Dynamics of bacteriophage isolation from the body of phage treated persons

Sapir I.B. (1939)
Observations of and comments on phage treatment of dysentery

Duration of blood stool, days	Number of pts
1	26
5	70
7	4
Total	100

Ansheles I.M. (1939)
Experience of the therapeutic and prophylactic application of dysentery bacteriophage in Leningrad in 1937



Entire city Control districts Study districts

Incident rates per 10 000 people for July-September

Application of bacteriophages for treatment and prophylaxis of foodborne infections (USSR and Russia, 1960s-2000s)

Author and publication year	Name of bacteriophage preparation	Etiologic agent	Nosology	Number of patients	Efficiency of bacteriophage therapy	Details of research
Akimkin et al., 2010	Salmonella bacteriophage®	Salmonella	Nosocomial salmonellosis	459 patients and 2500 hospital staff	No facts of bacterial shedding in the hospital for 12 months after phage prophilaxis	2-4 tablets given 3 times a day for 5-7 days. The facts of self-recovery were reported because patients had their own bacteriophage strains in their organisms
Anpilov et al., 1984	tablets with ac… proof co…			…persons	…prevent outbreaks in enclosed collectives	…nce …m …in different geographic zones of the Soviet Union
Babalova et al., 1968	Dysentery bacteriophage®, in tablets	Shigella flexneri and S. sonne, E. coli	Dysentery	30769 children at the age of 0.5 to 7 years	Number of diseased persons in study group is 3.8 times lower than in the control group	1 tablet given every week for 109 days

The largest clinical trial of the period on prophylaxis of bacterial dysentery in Tbilisi involved 30 769 children...

Application of bacteriophages for treatment and prophylaxis of foodborne infections (USSR and Russia, 1960s-2000s) [continued]

Author and publication year	Name of bacteriophage preparation	Etiologic agent	Nosology	Number of patients	Efficiency of bacteriophage therapy	Details of research
Nilova et al., 2007	Staphyphage®, Proteus					Tests were performed to determine the Microgen)
Pogorelskaya et al., 1968	Coli-proteus bacteriophage®, Dysentery bacteriophage®	Entero-pathogenic E.coli, Pr. vulgaris, Pr. mirabilis, Shigella flexneri	Acute intestinal infection	3547 children at the age of 1 to 3 years	Phage prophylaxis during epidemic season reduced the incidence of intestinal infections	Multiple regular administration of phages (16-20 times a year)
Shahmardanov et al., 1997	Pyobacteriophage polyvalent®	Shigella flexneri and S. sonne, S. enteritidis	Dysentery, salmonellosis	29 41	Reduction in the length of diarrhea and alleviation of pains in the abdomen as compared with the standard therapy	Daily dose is 30 ml
Solodovnikov et al., 1970	Polyvalent dysentery bacteriophage®, in tablets with acid-proof coating	Shigella flexneri and S. sonne	Dysentery, acute intestinal infection	6522 children at the age of 1 to 7 years	Number of diseased persons in study group is 2.1-2.5 times lower than in the control group	1-2 tablets given every week from June to October

Incidence rate in children with microbiologically proven dysentery and colibacillosis treated with a combined (dysentery and coli-proteus) bacteriophage was more than 3 times less than in the untreated group…

Application of bacteriophages for treatment and prophylaxis of foodborne infections (USSR and Russia, 1960s-2000s) [continued]

Author and publication year	Name of bacteriophage preparation	Etiologic agent	Nosology	Number of patients	Efficiency of bacteriophage therapy	Details of research
Anpilov et al., 1984	Polyvalent dysentery bacteriophage®, in tablets with acid-proof coating	Shigella flexneri	Dysentery	3000 persons	Effective remedy to prevent outbreaks in enclosed collectives	2 tablets given once every 3 days from June to October in different geographic zones of the Soviet Union
Babalova et al., 1968	Dysentery			30760 children at	Number of diseased persons in study	
Voroshilova et al., 2010	Enterobacter purified	E. agglomerans	infections		therapy by 20-40%	
Litvinova et al., 1978	Coli-proteus bacteriophage®	E. coli, Proteus	Neonatal sepsis	500 premature children	Administration of bacteriophage allowed to reduce the lethality	Dose of 2 ml/kg administered twice a day during 3 days. The course is repeated after the interval of 3-4days
Milutina, 1990	Salmonella bacteriophage®, Dysentery bacteriophages®	S. Typhimurium, S. Enteritidis, Shigella flexneri	Salmonellosis, dysentery	45 children under the age of 12 months; 67 children above the age of 12 months	Recovery effect of salmonellosis phage is 81.8-85.4 %, and that of dysentery phage, 90.9%	Synergism of phage and antibiotic therapy was reported

Incidence rates in the study and control groups during the rise of epidemic varied statistically, reaching in a number of military formations in the Far East a difference of 9.5 times…

Application of bacteriophages for treatment and prophylaxis foodborne infections (USSR and Russia, 1960s-2000s) [continued]

Author and publication year	Name of bacteriophage preparation	Etiologic agent	Nosology	Number of patients	Efficiency of bacteriophage therapy	Details of research
Akimkin et al., 2010	Salmonella bacteriophage®	Salmonella	Nosocomial salmonellosis	459 patients and 2500 hospital staff	No facts of bacterial shedding in the hospital for 12 months after phage prophilaxis	2-4 tablets given 3 times a day for 5-7 days. The facts of self-recovery were reported because patients had their own bacteriophage strains in their
					than traditional antibiotic therapy by 20-40%	
Drozdova et al, 1988	Coli bacteriophages FM_{17}	E.coli M17	Experimental model of E.coli infection	28 children (0-14 y.o.) and 27 adults	Phage is effective in prevention of indoor transfer of intestinal infection	Surfaces previously contaminated by bacteria were treated with bacteriophage for 6 days

Not only the patients were treated with phages, but also the staff of the military hospital (3000 in total)……

…application of phage sprays in hospital environments proved effective in prevention of indoor transfer of acute intestinal infections

BACTERIOPHAGES AS PHARMACEUTICALS MANUFACTURED BY MICROGEN IN MODERN RUSSIA













A new class of probiotics – phagebiotics

No side effects typical for classical probiotics: infections, metabolic activities, immune stimulation, gene transfer and gastrointestinal ones

acteriophage 1:2, 66-85; May/June 2011; © 2011 Landes Bioscience

Phage treatment of human infections

Stephen T. Abedon,[1] Sarah J. Kuhl,[2] Bob G. Blasdel[1,3] and Elizabeth Martin Kutter[3,*]

Research Article

Bacteriophage Administration Reduces the Concentration of *Listeria monocytogenes* in the Gastrointestinal Tract and Its Translocation to Spleen and Liver in Experimentally Infected Mice

Volker Mai,[1,2] Maria Ukhanova,[2] Lee Visone,[1] Tamar Abuladze,[3] and Alexander Sulakvelidze[2,3]

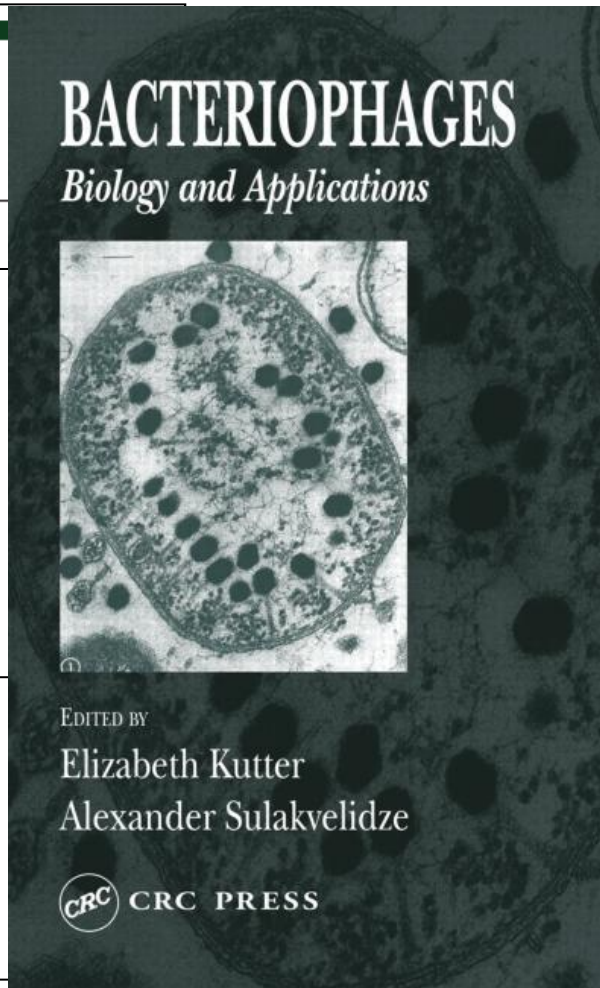
EDITOR'S CORNER

Bacteriophage 1:1, 1-2; January/February 2011; © 2011 Landes Bioscience

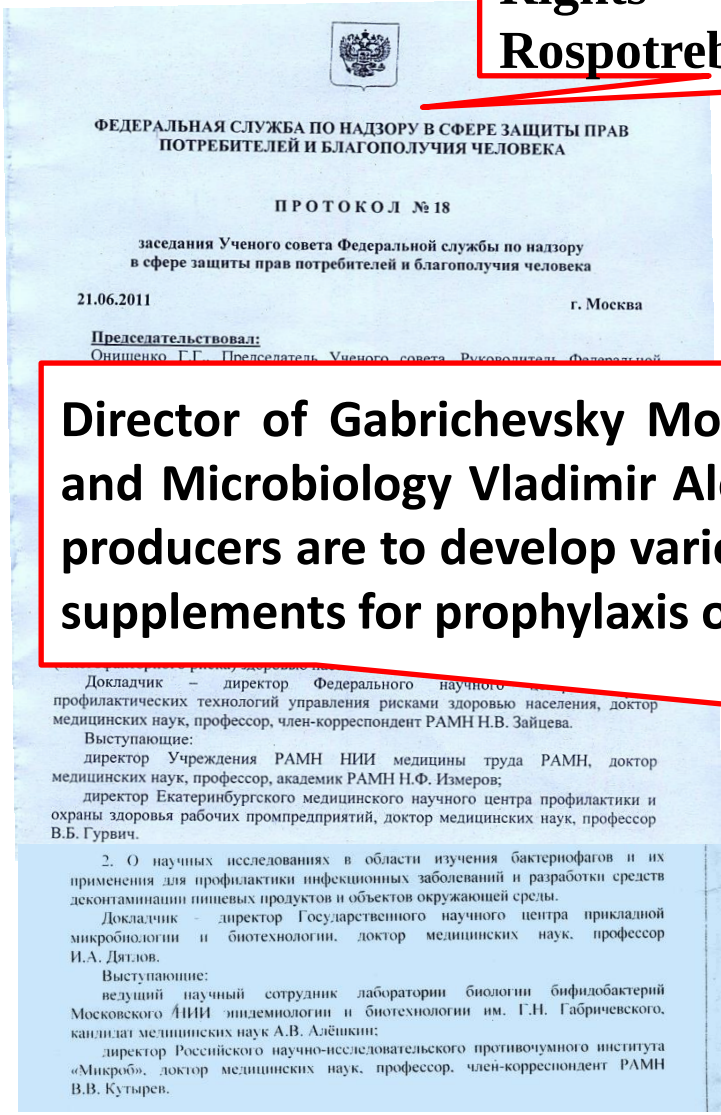
Bacteriophage

A new journal for the most ubiquitous organisms on Earth

Alexander Sulakvelidze



Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing – Rospotrebnadzor



ФЕДЕРАЛЬНАЯ СЛУЖБА ПО НАДЗОРУ В СФЕРЕ ЗАЩИТЫ ПРАВ ПОТРЕБИТЕЛЕЙ И БЛАГОПОЛУЧИЯ ЧЕЛОВЕКА

ПРОТОКОЛ № 18

заседания Ученого совета Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека

21.06.2011 г. Москва

Председательствовал:

Докладчик – директор Федерального научного ... профилактических технологий управления рисками здоровью населения, доктор медицинских наук, профессор, член-корреспондент РАМН Н.В. Зайцева.

Выступающие:

директор Учреждения РАМН НИИ медицины труда РАМН, доктор медицинских наук, профессор, академик РАМН Н.Ф. Измеров;

директор Екатеринбургского медицинского научного центра профилактики и охраны здоровья рабочих промпредприятий, доктор медицинских наук, профессор В.Б. Гурвич.

2. О научных исследованиях в области изучения бактериофагов и их применения для профилактики инфекционных заболеваний и разработки средств деконтаминации пищевых продуктов и объектов окружающей среды.

Докладчик – директор Государственного научного центра прикладной микробиологии и биотехнологии, доктор медицинских наук, профессор И.А. Дятлов.

Выступающие:

ведущий научный сотрудник лаборатории биологии бифидобактерий Московского НИИ эпидемиологии и биотехнологии им. Г.Н. Габричевского, кандидат медицинских наук А.В. Алёшкин;

директор Российского научно-исследовательского противочумного института «Микроб», доктор медицинских наук, профессор, член-корреспондент РАМН В.В. Кутырев.

РосНИПЧИ «Микроб» Роспотребнадзора В.В. Кутыреву, директору Московского НИИЭМ им. Г.Н. Габричевского Роспотребнадзора В.А.Алешкину:

4.1 в 2012 году сформировать научную тематику, направленную на поиск и конструирование вирулентных бактериофагов с высокими литическими свойствами против основных патогенных микроорганизмов, имеющих значение в пищевой промышленности и клинической практике;

4.2 в целях создания системы оценки и обеспечения безопасности для человека при использовании бактериофагов в лечебных и профилактических целях, провести работу по изучению зарубежного опыта оценки безопасности фагов и представить свои рекомендации по созданию алгоритма их изучения. Вынести данный вопрос на заседание ученого совета Роспотребнадзора в 2011 году.

Director of Gabrichevsky Moscow Research Institute for Epidemiology and Microbiology Vladimir Aleshkin in co-operation with bacteriophage producers are to develop various forms of phage-based probiotic dietary supplements for prophylaxis of foodborne infections

7. Директору Московскому НИИЭМ им. Г.Н. Габричевского В.А. Алешкину совместно с производителями фаговых препаратов направить усилия на разработку пищевых добавок на основе бактериофагов, а также в комплексе с бактериоцинами для применения в пищевой промышленности при производстве полуфабрикатов и готовых продуктов. Разработать варианты БАД на основе бактериофагов для декретированных категорий работников.

Председатель Ученого совета,
Руководитель Федеральной
службы по надзору в сфере
защиты прав потребителей
и благополучия человека,
академик РАМН

Г.Г. Онищенко

**Head of Federal Service,
Chief State Sanitary Physician
of the Russian Federation G.G. Onishchenko**

Phage-based probiotic dietary supplement “FOODPHAGE”



ДЕКЛАРАЦИЯ О СООТВЕТСТВИИ

Федеральное бюджетное учреждение науки "Московский научно-исследовательский институт эпидемиологии и микробиологии им. Г.Н. Габричевского" Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека (ФБУН МНИИЭМ им. Г.Н. Габричевского Роспотребнадзора)

Зарегистрировано: Межрайонной инспекцией Федеральной налоговой службы № 46 по г. Москве. Свидетельство о внесении записи в ЕГРЮЛ от 29.03.2011 г.; ОГРН № 1037739396507

125212, г. Москва, ул. Адмирала Макарова, д. 10, телефон (495) 452-18-16, факс (495) 452-18-16

в лице директора Алешкина Владимира Андриановича

заявляет, что

Специализированный пищевой продукт для диетического (профилактического) питания "ФУДФАГ" (FOODPHAGE).

выпускаемая по ТУ 9197-163-78095326-2012 "Продукт диетического (профилактического) питания "ФУДФАГ" (FOODPHAGE). Технические условия".

Серийный выпуск

Код ОК 005-93 (ОКП): 91 9769

Код ТН ВЭД России

соответствует требованиям

СанПиН 2.3.2.1078-01 "Гигиенические требования безопасности и пищевой ценности пищевых продуктов". Приложение 1. Индексы п.п. 1.10.10., 1.10.10.1., 1.9.11.1, 1.10.8.; ГОСТ Р 51074-2003 "Продукты пищевые. Информация для потребителя. Общие требования. р.3. СанПиН 2.3.2.1290-03 "Гигиенические требования к организации производства и оборота биологически активных добавок к пище (БАД)".

Декларация принята на основании

свидетельства о государственной регистрации № RU.77.99.19.004.E.001234.02.13 от 20.02.2013 г., выданного Роспотребнадзором (бланк № 0236510); протокола лабораторных исследований № 317 от 30.01.2013 г., выданного АИЦ Филиал ФБУЗ "Центр гигиены и эпидемиологии в городе Москве" в САО города Москвы (аттестат аккредитации № РОСС RU.0001.21АВ38).

Дата принятия декларации: 28.02.2013

Декларация о соответствии действительна до: 28.02.2016

Директор
В.А. Алешкин
инициалы, фамилия

подпись

м.п.

Сведения о регистрации декларации о соответствии

ОРГАН ПО СЕРТИФИКАЦИИ ПРОДУКЦИИ И УСЛУГ ОБЩЕСТВА С ОГРАНИЧЕННОЙ ОТВЕТСТВЕННОСТЬЮ "КООРДИНАЦИОННЫЙ ЦЕНТР СИСТЕМ ДОБРОВОЛЬНОЙ СЕРТИФИКАЦИИ"

121170, г. Москва, Кутузовский проспект, д. 36, стр. 8, тел. (495) 780-54-47, факс (495) 780-54-47

Аттестат рег. № РОСС RU.0001.10АИ53 выдан Федеральное агентство по техническому регулированию и метрологии

Дата регистрации 28.02.2013, регистрационный номер декларации РОСС RU.АИ53.Д01357

Е.И. Свешова
инициалы, фамилия руководителя органа по сертификации

подпись

м.п.



ЕВРАЗЭС

ТАМОЖЕННЫЙ СОЮЗ
РЕСПУБЛИКИ БЕЛАРУСЬ, РЕСПУБЛИКИ КАЗАХСТАН
И РОССИЙСКОЙ ФЕДЕРАЦИИ

Федеральная служба по надзору в сфере защиты прав потребителей и благополучия человека
Главный государственный санитарный врач Российской Федерации
Российская Федерация

(уполномоченный орган Стороны, руководитель уполномоченного органа, наименование административно-территориального образования)

СВИДЕТЕЛЬСТВО
о государственной регистрации

№ RU.77.99.19.004.E.001234.02.13 от 20.02.2013 г.

Продукция:
специализированный пищевой продукт для диетического (профилактического) питания "ФУДФАГ" (FOODPHAGE). Изготовлена в соответствии с документами: ТУ 9197-163-78095326-2012.
Изготовитель (производитель): ЗАО "ИГТех", 125212, Г. Москва, ул.Адмирала Макарова, д. 10 стр. 1, ФБУН "МНИИЭМ им. Г.Н. Габричевского" Роспотребнадзора, 125212, г. Москва, ул. Адмирала Макарова, д. 10, Российская Федерация. Получатель: "ФБУН МНИИЭМ им. Г.Н. Габричевского Роспотребнадзора", 125212, г. Москва, ул. Адмирала Макарова, д. 10, Российская Федерация.

(наименование продукции, нормативные и (или) технические документы, в соответствии с которыми изготовлена продукция, наименование и место нахождения изготовителя (производителя), получателя)

соответствует
Единым санитарно-эпидемиологическим и гигиеническим требованиям к товарам, подлежащим санитарно-эпидемиологическому надзору (контролю)

прошла государственную регистрацию, внесена в Реестр свидетельств о государственной регистрации и разрешена для производства, реализации и использования
для реализации населению в качестве продукта диетического (профилактического) питания для декретированных категорий работников

Настоящее свидетельство выдано на основании (перечислить рассмотренные протоколы исследований, наименование организации (испытательной лаборатории, центра), проводившей исследования, другие рассмотренные документы):
взамен свидетельства о государственной организации № RU.77.99.19.004.E.000122.01.13 от 14.01.2013 г., экспертного заключения ФГБУ "НИИ Питания" РАМН № 72/Э-1282/б-12 от 13.12.2012 г., протоколов лабораторных исследований ФБУН ГНЦ ПМБ № 1 от 31.01.2013 г., ФБУЗ ЦГиЭ в г. Москве Филиал ФБУЗ "ЦГиЭ в г. Москве" в САО г. Москвы № 317 от 30.01.2013 г.

Срок действия свидетельства о государственной регистрации устанавливается на весь период изготовления продукции или поставок подконтрольных товаров на территорию таможенного союза

Подпись, ФИО, должность уполномоченного лица, выдавшего документ, и печать органа (учреждения), выдавшего документ

Г.Г. Онищенко

(Ф. И. О., подпись)

№0236510

М. П.

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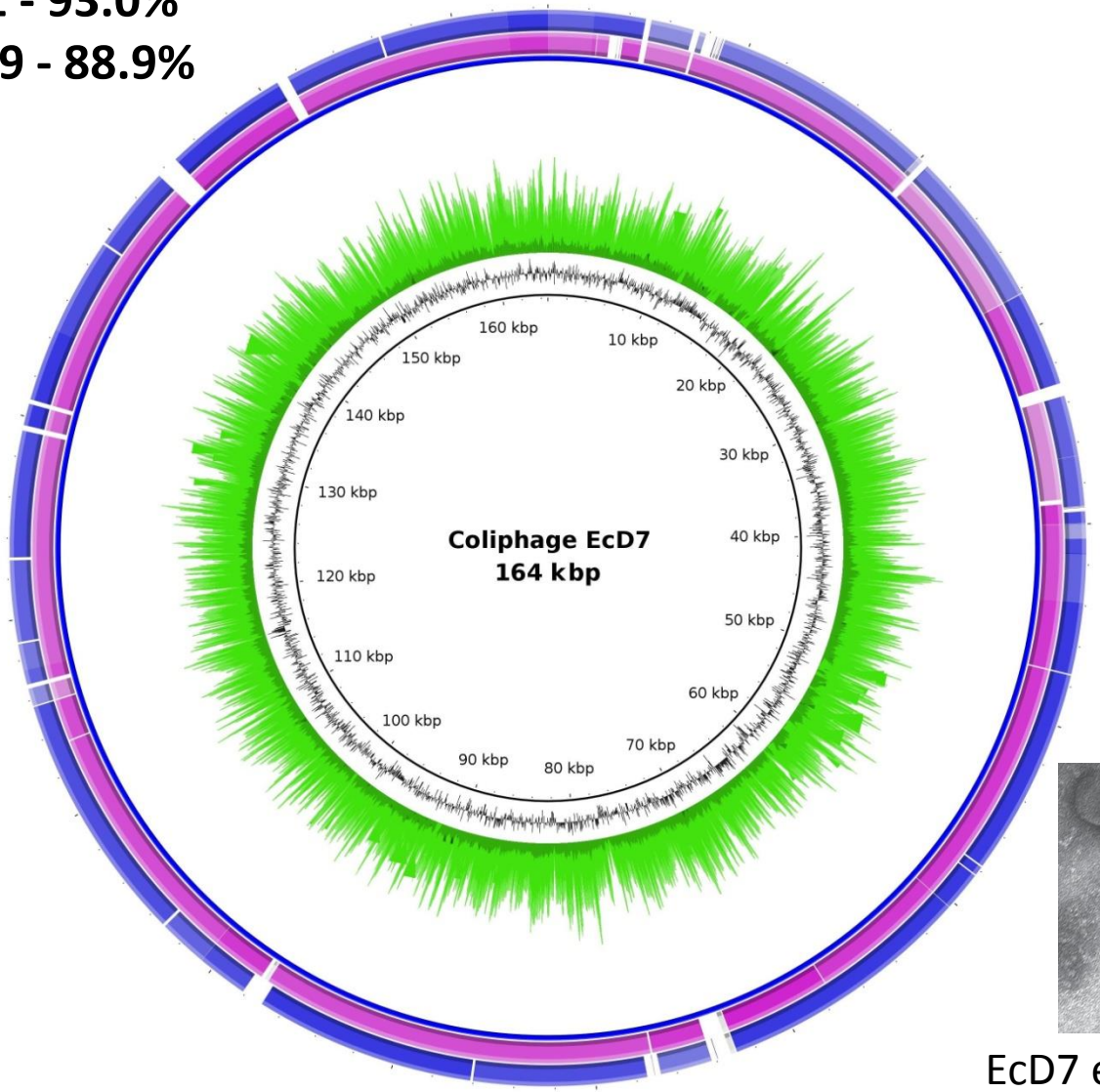


Characteristics of the bacteriophage cocktail included in probiotic dietary supplement "FOODPHAGE"

Phage	CH1	EcD7	V18	SE40	ST11	SI3	Lm1
Host species	*S. aureus*	*E. coli* O104:H4	*E. coli* O157:H7	*S. enteritidis*	*S. typhimurium*	*S. infantis*	*L. monocytogenes*
Initial source & place of isolation	patient's wound, Chelyabinsk	chicken feces, Moscow Region	cowshed sewage, Moscow Region	soil, Moscow Region	chicken feces, Kaluga Region	cattle feces, Moscow Region	sheep feces, Astrakhan Region
Host range activity (%/bacterial cultures)	92/ MRSA strains	100/ *E. coli* O104:H4 O104:H12 O157:H7 etc., 70/ *Sh. sonnei* & *flexneri*	100/ *E. coli* O157:H7 O157:H^-	85/ *S. enteritidis*	100/ *S. typhimurium*	64/ *S. infantis*	92/ *L. monocytogenes*
Presence of known toxins-related genes	No α-toxin gene	No rfb, stx1, stx2, eae & fliC genes	No rfb, stx1, stx2, eae & fliC genes	No stA, ltB genes	No stA, ltB genes	No stA, ltB genes	No LLO-toxin gene
Activity (titre), pfu/ml	10^{12}	10^{12}	10^{11}	10^{13}	10^{12}	10^{12}	10^{10}

A comparison of the genome of the coliphage EcD7 with the affine phages of the T4-like viruses Phi1 and RB49

Phage genome identity:
EcD7 against Phi1 - 93.0%
EcD7 against RB49 - 88.9%





EcD7 electron micrograph

A comparison of the genome of the coliphage V18 with the affine phages of the Myoviridae rv5, vB_EcoM-FV3, 2 JES-2013 and vB_EcoM_FFH2

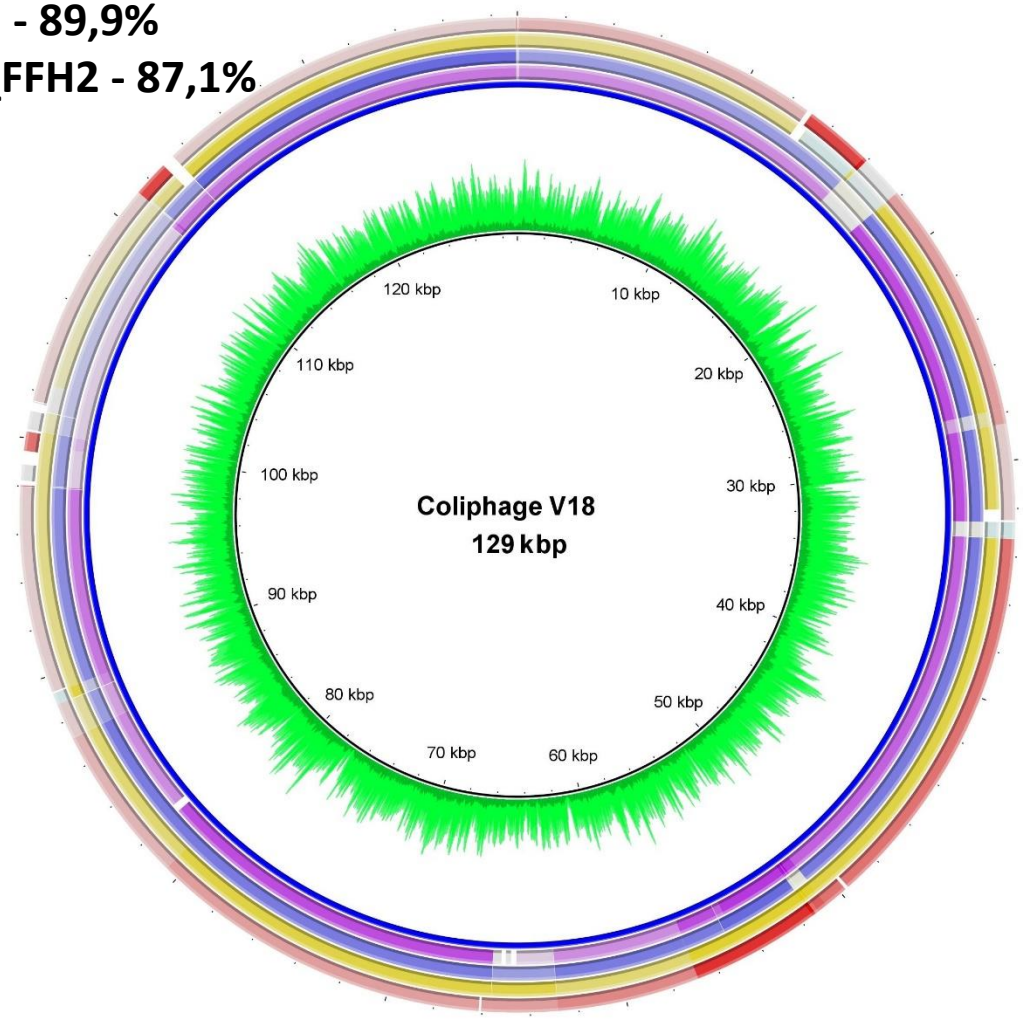
Phage genome identity:

V18 against rv5 - 88,6%

V18 against vB_EcoM-FV3 - 83,7%

V18 against 2 JES-2013 - 89,9%

V18 against vB_EcoM_FFH2 - 87,1%





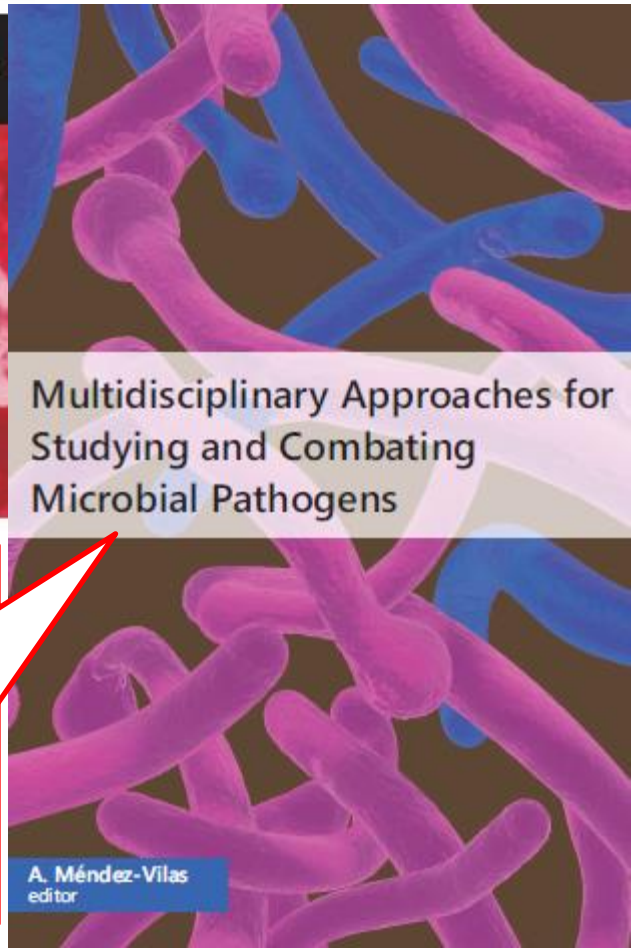
V18 electron micrograph

Bacteriophages as Probiotics and Decontaminating Agents for Food Products , in: Bacteriophages: Biology, Applications and Role in Health and Disease, Clark Denton and Richard J. Crosby (Ed.), pp. 93-110





Bacteriophage cocktail in prophylaxis against food-borne infections, in: Worldwide research efforts in the fighting against microbial pathogens, A.Mendez-Vilas (Ed.), pp. 100-104



Effectiveness of phage-based probiotic dietary supplement in the prevention of E.coli traveler's diarrhea: a small-scale study, in: Multidisciplinary Approaches for Studying and Combating Microbial Pathogens, A.Mendez-Vilas (Ed.), pp. 81-84

Design of a pilot study on modeling of E.coli foodborne infection

Groups	Study	Control
Lab animals – mice		
Number	20	20
Initial control for absence of lactose negative E. coli in the 1st day of experiment		
Infestation on 2nd-4th day of experiment	Once in 24 hours intragastrically 0.5 ml E. coli K12 C600 in titer 5×10^7 CFU/ml	
Prophylaxis from 1st to 5th day	Once in 24 hours 0.5 ml, FoodPhage	Once in 24 hours 0.5 ml, saline solution
Microbiological analysis - identification of lactose negative E. coli with subsequent PCR-analysis using specific primer for E.coli K12 C600 on 6th day		
Humans – healthy volunteers		
Number	30	15
Initial control for absence of lactose negative E. coli in the 1st day of experiment		
Infestation on 2nd-4th day of experiment	Once in 24 hours per os 20 ml E. coli K12 C600 in titer 5×10^7 CFU/ml	
Prophylaxis from 1st to 5th day	Twice in 24 hours 50 ml, FoodPhage	Twice in 24 hours 50 ml, saline solution
Microbiological analysis - identification of lactose negative E. coli with subsequent PCR-analysis using specific primer for E.coli K12 C600 on 6th day		

Microbiological test results for feces

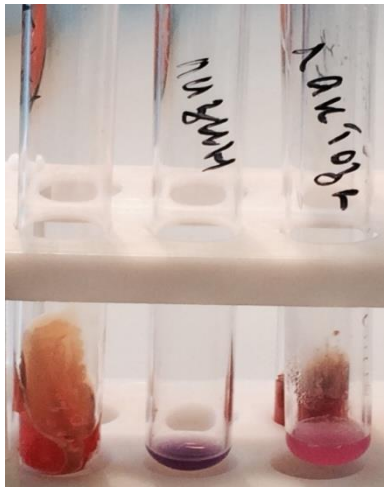
GROUPS	1st DAY (prior to infestation)	6th DAY
Identified microorganisms	Lactose-negative E. coli, CFU/ml	
Lab animals – mice		
Control	0	≤ 10^4
Study	0	0
Humans – healthy volunteers		
Control	0	≤ 10^6
Study	0	0

E. coli Lac+

Endo agar



TSI agar | Lysine agar | Lactose agar

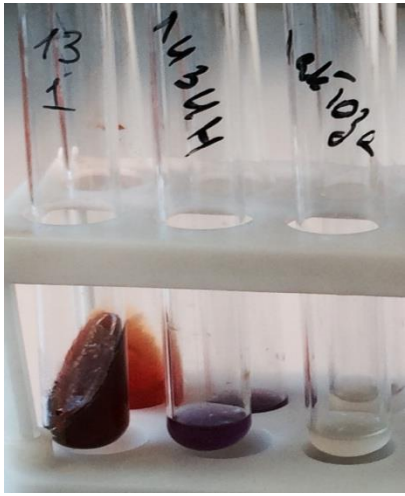


E. coli Lac-

Endo agar



TSI Agar | Lysine agar | Lactose agar



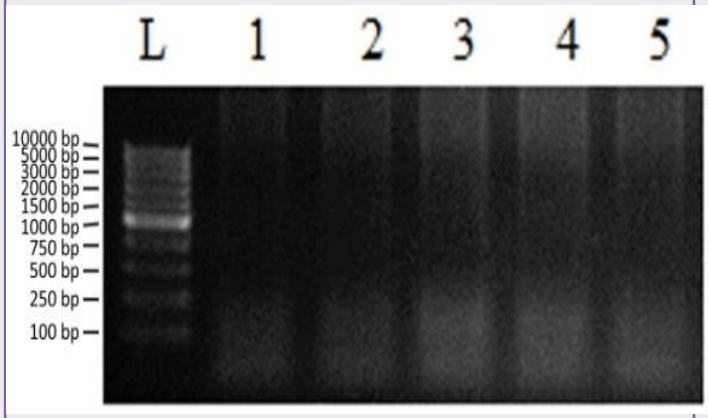
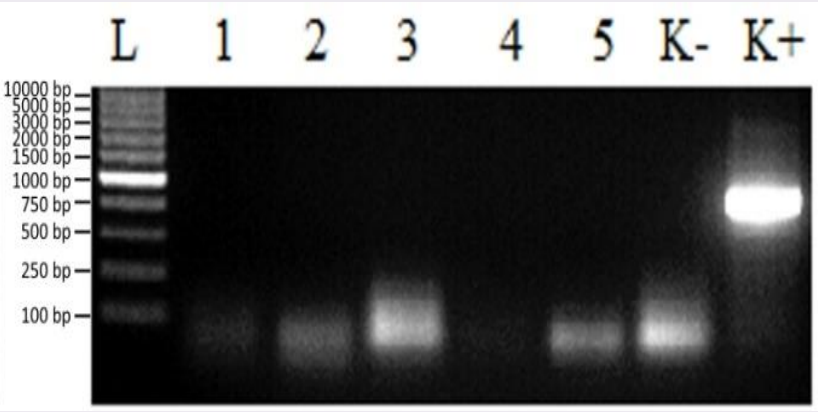
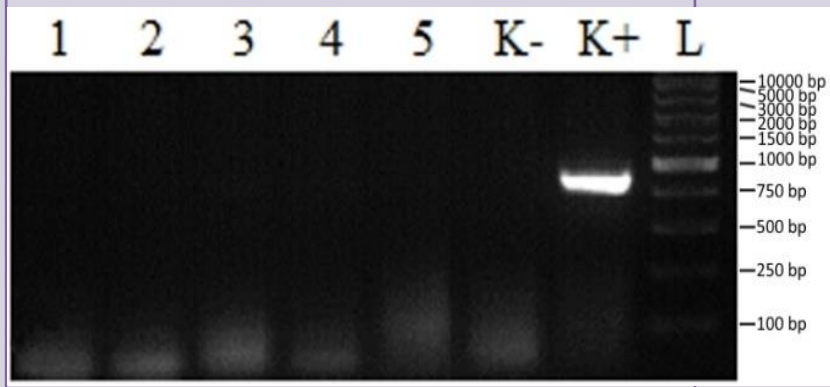
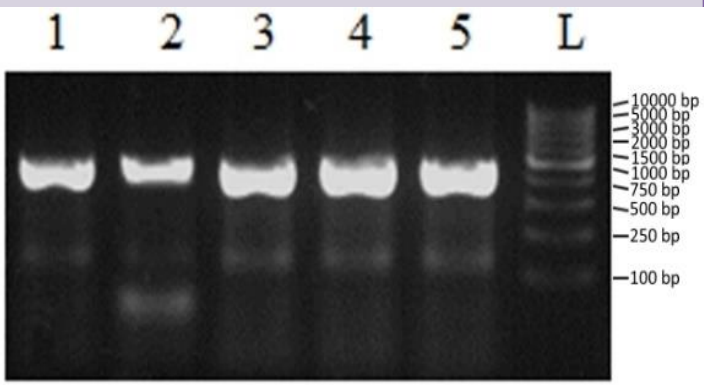
Identification of Echerichia bacteriophage in stools of study groups on 6th-7th day (spot-test)

Day of experiment	7 (48 h after intake of FoodPhage)	8 (72 h after intake of FoodPhage)
Per cent identified	100% of cases	20% of cases





PCR-test results

Primers for amplification	**K12-R (59-ATCCTGCGCACC AATCAACAA-39), K12IS-L (59-CGCGATGGAAGATGCTCTGTA-39). Amplicon length 969 bp**	
Day of experiment	**1 (prior to infestation)**	**6**
Study group		
Control group		

1-5 – 1st-5th dilutions of sample, K- and K+ – negative and positive controls, L– ladder, 1 kb

Is FoodPhage really active against global foodborne infections?

spot-test in the double-layer agar assay

#	super clear
+	clear
"+/-"	turbid

Strain	Serogroup	Virulence genes	EcD7	V18	FoodPhage	Strain	Serogroup	Virulence genes	EcD7	V18	FoodPhage
ED155	O126	vtx1	-	#	#	ED479b	O157	eae vtx1 vtx2	+	#	#
ED159	O157	eae vtx1 vtx2	+	#	#	ED499	O157	eae vtx2	+	#	#
ED179	O157	eae vtx2	+	#	#	ED510	O128	vtx2	-	-	#
ED207	O157	eae vtx1 vtx2	+	+	"+/-"	ED529	O15	vtx2	-	+	+
ED209	O157	eae vtx2	+	+	+	ED548	O146	vtx1 vtx2	+	+	“+/-”
ED238	O121	eae vtx2	+	-	+	ED551	O26	eae vtx2	-	+	+
ED257	O157	eae vtx1 vtx2	+	+	#	ED566	O145	eae vtx2	"+/-"	-	+
ED259	O103	eae vtx1	"+/-"	"+/-"	#	ED581	O111	eae vtx1 vtx2	-	+	+
ED273	O157	eae vtx2	+	+	#	ED602	O121	eae vtx2	#	-	#
ED279	O157	eae vtx2	+	+	#	ED645	O145	eae vtx2	-	-	+
ED280	O157	eae vtx2	+	+	#	ED672	O111	eae vtx1 vtx2	-	+	+
ED287	O103	eae vtx1	"+/-"	"+/-"	+	ED676	O26	eae vtx2	-	-	+
ED314	O157	eae vtx1 vtx2	+	#	+	ED677	O103	eae vtx2	-	-	+
ED320	O157	eae vtx1 vtx2	"+/-"	#	+	ED678	O114	eae vtx2	-	+	+
ED326	O157	eae vtx2	+	#	+	ED681	O128	eae vtx2	-	-	-
ED340	O157	eae vtx2	"+/-"	#	+	ED692	O55	eae vtx2	-	"+/-"	"+/-"
ED350	O157	eae vtx2	+	+	#	ED702	O55	eae vtx2	-	+	“+/-”
ED391	O157	eae vtx2	+	+	#	ED703	O104	aggR vtx2	+	+	#
ED422	O157	eae vtx1 vtx2	+	#	+	ED731	O128	vtx1 vtx2	-	-	+
ED438	O157	eae vtx1 vtx2	+	+	+	ED745	O26	eae vtx1	-	+	"+/-"
ED446	O55	eae vtx-	-	+	+	ED771	O104	aggR vtx2	#	+	#
ED450	O157	eae vtx1 vtx2	"+"	+	#	ED792	O125	eae vtx2	-	-	+
ED473	O145	eae vtx2	"+/-"	-	+	ED841	O104	aggR vtx2	#	-	#
ED476	O111	eae vtx1 vtx2	-	+	+	ED898	O121	eae vtx2	+	-	+
ED478	O156	eae vtx1	+	#	#	ED924	O127	aggR vtx2	"+/-"	+	#
ED479a	O157	eae vtx1 vtx2	"+"	#	#						

Conclusions

- In these animals and human small-scale trials we have demonstrated the possibility to use the phage-based probiotic dietary supplement FoodPhage for prophylaxis of a non-pathogenic E. coli infection
- These results have great significance because two original coliphages included in this cocktail have a broad host-range including O26, O55, O103, O104, O121, O125, O127, O128, O145, O146, O157 E. coli strains which cause severe cases of foodborne infections

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The Program Committee of the conference invites scientists and specialists of your organization to take an active part in the **Third International Scientific Conference entitled "Bacteriophages: Theoretical and Practical Aspects of Their Application in Medicine, Veterinary and Food"** which will be held in Moscow **13-15 October 2016**.

Thank you for your attention!

