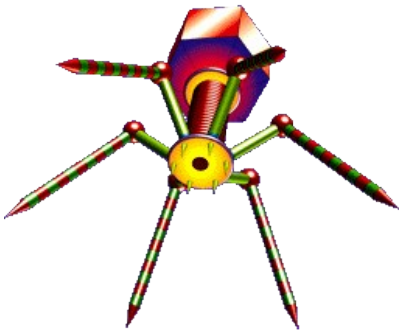

Bacteriophage Ecology Group (BEG) News

Dedicated to the ecology and evolutionary biology of the parasites of unicellular organisms (UOPs)

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January 1, 2001 issue (volume 7)

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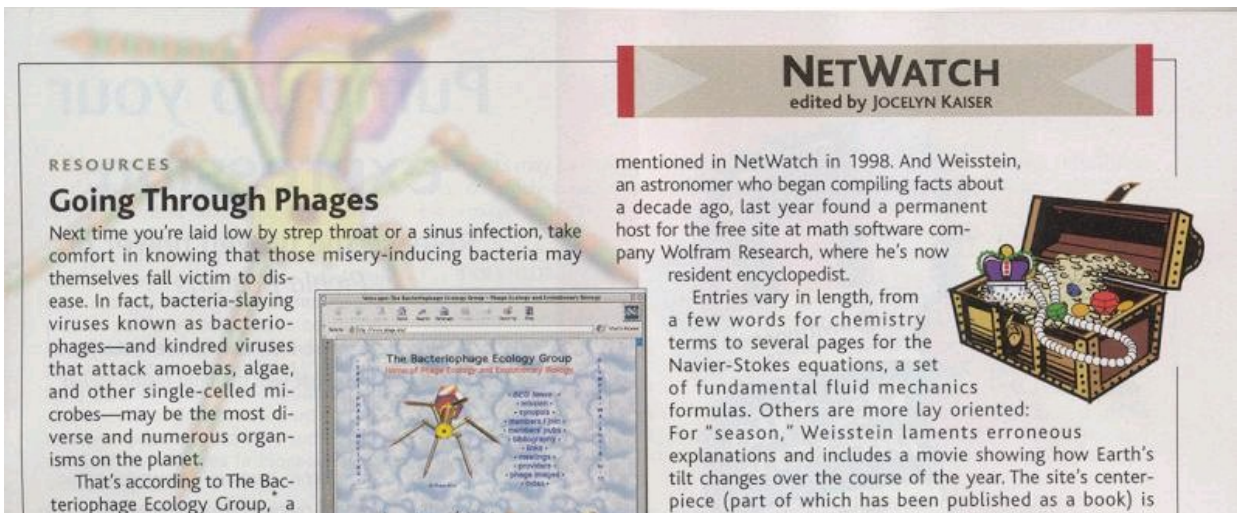
Editorial

Editorials should be written on subjects relevant to The Bacteriophage Ecology Group as an organization, to *BEG News* (either the concept or a given issue of *BEG News*), or the science of Bacteriophage Ecology. While my assumption is that I will be writing the bulk of these editorials, I wish to encourage as many people as possible to seek to relieve me of this duty, as often as possible. Additionally, I welcome suggestions of topics that may be addressed. Please address all correspondences to abedon.1@osu.edu or to "Editorials," *Bacteriophage Ecology Group News*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Please send all submissions as Microsoft Word documents, if possible (I'll let you know if I have trouble converting other document formats), and in English.

Science NetWatch October 13, 2000



NETWATCH
edited by JOCELYN KAISER

RESOURCES

Going Through Phages

Next time you're laid low by strep throat or a sinus infection, take comfort in knowing that those misery-inducing bacteria may themselves fall victim to disease. In fact, bacteria-slaying viruses known as bacteriophages—and kindred viruses that attack amoebas, algae, and other single-celled microbes—may be the most diverse and numerous organisms on the planet.

That's according to The Bacteriophage Ecology Group, a

mentioned in NetWatch in 1998. And Weisstein, an astronomer who began compiling facts about a decade ago, last year found a permanent host for the free site at math software company Wolfram Research, where he's now resident encyclopedist.

Entries vary in length, from a few words for chemistry terms to several pages for the Navier-Stokes equations, a set of fundamental fluid mechanics formulas. Others are more lay oriented: For "season," Weisstein laments erroneous explanations and includes a movie showing how Earth's tilt changes over the course of the year. The site's centerpiece (part of which has been published as a book) is

site started 4 years ago by microbiologist Stephen Abedon of Ohio State University, Mansfield. Born of his frustration at the lack of cohesion among the biologists who study bacteriophage ecology and evolution, the site will encourage collaboration and help define this emerging—and sprawling—field, Abedon hopes. It spans everything from studying the role of phages in antibiotic resistance to trying to enlist them in combating human staph infections.

One major site offering is a periodic newsletter that covers topics such as the use of phages as models for human viruses. You can also track down kindred researchers from around the world or troll a 3500-paper bibliography and list of recent publications. Other features include a passel of phage-themed links and a gallery of micrographs and cartoons. Sit back and enjoy the animation showing a lunar lander–like phage injecting its DNA into a cell.

www.phage.org

TUTORIALS

Fish Stories

To learn the life histories of their slippery subjects, fisheries scientists pull out the animals' otoliths—small ear bones with microscopic growth rings. The otoliths' shape and elemental content reveal everything from a fish's species and age to its migratory wanderings. This site offers a thorough introduction to otoliths, from how the growth rings form to various analytical techniques (such as radiochemical dating and laser assays). A section for students includes directions for a high school–level otolith class project.

www.mar.dfo-mpo.gc.ca/science/mfd/otolith/english/daily.htm

ENCYCLOPEDIAS

Growing a Book of Science

To look up, say, "brown dwarf," "pendulum," or "Navier-Stokes equations," you could consult any number of free Web dictionaries. But these corporate sites often lack the breadth, detail, and occasional quirkiness of those built by lone fact aficionados. Those seeking such an experience may want to check out Eric Weisstein's Treasure Troves of Science. This storehouse of definitions for math, astronomy, physics, and other topics has grown considerably since it was MathWorld, which offers definitions, diagrams, and references for nearly 10,000 terms—from the A-Cordial graph to the Zsigmondy theorem. Especially popular among students are the brief biographies of more than 1000 scientists. Frustrated by a missing entry? Take up Weisstein's invitation to contribute.

www.treasure-troves.com

REVIEWS

Neandertal Nook

A flurry of recent evidence fueling a debate over whether Neandertals were related to modern humans inspired Neandertals and Modern Humans: A Regional Guide. The site is run by Scott J. Brown, "an independent anthropological researcher and writer" who believes the stocky, low-browed hominids who died out about 30,000 years ago "were far more intelligent and capable than many anthropologists have suspected." Despite his own views, Brown has attempted to evenhandedly summarize evidence from Europe and Asia—such as genetic analysis of bones, and a Neandertal child's skull that's said to have modern traits. The well-referenced site also includes links to relevant books, journals, museums, societies, and research institutions.

www.neanderthal-modern.com

CATALOGS

Meteorite Stash

About 60 meteorites have been found in Canada, including Tagish Lake; fragments from a fireball over the Yukon last January may be the most primitive meteorite ever identified (see Report on p. 320). The Provincial Museum of Alberta hosts a site describing where and how each of the country's meteorites was discovered.

www.geo.ucalgary.ca/cdnmeteorites

Send Internet news and great Web site suggestions to netwatch@aaas.org

CREDITS (BOTTOM LEFT) BEDFORD INST. OF OCEANOGRAPHY, CANADA; (BOTTOM RIGHT) C. ZOLLIKOFER/UNIV. OF ZÜRICH, IRCHEL

Editorial Archive

- BEG: What we are, Where we are, Where we're going
- When Grown *In Vitro*, do Parasites of Multicellular Organisms (MOPs) become Unicellular Organism Parasites (UOPs)?
- Bacteriophages as Model Systems
- 2000 and Sun: A Phage Odyssey
- Lytic, Lysogenic, Temperate, Chronic, Virulent, Quoi?
- Which Ecology are You?
- *Science* NetWatch October 13, 2000

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New BEG Members

The BEG members list can be found at www.phage.org/beg_members.htm as well as on the BEG home page. As we add new members, these individuals will be introduced in this section. Note that, in fact, there are two ways of "joining" BEG. One, the "traditional" way, is to have your name listed on the web page and on the list server. The second, the "non-traditional" way, is to have your name only listed on the list server. The latter I refer to as "non-members" on that list. Members, e.g., individuals listed on the BEG home page, should be limited to individuals who are actively involved in science and who can serve as a phage ecology resource to interested individuals. If you have an interest in phage ecology but no real expertise in the area, then you should join as a non-member. To join as a member, please contact BEG using the following link: abedon.1@osu.edu. Include:

- your name
- your e-mail address
- your snail-mail address
- the URL of your home page (if you have one)
- a statement of whether or not you are the principal investigator
- a statement of your research interests (or phage ecology interests)
- a list of your phage ecology references, if any

Note that it is preferable that you include the full reference, including the abstract, if the reference is not already present in the BEG bibliography. Responsibility of members includes keeping the information listed on the BEG members list up to date including supplying on a reasonably timely basis the full references of your new phage ecology publications. Reprints can also be sent to *The Bacteriophage Ecology Group*, care of Stephen Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. To join BEG as a non-member, please contact BEG using the following link: abedon.1@osu.edu and minimally include your name and e-mail

address.

Please welcome our newest members

name (home page links)	status	e-mail	address
Cynthia Eayre	PI	ceayre @asrr.arsusda.gov	USDA ARS, 2021 S. Peach, Fresno, CA 93727
	interests:	I am a plant pathologist working on soilborne diseases of fruit and nut trees and strawberry. In the past I have isolated and tested phage for control of *Erwinia* soft rot of potatoes. Currently, I am starting a project to look for phage of *Agrobacterium tumefaciens*, cause of crown gall of walnuts and many other trees. As part of the crown gall phage project, I am interested in isolating phage, testing host range, and the effect of phage of rhizosphere populations of *Agrobacterium*, and survival of the phage on trees harvested from the nursery and shipped to growers for planting. (contents | BEG members | top of page)	
Abraham Eisenstark	PI	eisenstarkA @missouri.edu	Cancer Research Center, 3501 Berrywood Drive, Coulmbia, MO. 65201
	interests:	(contents | BEG members | top of page)	
Ipek Kurtboke	PI	micropeace @hotmail.com	LPO Box 2093, Hawthorn, Melbourne, Victoria 3122, Australia
	interests:	The use of phages as ecological and taxonomical tools, in anti-viral research, and in bacteriophage therapy. I was the organizer of the ACTINOPHAGE workshop in the 11th International Symposium on the Biology of Actinomycetes held in Crete, Greece in October, 1999. My call for an international study on Actinophages has been accepted and similar workshops will be conducted in the forthcoming conferences. I would like to encourage the group members to inform me about their research work on actinophages if they are interested to be involved in this international study. (contents | BEG members | top of page)	
Brennan O'Banion	---	virukill @aol.com	University of Kentucky, 161 Oliver Raymond Bldg., Lexington, Ky 40506
	interests:	Iodine disinfection kinetics using the serotype 2, male-specific bacteriophage GA which shows a higher resistance to inactivation when compared to phages MS-2, Q beta, Phi-X174, and PRD1. Isoelectric focusing will be used to determine if a relationship can be found to tie resistance to the degree of conformational change, after iodination, in the molecular constituents of viral capsids. (contents | BEG members | top of page)	
Ro Osawa	PI	osawa @ans.kobe-u.ac.jp	Department of Bioscience, Graduate School of Science and Technology, Kobe University, Rokko-dai 1-1, Nada-ku, Kobe City Japan 657-8501
	interests:	Ecology of Shiga-toxin converting phages. (contents | BEG members | top of page)	
Raúl Ricardo Raya	PI	rraya @cerela.org.ar	CERELA - Chacabuco 145, 4000 - S M Tucuman, Tucuman Argentina
	interests:	Genetics and molecular biology of thermophillic *Lactobacillus*. (contents | BEG members | top of page)	
Mark E Rapson	---	mrapson @bio.warwick.ac.uk	University Of Warwick, Coventry, West Midlands CV4 7AL UK
	interests:	Isolation and Development of Staphylophage to control Surgical Wound Infections by MRSA. (contents | BEG members | top of page)	
Grégory Resch	---	reschg @hotmail.com	Institut de M□decine Dentaire, Hebelstrasse, 3, 4056 BALE (SUISSE)
	interests:	The topic of my thesis work is the Aaphi23 phage family from the Oral pathogen *Actinobacillus actinomycetemcomitans*. I'm currently sequencing the 44kbs genome of one representative of this family. (contents | BEG members | top of page)	
Miguel A. Talledo	PI	d190075 @unmsm.edu.pe	Universidad Nacional Mayor de San Marcos, Faculty of Biological Sciences, Laboratory of Microbiology and Microbial Biotechnology, Unidad Vecinal Mirones, Block 4A-300, Lima 1 Peru
	interests:	I study the *Vibrio cholerae* phages found here in Lima, as well as the possible diversity of *Vibrio* phages in our coasts (South America Pacific Ocean). We are at the initial stages of this study, mainly isolation of *Vibrio* phages and hoping to get into phage characterization soon. (contents | BEG members | top of page)	
Steven W. Wilhelm	PI	Wilhelm @utk.edu	Department of Microbiology, The University of Tennessee, Knoxville, TN 37996-0845
	interests:	Activity of viruses (bacteriophage and cyanophage) in marine systems.	
Yukio Yoshizawa	PI	yukio.yoshizawa @jikei.ac.jp	Radioisotope Reseach Center, Jikei Unoversity School of Medicine, 3-25-8 Nishishimbashi, Minato-ku, Tokyo 105-8461, JAPAN
	interests:	Phage-conversion of bacterial toxins, especially that of *Staphylocuccus aureus*. (contents | BEG members | top of page)	

New Links

Links relevant to The Bacteriophage Ecology Group fall into a number of categories (e.g., see Bacteriophage Ecology Links at www.phage.org/beg_links.htm). Listed below are new links found on that page. If you know of a link that should be included on this page, or the whereabouts of a now-dead link, please let me know.

New Bacteriophage Ecology (Etc.) Links

- A Balm for Sunburned Viruses
- Microbial Gene Transfer: An Ecological Perspective
- Phage in Continuous Culture
- Continuous Culture Model to Examine Factors That Affect Transduction among Pseudomonas aeruginosa Strains in Freshwater Environments
- Characterisation of Phage of Staphylococcus Species and Evaluation of Their Therapeutic Potential
- Phage therapy: The peculiar kinetics of self-replicating pharmaceuticals
- FDA.gov "bacteriophage" search
- FDA.gov "phage" search

New Features

In this section I highlight new or updated features of the BEG site. If you have any ideas of how either the BEG site or *BEG News* might be improved, please let me know.

Phage Jobs Link:

There is now a link from the BEG splash page to the current issue of BEG News job section.

Phage Members List:

I've redone the BEG splash page members list. The old multicolumn list was getting unwieldy as our membership has expanded. Let me know what you think of the new list or if you have any suggestions of how I might improve it.

Bacteriophage Ecology Web Ring:

If you scroll to the bottom of the BEG splash page you will find that BEG is now the hub of the "Bacteriophage Ecology" web ring. Unfortunately, I seem to have joined this Yahoo-based service twice. Please join the ring (if we don't have enough members we will be deleted), but please join the first-listed ring, not the second (even though they otherwise appear to be identical). To join the ring directly, go to this web page: http://edit.webring.yahoo.com/cgi-bin/membercgi?ring=bacteriophageeco&addsite. Thanks!

Upcoming Meetings

The BEG Meetings link will continue. Reminders of upcoming meetings will be placed in this section of *BEG News*. If you know of any meetings that might be of interest to BEG members, or would like to recap a meeting that you've attended, then please send this information for posting to abedon.1@osu.edu or to "BEG Meetings," *Bacteriophage Ecology Group News*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906.

Evergreen International Phage Meeting

Next Summer's phage meeting has been scheduled for August 8-13, 2001. The web page for this meeting can be found at http://www.evergreen.edu/user/T4/2001Meet.html. As always, this will be *the* meeting that brings together phage people with the widest possible array of interests - from the ecological to the molecular - in a setting of rain forest spender in the city that *Time Magazine* dubbed the "Hippest town in the West".

Jobs

Postdoctoral Position Bacterial-Bacteriophage Genetics

A two-year postdoctoral position in bacterial-bacteriophage genetics is available to investigate inter-species gene transfer within the *Burkholderia cepacia* complex using bacteriophages with inter-species host range. This is a Cystic Fibrosis Foundation funded position and is a collaborative project between the laboratories of John J. LiPuma, M.D. (U. Michigan-Medical School) and Carlos F. Gonzalez, Ph.D.

(Texas A&M University). Candidates should have previous training in molecular biology, bacterial genetics, biochemistry, or virology. Send curriculum vitae, names, telephone numbers, and e-mail addresses of three references to Carlos F. Gonzalez, Department of Plant Pathology and Microbiology, 120 Peterson Bldg., Texas A&M University, College Station, Texas, 77843, Office: 979-845-8462, Fax: 979-845-6483; email cf-gonzalez@tamu.edu.

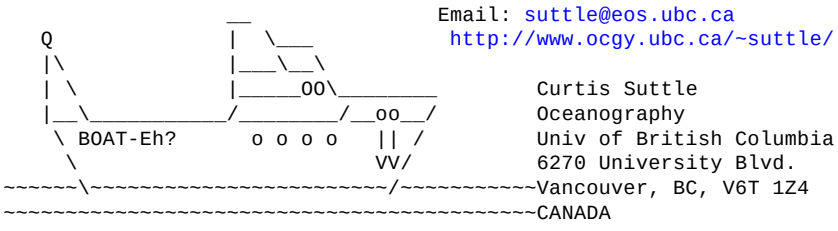
POSITION ANNOUNCEMENT: AVAILABILITY: IMMEDIATE

Post-doctoral position in *Salmonella* and enterohemorragic *E.coli* (EHEC) phage ecology and phage therapy: Position available to investigate the natural history, field ecology and diagnostic and/or therapeutic potential of bacteriophages specific for *Salmonella typhimurium* and EHEC O157, O111, and O26 in the livestock production environment. Will involve both lab and field based research. Ideal candidate will be a PhD microbiologist with experience in isolating and characterizing bacteriophages from the field. Previous work experience with Salmonella and EHEC is not necessary. Two year position with annual extensions possible. Annual salary of approx. $38,000 + benefits. Starting date: negotiable, but prefer between prior to Sept 2000. Interested candidates should contact Jim Keen, Animal Health Research Unit, USMARC, Clay Center, NE 68933; Ph: 402-762-4343; Email: keen@email.marc.usda.gov for additional information.

Postdoctoral Positions - Viruses, Sediments and Harmful Algal Blooms

Two postdoctoral positions are available immediately to participate in research programs investigating viruses that infect the toxic bloom-forming alga *Heterosigma akashiwo*. The first project is investigating the use of algal and viral biomarkers in sediment cores to reconstruct the frequency and extent of occurrence of blooms of this toxic alga,. Harmful algal blooms are a worldwide problem with enormous ecological and economic consequences. There is evidence the incidence and severity of blooms has increased in the last few decades as the result of environmental change. As well, the introduction and range expansion of harmful algal species is of great concern, but is often hard to document. The successful candidate will develop and apply quantitative PCR-based methods to determine the distribution in sediment cores of cysts and viruses that are specific to *Heterosigma akashiwo* in order to hindcast the occurrence of toxic algal blooms. The second position will join a project that is investigating the role of viruses in controlling the bloom dynamics of this alga in the water column. You will join an active laboratory of about 15 researchers investigating natural viral communities and viral mediated processes.

Applicants should provide a cover letter, CV and contact information for two references. Applicants must be within 3 years of having received a PhD. For further information, please contact Curtis Suttle, Department of Earth & Ocean Sciences Oceanography), University of British Columbia, 6270 University Blvd, Vancouver, BC, V6T 1Z4 Canada. Phone (604) 822-8610; Fax (604) 822-6091. Applications By Email Are Preferred



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Submissions

Submissions are non-editorial items describing or highlighting some aspect of bacteriophage ecology including news pieces, historical pieces, reviews, and write-ups of research. Peer review of submissions is possible and a desire for peer review should be indicated. Send all submissions to abedon.1@osu.edu or to "Submissions", Bacteriophage Ecology Group News, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Please send all submissions as Microsoft Word documents, if possible (I'll let you know if I have trouble converting any other document formats), and in English.

How big is 10^{30}?

Whitman *et al*. (1998) argue that there are between 10^{30} and 10^{31} prokaryotic cells on our planet. If we assume numerically one virus for every prokaryote host, then we conservatively (e.g., Bergh *et al.*, 1989) reach a total worldwide abundance of 10^{30} virus-like particles. Is 10^{30} virus-like particles a reasonable estimation? What does a number like that mean? Astronomers, for instance, can account for "only" about 10^{22} stars in the entire universe (Turner, 2000). The size of the universe is something on the order of 1.5 x 10^{10} light years across (depending on whose estimation of the age of the universe you choose to believe), while a light year is about 10^{13} kilometers (9,460,800,000,000, actually). That means that the universe is something like to 10^{29} mm wide (10^{10} x 10^{13} x 10^{6} mm/kilometer). If phages were one mm wide, then 10^{30} phages placed end to end would form a single line that would stretch ten-times across the entire universe! Of course, phages (and bacteria) are something less than one mm wide.

If we go to a site called About Big Numbers (ABN) we find that 10^{30} is approximately the mass of the sun (in pounds; 4 x 10^{30}) and about 1/100th of the volume of the sun (in cubic inches; 86 x 10^{30}), and that there are over 10^{47} atoms of water on Earth's surface. Of greater relevance to our subject, though, About Big Numbers claims that 10^{36} is the "Maximum number of living things the Earth can accommodate." Therefore 10^{30} would only be claiming that the Earth's smallest "organisms" would numerically represent only one-millionth of the Earth's total organismal capacity. From that perspective, 10^{30} phages strike me as quite reasonable, perhaps even on the low side.

The chemist in me wants to know how many moles 10^{30} represents. Avagadro's number is 6.022 x 10^{23} atoms, molecules, or

particles per mole. 10^{30} / 6.022 x 10^{23} = 1.66 x 10^{6} or over one million moles of bacteriophage! Given the examples in the above paragraph, my first impulse would be to compare this number with the number of moles that make up the Sun. Since the sun consists mostly of hydrogen gas (with a molecular weight of 2) and there are 454 grams per pound, then there are approximately 10^{33} moles of hydrogen making up the sun! This would mean that the sun has nearly 10^{27} molecules for every phage on Earth. However, far more humbling, the volume of the sun would accommodate approximately one million earth-size balls (ABN). That would be a lot of heat-inactivated phages!

Dubin *et al.* (1970) provide an estimation of the molecular weights of phages T4, T5, and T7 of 192, 109, and 50 x 10^{6} dalton, which we'll assume on average is something like 10^{8} grams per mole of phage. This is approximately the mass of a single blue whale, i.e., 100 short tons (see: MegaConverter 2 for the conversion to grams). 10^{8} grams per mole translates to about 10^{14} grams of phages (10^{6} moles) found on the Earth. That's about equal to the total mass of humanity (6 x 10^{9} people at 50 kilograms per person), and is slightly more than the total mass of the approximately 10^{8} cows in the U.S. (ABN) where 10^{6} grams is one metric ton and a good-sized cow is about half a metric ton. The mass of the whole Earth is approximately 5 x 10^{28} grams (ABN), so we need not worry about running out of planet to make our phages. In fact, a single mole of an average-sized bacterium weighs approximately 5 x 10^{11} grams (30 x 10^{12} "average-sized" bacteria per ounce; ABN) which means that 10^{30} phages is equivalent in mass to 200 moles of bacteria (10^{14} / 5 x 10^{11}), or about 10^{26} individual cells. Numerically, 10^{26} is 10 orders of magnitude less than the above-noted guestimate for Earth's total organismal capacity.

So if there may be 10^{30} phages then there are ~10^{6} moles of phages or something like 10^{14} grams in total. What phage density would be necessary to account for such numbers? Estimations of the surface area of the Earth can vary depending upon whether Earth is truly a sphere (in fact, the poles are flattened) or whether one insists on taking into account the degree to which that surface is rough (which can dramatically increase the Earth's surface area). For our purposes we will assume that Earth is a perfectly smooth sphere with a diameter of 1.28 x 10^{7} meters at the equator. The surface area of a sphere is $4\pi r^2$ and 4 x 3.14 x (1.28 x 10^{7} /2)^2 = 5 x 10^{14} square meters or 5 x 10^{18} square centimeters. The density of phages therefore is 10^{30} / 5 x 10^{18} = 2 x 10^{11} which, to be conservative, we'll call 5 x 10^{11}. To account for 10^{30} total phages this is the number that would have to be present per ml to a depth of 1 cm over the surface of the entire world's oceans. A more reasonable density is 10^{6} phages per ml (or, at least, of virus-like particles), total count (Wommack & Colwell, 2000). Diluting 5 x 10^{11} phages per ml to 10^{6} phages per ml requires a depth of 500,000 cm which is 5,000 meters of 10^{6} phages per ml to account for 10^{30} phages worldwide. 5,000 meters is within the range of the average depth of the world's oceans, which is about 4 kilometers. So 10^{30} represents an assumption of approximately 10^{6} virus particles per ml over (and under) the entire world's oceans.

More precisely, assuming 10^{6} virus-like particles per ml (source of ocean data = OceanLink Answers to Oceanography Questions):

Ocean	Area	Ave. depth	Volume	Phages
Atlantic	8.20 x 10^{17} cm^2	3.33 x 10^{5} cm	3.22 x 10^{23} cm^3	3.22 x 10^{29}
Indian	7.36 x 10^{17} cm^2	3.89 x 10^{5} cm	2.92 x 10^{23} cm^3	2.92 x 10^{29}
Pacific	1.66 x 10^{18} cm^2	4.28 x 10^{5} cm	7.24 x 10^{23} cm^3	7.24 x 10^{29}
Total				**1.33 x 10^{30}**

Thus, a total of 10^{30} phages is, in fact, a reasonable and entirely plausible worldwide estimation of total virus particles.

MicroDude, a.k.a., **Stephen T. Abedon**
is the Developer and Editor of The Bacteriophage Ecology Group web site which is dedicated to the ecology and evolutionary biology of the parasites of unicellular organisms (UOPs)

REFERENCES

1. **Bergh, O., K.Y. Borsheim, G. Bratbak, and M. Heldal**. 1989. High abundance of viruses found in aquatic environments. *Nature* **340**:467-468.

2. **Dubin, S.B., G.B. Benedek, F.C. Bancroft, and D. Freifelder**. 1970. Molecular weights of coliphages and coliphage DNA. II. Measurement of diffision coefficients using optical mixing spectroscopy, and measurement of sedimentation coefficients. *Journal of Molecular Biology* **54**:547-556.

3. **Turner, M.S.** 2000. More than meets the eye. *The Sciences* **November/December**:32-37.

4. **Whitman, W.B., D.C. Coleman, and W.J. Wiebe**. 1998. Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences, USA* **95**:6578-6583.

5. **Wommack, K.E. and R.R. Colwell**. 2000. Virioplankton: viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews* **64**:69-114.

Letters & Questions

Letters should consist of comments, short statements, or personal editorials. Send all letters to abedon.1@osu.edu or to "Letters", Bacteriophage Ecology Group News, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Please send all letters in English and all mailed or attached letters as Microsoft Word documents, if possible (I'll let you know if I have trouble converting any other document formats). In addition, to standard letters, BEG receives questions on a regular basis that may be addressed by BEG members. These questions are listed below. Anybody interested in answering these questions through *BEG News*, e-mail me at the following address: abedon.1@osu.edu. Alternatively, answer by clicking the authors name. Please note that these questions have not been edited for grammar, spelling, or clarity.

John: I found your website recently and was impressed. I am a microbiologist working for a company that manufactures cultured dairy products. My boss and I differ over how to interpret plate counts of our lactic starter culture. Would you be willing to address a question concerning bacterial plate counts, or at least respond and point me toward references that might help me?

Our company occasionally makes changes in the way it propagates lactic acid bacteria starter. We usually determine cell numbers in starter before and after making changes to see whether the culture has been affected by the process change. We use plate counts (pour plates) to determine numbers. My boss and I differ in opinion concerning inherent accuracy of plate counts on starter culture.

I was preceded in my job by a microbiologist who believed that cell numbers in two populations of starter (as compared using plate counts) were probably not really different unless the cell numbers differed by at least 4-5 times. That is, a control starter with 2.0e8 is not different than a culture containing twice as many cells, i.e. 4.0e8. But this control culture would likely be different from starter that contains 5 times as many cells (1.0e9). My boss feels plate counts are more accurate than this.

If you have time, could you please respond to my question. I would greatly appreciate suggestions regarding where I could find out more about day-to-day reproducibility and accuracy of the plate count method. Should cell counts differ by 4-5 times before you consider the two populations truly different? My e-mail address is: meilinger@primary.net.

Steve: OK, let me try to take a stab at this. First of all, in terms of statistical variance, one should expect on a plate-by-plate basis something like an error approximately equal to the square root of the actual (mean, I suppose) plate count. Thus, if you plated out the same culture using two different dilution series you should see error that is much less than five-fold. However, that answer does not address the specifics of enumerating lactic acid starter culture. It is possible that these cultures are very dependent on plating conditions. Alternatively, are we talking about log-phase bacteria (or, indeed, different or multiple strains)? If the bacteria are well past log phase then it is possible that plate counts will not accurately reflect (undercount, in fact) the number of bacteria that are viable if revived under less harsh conditions (e.g., in broth). It could be that plating conditions differ from day to day with regard to post log-phase bacteria.

I'm pondering how one would actually go about testing these ideas. Perhaps you could compare total cell count with viable count over the course of a number of days. My guess is that your boss is correct in assuming that plate counts are indeed more accurate than your predecessor supposed. It would be interesting to contact your predecessor find out the basis of the supposition.

Mind if I place this question in the next issue of Bacteriophage Ecology Group News? Can I use your name and e-mail address?

John: Thank you for your very quick reply. I would be pleased if you placed my question in Bacteriophage Ecology Group News, and you can use my name. My company is somewhat paranoid about discussing research on public sites. People could respond to me at my home e-mail address of meilinger@primary.net or, if you'd be willing, through you. But, I don't want to burden you with this responsibility simply because I asked for your help on this matter.

After I sent you the e-mail, I also began to realize that my question isn't as straightforward as I first assumed. I'm questioning the plate-to-plate variation inherent in the plate count method, but also concerned with differences between tests that are being conducted at different times. Media used in these tests varies somewhat (we propagate in pasteurized milk that isn't standardized for solids) as do other factors that could affect growth of the starter. The culture is multi-strain, and 'crude' estimations of strain ratios suggest that lots of the starter coming directly from the manufacturer also varying somewhat compositionally.

Another bit of information that I forget to provide initially concerns determination of ratios of stains in our culture. We have started to use a method published by the International Dairy Federation to selectively enumerate *Leuconostoc* and *Lactococcus lactis* ssp. lactis biovar diacetylactis. These 'bugs' utilize citrate and are considered the 'flavor-producing' microorganisms in most mesophilic dairy fermentations. The ratio of these organisms to the total population of lactic acid bacteria in the culture supposedly gives an indication of the potential of that culture to provide flavor compounds in addition to producing acid. In the IDF method, *Diacetylactis* and *Leuconostoc* are distinguished from non-citrate fermenting lactics by zones of clearing around colonies grown on a media containing calcium citrate called Leesment agar . *Leuconostoc* are further differentiated from *Diacetylactis* by zones of clearing and breakdown of X-gal, a lactose analogue that turns the colonies blue when utilized. Colony counts of these two groups cannot be made within the traditional 25-250 colonies per petri plate because clearing zones and the blue coloring 'spread' and can obscure exactly which colonies are producing the desired reactions. Therefore, 'D' and 'L' counts must usually be made when colony forming units are at lower concentrations, e.g. 5-30 per plate. I'm sure this also decreases the accuracy of the method. I do replicate plates, but I'm not sure this guarantees that my calculated ratios are all that accurate and reliable.

Thank you again for your help. I really appreciate your insight on these questions.

Steve: Even the simplest of things in biology are hopelessly complex, eh?

John: Amen.

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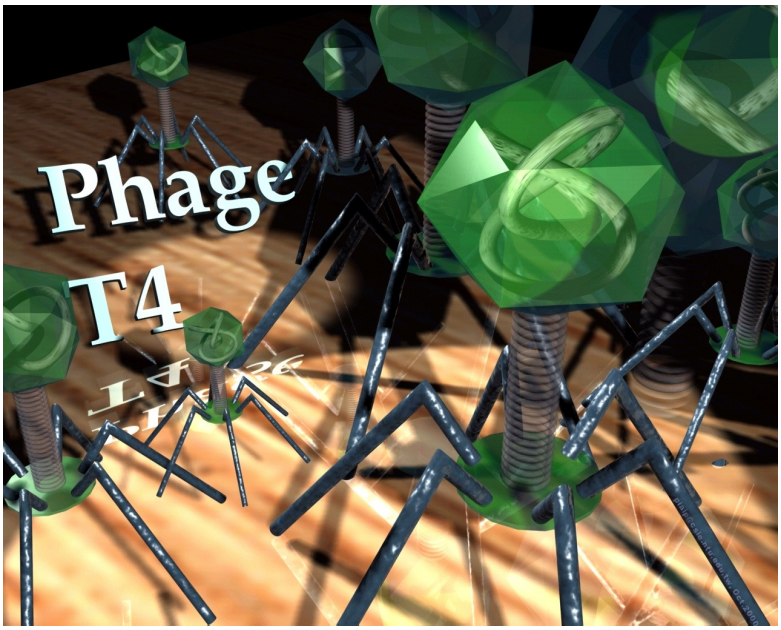
Phage Images

Please send any phage images that you would like to present in this section to "Phage Images," *The Bacteriophage Ecology Group*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Alternatively, you may scan the images yourself and send them as an attachment to abedon.1@osu.edu. Please save all scans in gif or jpg formats and preferably with an image size (in terms of width, height, and kbytes) that will readily fit on a standard web page.



Phage Image Archive

- BEG Phage Images Page
- The Face of the Phage
- Bacteriophage T2 by H.-W. Ackermann
- SSV1-Type Phage
- Saline Lake Bacteriophage - David Bird
- Coliphage LG1 - Larry Goodridge
- Bacteriophage HK97 - Bob Duda
- Phage T4 (art) - Francis S. Lin

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New Publications

New bacteriophage publications are listed below. Each quarter not-yet-listed publications from the previous two years will be presented along with their abstracts. The indicator "???" denotes, of course, that specific information is not yet in the BEG Bibliography. Please help in the compilation of the BEG Bibliography by supplying any updated information, correcting any mistakes, and, of course, sending the references to your bacteriophage ecology publications, as well as the references to any bacteriophage ecology publications that you know of but which are not yet in the bibliography (send to abedon.1@osu.edu or to "BEG Bibliography," *Bacteriophage Ecology Group News*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906). Also, be sure to indicate any listed publications that you feel should not be presented in the BEG Bibliography. This list is also present with available abstracts at the end of *BEG News*.

1. Seasonal population dynamics and interactions of competing bacteriophages and their host in the rhizosphere. Ashelford, K. E., Norris, S. J., Fry, J. C., Bailey, M. J., Day, M. J. (2000). *Applied and Environmental Microbiology* 66:4193-4199. [PRESS FOR ABSTRACT]

2. Experimental design optimization of filamentous phage transfection into mammalian cells by cationic lipids. Aujame, L., Seguin, D., Droy, C., Hessler, C. (2000). *BioTechniques* 28:1202-1213. [PRESS FOR ABSTRACT]

3. Evolution of life's fringes. Balter, M. (2000). *Science* 289:1866-1867. [PRESS FOR ABSTRACT]
[PRESS FOR ABSTRACT]

4. A comparison of methods for counting viruses in aquatic systems. Bettarel, Y., Sime-Ngando, T., Amblard, C., Laveran, H. (2000). *Applied and*

Environmental Microbiology 66:2283-2289. [PRESS FOR ABSTRACT]

5. The relative importance of competition and predation varies with productivity in a model community. Bohannan, B. J. M., Lenski, R. E. (2000). *American Naturalist* 156:329-340. [PRESS FOR ABSTRACT]

6. A high yielding mutant of mycobacteriophage L1 and its application as a diagnostic tool. Chatterjee, S., Mitra, M., Das, Gupta SK (2000). *FEMS Microbiology Letters* 188:47-53. [PRESS FOR ABSTRACT]

7. Evaluation of CD4+ T cell function In vivo in HIV-infected patients as measured by bacteriophage phiX174 immunization. Fogelman, I, Davey, V, Ochs, H. D., Elashoff, M., Feinberg, M. B., Mican, J., Siegel, J. P., Sneller, M., Lane (2000). *Journal of Infectious Diseases* 182:435-441. [PRESS FOR ABSTRACT]

8. Characterization of natural isolates of *Lactobacillus* strains to be used as starter cultures in dairy fermentation. Hebert, E. M., Raya, R. R., Tailliez, P., de, Giori GS (2000). *International Journal of Food Microbiology* 59:19-27. [PRESS FOR ABSTRACT]

9. Ultraviolet radiation effects on bacterioplankton and viruses in marine ecosystems. Jeffrey, W. H., Kase, J. P., Wilhelm, S. W. (2000). pp. 206-236 in De Mora, S. J., et al. (eds.) *Effects Of UV Radiation On Marine Ecosystems*. Cambridge University Press, Cambridge. [no abstract]

10. Bacteriophage infections in the industrial acetone butanol (AB) fermentation process. Jones, D. T., Shirley, M., Wu, X, Keis, S. (2000). *J Mol Microbiol Biotechnol* 2:21-26. [PRESS FOR ABSTRACT]

11. [A method for detection of coliphages in the drinking water]. Kashkarova, G. P., Dorodnikov, A. I. (2000). *Gigiena i Sanitariia* 66-68. [no abstract]

12. [Effect of chitosan derivatives on the development of phage infection in cultured *Bacillus thuringiensis*]. Kochkina, Z. M., Chirkov, S. N. (2000). *Mikrobiologiia* 69:266-269. [PRESS FOR ABSTRACT]

13. Antibacterials that are used as growth promoters in animal husbandry can affect the release of Shiga-toxin-2-converting bacteriophages and Shiga toxin 2 from *Escherichia coli* strains. Kohler, B., Karch, H., Schmidt, H. (2000). *Microbiology* 146 (Pt 5):1085-1090. [PRESS FOR ABSTRACT]

14. Impact of acid on survival of *Vibrio vulnificus* and *Vibrio vulnificus* phage. Koo, J., Depaola, A., Marshall, D. L. (2000). *Journal of Food Protection* 63:1049-1052. [PRESS FOR ABSTRACT]

15. [Variants of *Yersinia pestis* resistant to a diagnostic bacteriophage and the problems related to them]. Lebedeva, S. A. (2000). *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* 99-104. [PRESS FOR ABSTRACT]

16. Influence of salts on virus adsorption to microporous filters. Lukasik, J., Scott, T. M., Andryshak, D., Farrah, S. R. (2000). *Applied and Environmental Microbiology* 66:2914-2920. [PRESS FOR ABSTRACT]

17. Bacterial growth rate and marine virus–host dynamics. Middleboe, M. (2000). *Microbial Ecology* 40:114-124. [PRESS FOR ABSTRACT]

18. Characterization of a shiga toxin 2e-converting bacteriophage from an *Escherichia coli* strain of human origin. Muniesa, M., Recktenwald, J., Bielaszewska, M., Karch, H., Schmidt, H. (2000). *Infection and Immunity* 68:4850-4855. [PRESS FOR ABSTRACT]

19. Bacteriophage phiYeO3-12, specific for *Yersinia enterocolitica* serotype O:3, is related to coliphages T3 and T7. Pajunen, M., Kiljunen, S., Skurnik, M. (2000). *Journal of Bacteriology* 182:5114-5120. [PRESS FOR ABSTRACT]

20. Phage therapy: The peculiar kinetics of self-replicating pharmaceuticals. Payne, R. J. H., Phil, D., Jansen, V. A. A. (2000). *Clinical Pharmacology and Therapeutics* 68:225-230. [PRESS FOR ABSTRACT]

21. Selection of tumor-specific internalizing human antibodies from phage libraries. Poul, M. A., Becerril, B., Nielsen, U. B., Morisson, P., Marks, J. D. (2000). *Journal of Molecular Biology* 301:1149-1161. [PRESS FOR ABSTRACT]

22. Detection of phages infecting *Bacteroides fragilis* HSP40 using a specific DNA probe. Puig, M., Jofre, J., Girones, R. (2000). *Journal of Virological Methods* 88:163-173. [PRESS FOR ABSTRACT]

23. Tissue-specific gene expression identifies a gene in the lysogenic phage Gifsy-1 that affects *Salmonella enterica* serovar typhimurium survival in Peyer's patches. Stanley, T. L., Ellermeier, C. D., Slauch, J. M. (2000). *Journal of Bacteriology* 182:4406-4413. [PRESS FOR ABSTRACT]

24. Construction of lux bacteriophages and the determination of specific bacteria and their antibiotic sensitivities. Ulitzur, S., Kuhn, J. (2000). *Methods in Enzymology* 305:543-557. [no abstract]

25. Clay minerals protect bacteriophage PBS1 of *Bacillus subtilis* against inactivation and loss of transducing ability by UV radiation. Vettori, C., Gallori, E., Stotzy, G. (2000). *Canadian Journal of Microbiology* 46:770-773. [PRESS FOR ABSTRACT]

26. Bacterial carbon production in Lake Erie is influenced by viruses and solar radiation. Wilhelm, S. W., Smith, R. E. H. (2000). *Canadian Journal of Fisheries and Aquatic Sciences* 57:317-326. [PRESS FOR ABSTRACT]

27. Toward selection of internalizing antibodies from phage libraries. Becerril, B., Poul, M. A., Marks, J. D. (1999). *Biochemical & Biophysical Research Communications* 255:386-393. [PRESS FOR ABSTRACT]

28. Vaginal bacterial phaginosis? Blackwell, A. L. (1999). *Sexually Transmitted Infections* 75:352-353. [PRESS FOR ABSTRACT]

29. [An accelerated method for detecting coliphages in the drinking water]. Dmitrieva, R. A., Doskina, T. V., Nedachin, A. E., Sidorenko, S. G. (1999). *Gigiena i Sanitariia* 71-72. [no abstract]

30. Elimination of viruses, phages, bacteria and *Cryptosporidium* by a new generation Aquaguard point-of-use water treatment unit. Grabow, W. O., Clay, C. G., Dhaliwal, W., Vrey, M. A., Muller, E. E. (1999). *Zentralblatt fur Hygiene und Unweltmedizin* 202:399-410. [PRESS FOR ABSTRACT]

31. Virus removal by filtration. Graf, E. G., Jander, E., West, A., Pora, H., Aranha-Creado, H. (1999). *Developments in Biological Standarization* 99:89-94. [PRESS FOR ABSTRACT]

32. [Cryostabilization of biological properties of plague phages]. Kadetov, V. V., Kudriakova, T. A., Terent'ev, A. N., Kachkina, G. V., Borodina, T. N., Saiamov, S. R. (1999). *Voprosy Virusologii* 44:136-139. [PRESS FOR ABSTRACT]

33. Alternative origins for nannobacteria-like objects in calcite. Kirkland, Brenda L., Folk, Robert L., Lynch, F. L., McLean, Robert J. C., Molineux, Ian J., Rahnis, Michael A. (1999). *Geology (Boulder)* 27:347-350. [PRESS FOR ABSTRACT]

34. [The history of the discovery and study of *Brucella* bacteriophages]. Liapustina, L. V., Liamkin, L. I., Taran, I. F. (1999). *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* 123-124. [no abstract]

35. Bacteriophages in the evolution of pathogen-host interactions. Miao, E. A., Miller, S. I. (1999). *Proceedings of the National Academy of Sciences, USA* 96:9452-9454. [PRESS FOR ABSTRACT]

36. Model systems to study the parameters determining the success of phage antibody selections on complex antigens. Mutuberria, R., Hoogenboom, H. R., van, der Linden, de, Bruine AP, Roovers, R. C. (1999). *Journal of Immunological Methods* 231:65-81. [PRESS FOR ABSTRACT]

37. Microbial gene transfer: an ecological perspective. Paul, J. H. (1999). *Journal of Molecular Microbiology and Biotechnology* 1:45-50. [PRESS FOR ABSTRACT]

38. Diversity of *Bacteroides fragilis* strains in their capacity to recover phages from human and animal wastes and from fecally polluted wastewater. Puig, A., Queralt, N., Jofre, J., Araujo, R. (1999). *Applied and Environmental Microbiology* 65:1772-1776. [PRESS FOR ABSTRACT]

39. Resistance to DNA damage in natural viral communities from the Gulf of Mexico. Weinbauer, M. G., Wilhelm, S. W., Pledger, R. J., Mitchell, D. L., Suttle, C. A. (1999). *Aquatic Microbial Ecology* 17:111-120. [PRESS FOR ABSTRACT]

40. Biodistribution of filamentous phage-Fab in nude mice. Yip, Y. L., Hawkins, N. J., Smith, G., Ward, R. L. (1999). *Journal of Immunological Methods* 225:171-178. [PRESS FOR ABSTRACT]

41. Human enteric viruses in the water environment: a minireview. Bosch, A. (1998). *Int Microbiol* 1:191-196. [PRESS FOR ABSTRACT]

42. New cholera phages for *Vibrio cholerae* serovar O139 [letter]. Chakrabarti, A. K., Ghosh, A. N., Sarkar, B. L. (1998). *Journal of Infection* 36:131-132. [no abstract]

43. Removal of waterborne human enteric viruses and coliphages with oxidized coal. Cloete, T. E., Da, Silva E., Nel, L. H. (1998). *Current Microbiology* 37:23-27. [PRESS FOR ABSTRACT]

44. Virus occurrence and transport in a school septic system and unconfined aquifer. DeBorde, Dan C., Ball, Patrick N., Lauerman, Bruce, Woessner, William W. (1998). *Ground Water* 36:825-834. [PRESS FOR ABSTRACT]

45. Evolutionary relationships among putative RNA-dependent RNA polymerases encoded by a mitochondrial virus-like RNA in the Dutch elm disease fungus, Ophiostoma novo-ulmi, by other viruses and virus-like RNAs and by the Arabidopsis mitochondrial genome. Hong, Y., Cole, T. E., Brasier, C. M., Buck, K. W. (1998). *Virology* 246:158-169. [PRESS FOR ABSTRACT]

46. Fluorescent *Escherichia coli* C for enumeration of coliphages from environmental samples. Jothikumar, N., Cliver, D. O. (1998). *BioTechniques* 24:546-550. [no abstract]

47. Biologicheskiye sistemy kontrolya mutagennykh faktorov okruzhayushchey sredy. Pererva, T. P., Aleksandrov, Yu, Miryuta, A. Y., Miryuta, N. Y. (1998). *Dopovidi Natsional'noyi Akademiyi Nauk Ukrayini. Matematika, Prirodoznavstvo, Tekhnichni Nauki* 1998:188-192. [PRESS FOR ABSTRACT]

48. The origin and evolution of viruses (a review). Sinkovics, J., Horvath, J., Horak, A. (1998). *Acta Microbiologica et Immunologica Hungarica* 45:349-390. [PRESS FOR ABSTRACT]

49. Detection, quantification and morphological characterization of *Vibrio cholerae* indicator bacteriophages [Spanish]. Talledo, M. A., Gutiérrez, S., Merino, F., Rojas, N. (1998). *Rev. Peru. Biol.* 5:90-97. [no abstract]

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New Publications with Abstracts

For your convenience, a list of new publications without associated abstracts (but with links to abstracts) is found above. The list presented below is identical to the above list except that abstracts are included.

1. **Seasonal population dynamics and interactions of competing bacteriophages and their host in the rhizosphere. Ashelford, K. E., Norris, S. J., Fry, J. C., Bailey, M. J., Day, M. J. (2000). *Applied and Environmental Microbiology* 66:4193-4199.** We describe two prolonged bacteriophage blooms within sugar beet rhizospheres ensuing from an artificial increase in numbers of an indigenous soil bacterium, Further, we provide evidence of in situ competition between these phages, This is the first in situ demonstration of such microbial interactions in soil, To achieve this, sugar beet seeds were inoculated with *Serratia liquefaciens* CP6RS or its lysogen, CP6RS-ly-Phi 1. These were sown, along with uninoculated seeds, in 36 field plots arranged in a randomized Latin square. The plots were then sampled regularly over 194 days, and the plants were assayed for the released bacteria and any infectious phages, Both the lysogen and nonlysogen forms of CP6RS survived equally well in situ, contradicting earlier work suggesting lysogens have a competitive disadvantage in nature. A Podoviridae phage, identified as Phi CP6-4, flourished on the nonlysogen-inoculated plants in contrast to those plants inoculated with the lysogen. Conversely, the Siphoviridae phage Phi CP6-1 (used to construct the released lysogen) was isolated abundantly from the lysogen-treated plants but almost never on the

nonlysogen-inoculated plants. The uninoculated plants also harbored some Phi CP6-1 phage up to day 137, yet hardly any Phi CP6-4 phages were found, and this was consistent with previous years. We sinew that the different temporal and spatial distributions of these two physiologically distinct phages can be explained by application of optimal foraging theory to phage ecology. This is the first time that such in situ evidence has been provided in support of this theoretical model.

2. **Experimental design optimization of filamentous phage transfection into mammalian cells by cationic lipids. Aujame, L., Seguin, D., Droy, C., Hessler, C. (2000). *BioTechniques* 28:1202-1213.** A previous study showed that filamentous phage could be efficiently transfected into mammalian cells in the presence of the cationic lipid Transfectam. In the present study, we used an experimental plan based on a uniform network (Doehlert) matrix to estimate optimal transfection conditions in two different cell lines, CHO and Cos-7. Using the cationic lipid RPR120535b as a model, we show that optimal conditions can be determined much more readily than with standard response curves. Under optimal conditions as analyzed by FACS, up to 60% of Cos-7 and 50% of CHO cells can be transfected. Furthermore, a comparison of different lipids (Transfectam, RPR120535b, TC1-12 and GAP-DLRIE/DOPE) suggests that lipids with multiple amine groups are more efficient for the transfection of filamentous phage

3. **Evolution of life's fringes. Balter, M. (2000). *Science* 289:1866-1867.** Fresh evidence that viruses have existed for billions of years has scientists wondering what role these stripped-down microbes played in evolution.

4. **A comparison of methods for counting viruses in aquatic systems. Bettarel, Y., Sime-Ngando, T., Amblard, C., Laveran, H. (2000). *Applied and Environmental Microbiology* 66:2283-2289.** In this study, we compared different methods-including transmission electron microscopy-and various nucleic acid labeling methods in which we used the fluorochromes 4',6'-diamidino-2-phenylindole (DAPI), 4-[3-methyl-2,3-dihydro-(benzo-1, 3-oxazole)-2-methylmethyledene]-1-(3'-trimethyl ammoniumpropyl)-quinilinium diioide (YOPRO-1), and SYBR Green I, which can be detected by epifluorescence microscopy (EM), for counting viruses in samples obtained from freshwater ecosystems whose trophic status varied and from a culture of T7 phages. From a quantitative and qualitative viewpoint, our results showed that the greatest efficiency for all ecosystems was obtained when we used the EM counting protocol in which YOPRO-1 was the label, as this fluorochrome exhibited strong and very stable fluorescence. A modification of the original protocol in which YOPRO-1 was used is recommended, because this modification makes the protocol faster and allows it to be used for routine analysis of fixed samples. Because SYBR Green I fades very quickly, the use of this fluorochrome is not recommended for systems in which the viral content is very high ($>10^8$ particles/ml), such as treated domestic sewage effluents. Experiments in which we used DNase and RNase revealed that the number of viruses determined by EM was slightly overestimated (by approximately 15%) because of interference caused by the presence of free nucleic acids

5. **The relative importance of competition and predation varies with productivity in a model community. Bohannan, B. J. M., Lenski, R. E. (2000). *American Naturalist* 156:329-340.** Recent theory predicts that productivity can influence the relative importance of predation and competition in determining patterns in abundance, diversity, and community structure. In low-productivity systems, competition is predicted to be the major influence on community patterns, while at high productivity, the major influence is predicted to be predation. We directly tested this theory using a laboratory model community. Our model community consisted of the bacteriophage T2 (a virus that feeds on *Escherichia coli*) and two populations of *E. coli*, in glucose-limited chemostats. One *E. coli* population consisted of individuals that were sensitive to predation by T2 ("vulnerable" *E. coli*), and the other population consisted of individuals that were partially resistant to predation by T2 ("less vulnerable" *E. coli*). We manipulated productivity in this experiment by running replicate chemostats with different input concentrations of glucose. Our observations were consistent with theoretical predictions. We observed the decline of the more vulnerable prey population at higher productivity but not at lower productivity, and the decline of the less vulnerable prey population at lower productivity but not at higher productivity. However, the rate of decline in some replicates was slower than predicted, and extinctions were not observed during the experiments, contrary to theoretical predictions. We present some testable hypotheses that might explain the slow rate of decline observed.

6. **A high yielding mutant of mycobacteriophage L1 and its application as a diagnostic tool. Chatterjee, S., Mitra, M., Das, Gupta SK (2000). *FEMS Microbiology Letters* 188:47-53.** L1 is a lysogenic phage of mycobacteria, which along with L5 and D29 constitute a closely linked family of homoimmune mycobacteriophages. These phages can be potentially used for genetic engineering of mycobacteria and diagnosis of mycobacterial infection. The effectiveness of such phage based systems depends on the efficiency with which they infect and grow within target cells. While working with phage L1c1ts which is a temperature sensitive mutant of phage L1, we observed that high yielding phage stocks were generated by repeated passage through the host, Mycobacterium smegmatis. A plaque purified mutant L1-P2, obtained from one such high yielding stock, when analyzed further was found to infect host cells with increased efficiency. The DNA obtained from L1-P2 was examined by restriction digestion, and it was observed that spontaneous loss of DNA fragment from the right arm, which encodes early regulatory factors, had occurred. It has been further demonstrated that the high yielding property of the mutant phage could be utilized to increase the sensitivity of mycobacteriophage-based detection systems

7. **Evaluation of CD4+ T cell function In vivo in HIV-infected patients as measured by bacteriophage phiX174 immunization. Fogelman, I, Davey, V, Ochs, H. D., Elashoff, M., Feinberg, M. B., Mican, J., Siegel, J. P., Sneller, M., Lane (2000). *Journal of Infectious Diseases* 182:435-441.** Bacteriophage phiX174 immunization was used to measure CD4(+) T cell function in vivo in human immunodeficiency virus (HIV)-infected patients across all disease stages. Function was evaluated by measuring the ability of T cells to provide help to B cells in antibody production, amplification, and isotype switching. A total of 33 patients and 10 controls received 3 bacteriophage phiX174 immunizations 6 weeks apart. The patients' responses regarding bacteriophage-specific total antibody titers and IgG titers were quantitatively and qualitatively inferior to the controls' responses. Overall, 7 of 33 patients had normal T cell function. Baseline CD4 counts provided the strongest correlation with total antibody and IgG titers. HIV RNA had a weaker association with responses but had some predictive power among patients with a CD4 count >200 cells/microL. Bacteriophage phiX174 immunization seems to be a useful tool for measuring immune function in vivo, which suggests that most HIV-infected patients may have abnormal CD4(+) T cell function despite adequate antiretroviral treatment

8. **Characterization of natural isolates of *Lactobacillus* strains to be used as starter cultures in dairy fermentation. Hebert, E. M., Raya, R. R., Tailliez, P., de, Giori GS (2000). *International Journal of Food Microbiology* 59:19-27.** The technological relevant characteristics of five homofermentative lactobacilli strains, isolated from natural fermented hard cheeses, were studied. Isolates CRL 581 and CRL 654, from Argentinian artesanal hard cheeses, and isolates CRL 1177, CRL 1178, and CRL 1179, from Italian Grana cheeses, were identified as *Lactobacillus delbrueckii* subsp. lactis and *Lactobacillus helveticus*, respectively, by physiological and biochemical tests, SDS-PAGE of whole-cell proteins and sequencing of the variable (V1) region of the 16S ribosomal DNA. All strains showed high levels of beta-galactosidase activity. However, proteolytic activity varied widely among isolates. Strains CRL 581, CRL 654, and CRL 1177 hydrolyzed alpha- and beta-caseins and were able to coagulate reconstituted skim milk in less than 16 h at 42 degrees C. According to the substrate specificity, these proteinases have a caseinolytic activity comparable to that of the P(III)-type of lactococcal proteinases. No strains produced inhibitor substances (bacteriocin) and all were insensitive to attack by 14 *L. helveticus*- and *L. delbrueckii* subsp. lactis-specific bacteriophages

9. **Ultraviolet radiation effects on bacterioplankton and viruses in marine ecosystems. Jeffrey, W. H., Kase, J. P., Wilhelm, S. W. (2000). pp. 206-236 in De Mora, S. J., et al. (eds.) *Effects Of UV Radiation On Marine Ecosystems*. Cambridge University Press, Cambridge.**

10. **Bacteriophage infections in the industrial acetone butanol (AB) fermentation process. Jones, D. T., Shirley, M., Wu, X, Keis, S. (2000). *J Mol***

***Microbiol Biotechnol* 2:21-26.** The reported incidence and effects of bacteriophage infections occurring in the industrial acetone butanol (AB) fermentation processes operated in the USA, Japan, and Puerto Rico during the earlier part of the twentieth century is reviewed. The growth characteristics and solvent-producing ability of a lysogenic strain of Clostridium madisonii isolated from a phage infection in Puerto Rico was determined in molasses fermentation medium. The host strain harbours a large lysogenic phage belonging to the Siphoviridae and the growth rate of the lysogenic strain was found to be slower than the non-lysogenic parent strain and exhibited reduced solvent production. The history of phage infections that occurred in the South African AB process is documented along with the various remedial actions that were taken to restore production. A more detailed account of the last phage infection that occurred in 1980 involving a small pseudo-lysogenic phage belonging to the Podoviridae is given. This phage infected *Clostridium beijerinckii* P260 and a number of closely related industrial strains. Factory-scale fermentations contaminated by this phage were compared with equivalent laboratory-scale control fermentations. The effect of the phage infection in the full-scale and laboratory-scale fermentations were monitored. Results obtained in laboratory-based studies included an assessment of the effect of the multiplicity of infection and the timing of phage infection. The general effects and symptoms of phage infections in the industrial AB fermentation are reviewed including gross changes in the fermentation and changes in cell morphology. Common techniques used for the diagnosis of phage infections and approaches for controlling phage contamination in the AB fermentation are discussed. Prevention strategies included good factory hygiene, sterilisation, decontamination and disinfection, and the use of resistant strains immunised against specific phages

11. **[A method for detection of coliphages in the drinking water]. Kashkarova, G. P., Dorodnikov, A. I. (2000). *Gigiena i Sanitariia* 66-68.**

12. **[Effect of chitosan derivatives on the development of phage infection in cultured *Bacillus thuringiensis*]. Kochkina, Z. M., Chirkov, S. N. (2000). *Mikrobiologiia* 69:266-269.** The influence of chitosan fragments with different degrees of polymerization and some chemical chitosan derivatives on the infection of *Bacillus thuringiensis* by phage 1-97A was studied. It was shown that chitosan inhibits phage infection and inactivates phage particles. The extent of inhibition of phage infection inversely depended on the degree of polymerization of chitosan fragments. On the contrary, the extent of inactivation of phage virulence was proportional to the degree of polymerization. Chitosan derivatives did not inhibit the growth of bacilli. Deaminated chitosan derivatives at a concentration of 100 mg/ml efficiently inhibited phage reproduction, exhibiting no correlation between the degree of deamination and antiviral activity. The anionic derivative chitosan sulfate and N-succinate-6-O-sulfate did not inactivate phage, did not influence bacterial growth, and did not inhibit the process of viral infection

13. **Antibacterials that are used as growth promoters in animal husbandry can affect the release of Shiga-toxin-2-converting bacteriophages and Shiga toxin 2 from *Escherichia coli* strains. Kohler, B., Karch, H., Schmidt, H. (2000). *Microbiology* 146 (Pt 5):1085-1090.** Antibiotics are commonly used as growth promoters in animal husbandry worldwide. This practice has been linked to the emergence of particular antibiotic-resistant bacteria, and is now controversial. In this study, the ability of growth-promoting antibiotics to induce Shiga toxin (Stx)-converting bacteriophages from Stx-producing *Escherichia coli* (STEC) strains was investigated. Subinhibitory concentrations of the antibacterial growth promoters olaquindox, carbadox, tylosin and monensin were used for induction experiments. The amount of mature Stx-converting phage particles released from induced and non-induced cultures was determined, and the production of Stx was simultaneously measured by ELISA. Whereas the quinoxaline-1,4-dioxide-type antibiotics olaquindox and carbadox enhanced the release of Stx-converting phage particles from STEC cells, tylosin and monensin decreased phage induction. The production of Stx increased or decreased simultaneously with the amount of free phages. The results of this study show that particular antibacterial growth promoters can induce Stx phages. In vivo induction of Stx phages from lysogenic STEC may increase the amount of free phages in the intestine and therefore may contribute to the spread of STEC and development of new STEC pathotypes

14. **Impact of acid on survival of *Vibrio vulnificus* and *Vibrio vulnificus* phage. Koo, J., Depaola, A., Marshall, D. L. (2000). *Journal of Food Protection* 63:1049-1052.** Three strains of *Vibrio vulnificus* and *V. vulnificus* phages were tested for acid sensitivity at 21 degrees C. *V. vulnificus* strain 304 was more resistant to pH 4.0 than strains CVD-1 and A-9, whereas acid sensitivities of *V. vulnificus* strains at pH 3.0 and 2.0 were similar. V. vulnificus phage strain 110A-7 was more resistant to pH 4.0 than strain 153A-7, whereas acid sensitivities of phage strains at pH 3.5 and 3.0 were similar. Numbers of *V. vulnificus* and its phage were close to the limit of detection after 100 s at pH 2.0 and after 24 min at pH 3.0. Acid D-values at 21 degrees C decreased as pH decreased for both *V. vulnificus* and phages. D-values of phage strains at pH 3.5 were 10-fold greater than those of host strain at pH 4.0. D-values of phage strains were slightly greater than those of host strain at pH 3.0. These results suggest that *V. vulnificus* and its phage were very sensitive to pH of less than 3.0, although *V. vulnificus* phages were more resistant to acid than their host

15. **[Variants of *Yersinia pestis* resistant to a diagnostic bacteriophage and the problems related to them]. Lebedeva, S. A. (2000). *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* 99-104.** The data of literature on the pleiotropic variability of the resistance of *Y. pestis* mutants to diagnostic phage are presented. The conditions of reversion to the initial phenotype are characterized. The mechanisms of the appearance of such variability of Y. pestis, as well as problems arising in connection with this variability and linked with the pathogenic activity of Y. pestis, low effectiveness of the diagnostic methods used in the inspection of the natural foci of plaque, the reservation of microbes in nature during the periods between epidemics, are discussed

16. **Influence of salts on virus adsorption to microporous filters. Lukasik, J., Scott, T. M., Andryshak, D., Farrah, S. R. (2000). *Applied and Environmental Microbiology* 66:2914-2920.** We investigated the direct and indirect effects of mono-, di-, and trivalent salts (NaCl, MgCl(2), and AlCl(3)) on the adsorption of several viruses (MS2, PRD-1, phiX174, and poliovirus 1) to microporous filters at different pH values. The filters studied included Millipore HA (nitrocellulose), Filterite (fiberglass), Whatman (cellulose), and 1MDS (charged-modified fiber) filters. Each of these filters except the Whatman cellulose filters has been used in virus removal and recovery procedures. The direct effects of added salts were considered to be the effects associated with the presence of the soluble salts. The indirect effects of the added salts were considered to be (i) changes in the pH values of solutions and (ii) the formation of insoluble precipitates that could adsorb viruses and be removed by filtration. When direct effects alone were considered, the salts used in this study promoted virus adsorption, interfered with virus adsorption, or had little or no effect on virus adsorption, depending on the filter, the virus, and the salt. Although we were able to confirm previous reports that the addition of aluminum chloride to water enhances virus adsorption to microporous filters, we found that the enhanced adsorption was associated with indirect effects rather than direct effects. The increase in viral adsorption observed when aluminum chloride was added to water was related to the decrease in the pH of the water. Similar results could be obtained by adding HCl. The increased adsorption of viruses in water at pH 7 following addition of aluminum chloride was probably due to flocculation of aluminum, since removal of flocs by filtration greatly reduced the enhancement observed. The only direct effect of aluminum chloride on virus adsorption that we observed was interference with adsorption to microporous filters. Under conditions under which hydrophobic interactions were minimal, aluminum chloride interfered with virus adsorption to Millipore, Filterite, and 1MDS filters. In most cases, less than 10% of the viruses adsorbed to filters in the presence of a multivalent salt and a compound that interfered with hydrophobic interactions (0.1% Tween 80 or 4 M urea)

17. **Bacterial growth rate and marine virus–host dynamics. Middleboe, M. (2000). *Microbial Ecology* 40:114-124.** The dynamics of a marine virus–host system were investigated at different steady state growth rates in chemostat cultures and the data were analyzed using a simple model. The virus–host interactions showed strong dependence on host cell growth rate. The duration of the infection cycle and the virus burst size were found to depend on bacterial growth rate, and the rate of cell lysis and virus production were positively correlated with steady state growth rate in the cultures ($r^2 > 0.96, p < 0.05$). At bacterial growth rates of 0.02 to 0.10 h^{-1} in the chemostats the virus burst size increased from 12 ± 4 to 56 ± 4, and the latent period decreased from 2.0 to 1.7 h. Resistant clones of the host strain were present in the cultures from the beginning of the experiment and replaced the sensitive host cells following viral lysis in the cultures.

Regrowth of resistant cells correlated significantly ($r^2 = 1.000, p < 0.02$) with the lysis rate of sensitive cells, indicating that release of viral lysates stimulated growth of the non-infected, resistant cells. The constructed model was suitable for simulating the observed dynamics of the sensitive host cells, viruses and resistant clones in the cultures. The model was therefore used in an attempt to predict the dynamics of this virus–host interaction in a natural marine environment during a certain set of growth conditions. The simulation indicated that a steady state relationship between the specific viruses and sensitive and resistant bacterial clones may occur at densities that are reasonable to assume for natural environments. The study demonstrates that basic characterization and modeling of specific virus–host interactions may improve our understanding of the behavior of bacteria and viruses in natural systems.

18. **Characterization of a shiga toxin 2e-converting bacteriophage from an *Escherichia coli* strain of human origin. Muniesa, M., Recktenwald, J., Bielaszewska, M., Karch, H., Schmidt, H. (2000). *Infection and Immunity* 68:4850-4855.** An infectious Shiga toxin (Stx) 2e-converting bacteriophage (phiP27) was isolated from Stx2e-producing *Escherichia coli* ONT:H(-) isolate 2771/97 originating from a patient with diarrhea. The phage could be transduced to *E. coli* laboratory strain DH5alpha, and we could show that lysogens were able to produce biologically active toxin in a recA-dependent manner. By DNA sequence analysis of a 6,388-bp HindIII restriction fragment of phiP27, we demonstrated that the stx(2e) gene was located directly downstream of ileZ and argO tRNA genes. Although no analogue of an antiterminator Q encoding gene was present on this fragment, a lysis cassette comprising two holin genes which are related to the holin genes of *Pseudomonas aeruginosa* phage phiCTX and a gene homologous to the endolysin gene gp19 of phage PS3 were detected. The results of our study demonstrated for the first time that Stx2e can be encoded in the genome of an infectious bacteriophage

19. **Bacteriophage phiYeO3-12, specific for *Yersinia enterocolitica* serotype O:3, is related to coliphages T3 and T7. Pajunen, M., Kiljunen, S., Skurnik, M. (2000). *Journal of Bacteriology* 182:5114-5120.** Bacteriophage phiYeO3-12 is a lytic phage of *Yersinia enterocolitica* serotype O:3. The phage receptor is the lipopolysaccharide O chain of this serotype that consists of the rare sugar 6-deoxy-L-altropyranose. A one-step growth curve of phiYeO3-12 revealed eclipse and latent periods of 15 and 25 min, respectively, with a burst size of about 120 PFU per infected cell. In electron microscopy phiYeO3-12 virions showed pentagonal outlines, indicating their icosahedral nature. The phage capsid was shown to be composed of at least 10 structural proteins, of which a protein of 43 kDa was predominant. N-terminal sequences of three structural proteins were determined, two of them showing strong homology to structural proteins of coliphages T3 and T7. The phage genome was found to consist of a double-stranded DNA molecule of 40 kb without cohesive ends. A physical map of the phage DNA was constructed using five restriction enzymes. The phage infection could be effectively neutralized using serum from a rabbit immunized with whole phiYeO3-12 particles. The antiserum also neutralized T3 infection, although not as efficiently as that of phiYeO3-12. phiYeO3-12 was found to share, in addition to the N-terminal sequence homology, several common features with T3, including morphology and nonsubjectibility to F exclusion. The evidence conclusively indicated that phiYeO3-12 is the first close relative of phage T3 to be described

20. **Phage therapy: The peculiar kinetics of self-replicating pharmaceuticals. Payne, R. J. H., Phil, D., Jansen, V. A. A. (2000). *Clinical Pharmacology and Therapeutics* 68:225-230.** The specter of antibiotic-resistant bacteria has provoked renewed interest in the possible use of bacteriophages to control bacterial infections. We argue that clinical application of phage therapy has been held back by a failure to appreciate the extent to which the pharmacokinetics of self-replicating agents differ from those of normal drugs. For self-replicating pharmaceutical agents, treatment outcome depends critically on various density-dependent thresholds, often with apparently paradoxical consequences. An ability to predict these thresholds and associated critical time points is a necessity if phage therapy is to become clinically practicable.

21. **Selection of tumor-specific internalizing human antibodies from phage libraries. Poul, M. A., Becerril, B., Nielsen, U. B., Morisson, P., Marks, J. D. (2000). *Journal of Molecular Biology* 301:1149-1161.** Antibody internalization into the cell is required for many targeted therapeutics, such as immunotoxins, immunoliposomes, antibody-drug conjugates and for targeted delivery of genes or viral DNA into cells. To generate directly tumor-specific internalizing antibodies, a non-immune single chain Fv (scFv) phage antibody library was selected on the breast tumor cell line SKBR3. Internalized phage were recovered from within the cell and used for the next round of selection. After three rounds of selection, 40 % of clones analyzed bound SKBR3 and other tumor cells but did not bind normal human cells. Of the internalizing scFv identified, two (F5 and C1) were identified as binding to ErbB2, and one (H7) to the transferrin receptor. Both F5 and H7 scFv were efficiently endocytosed into SKBR3 cells, both as phage antibodies and as native monomeric scFv. Both antibodies were able to induce additional functional effects besides triggering endocytosis: F5 scFv induces downstream signaling through the ErbB2 receptor and H7 prevents transferrin binding to the transferrin receptor and inhibits cell growth. The results demonstrate the feasibility of selecting internalizing receptor-specific antibodies directly from phage libraries by panning on cells. Such antibodies can be used to target a variety of molecules into the cell to achieve a therapeutic effect. Furthermore, in some instances endocytosis serves as a surrogate marker for other therapeutic biologic effects, such as growth inhibition. Thus, a subset of selected antibodies will have a direct therapeutic effect.

22. **Detection of phages infecting *Bacteroides fragilis* HSP40 using a specific DNA probe. Puig, M., Jofre, J., Girones, R. (2000). *Journal of Virological Methods* 88:163-173.** Nine bacteriophage isolates of *Bacteroides fragilis*, obtained from urban sewage and pig faeces samples using four different host strains (HSP40, RYC4023, RYC2056 and RYC3318), were compared on the basis of morphology, host range, DNA restriction patterns, DNA hybridisation and protein composition. All the phages are siphovirus and, as judged from cleavage by restriction endonucleases, their genome is composed of double-stranded DNA of similar size (approximately 51-kb). Host range analysis differentiated two types of phages: (1) phages that clearly infect *B. fragilis* strains HSP40 (B40-2, B23-1, B23-2, B23-3 and B23-4, of which B40-8 is the phage type); and (2) the group of bacteriophages that were not infectious for HSP40 (B56-1, B56-2 and B18-1). Similarity in DNA restriction patterns and protein characteristics was found in the HSP40 infectious phages. Anti-B40-8 serum recognised only the proteins of the phages of this type. Although all phages showed similar major protein sizes, minor specific bands were detected. Bacteriophages B56-1, B56-2 and B18-1 showed heterogeneity in their DNA restriction profiles although some degree of DNA-DNA homology between all genomes was observed. Southern blot analysis with phage B40-8 DNA based probes identified a 1.5-kb DNA region homologous for all HSP40 phage isolates, but absent in the genome of the other phage isolates that did not infect this bacterial strain. The homologous region was used as a specific probe to specifically detect *B. fragilis* HSP40 phages

23. **Tissue-specific gene expression identifies a gene in the lysogenic phage Gifsy-1 that affects *Salmonella enterica* serovar typhimurium survival in Peyer's patches. Stanley, T. L., Ellermeier, C. D., Slauch, J. M. (2000). *Journal of Bacteriology* 182:4406-4413.** In vivo expression technology was used to identify *Salmonella enterica* serovar Typhimurium genes that are transcriptionally induced when the bacteria colonize the small intestines of mice. These genes were subsequently screened for those that are transcriptionally inactive during the systemic stages of disease. This procedure identified gipA, a gene that is specifically induced in the small intestine of the animal. The gipA gene is carried on the lambdoid phage Gifsy-1. Consistent with the expression profile, the sole defect conferred by a gipA null mutation is in growth or survival in a Peyer's patch. The gipA strain is wild type in its ability to initially colonize the small intestine and invade the intestinal epithelium. The mutant also survives and propagates at wild-type levels during the systemic stages of disease. The gipA open reading frame is homologous to a family of putative insertion sequence elements, although our evidence shows that transposition is not required for gipA function in the Peyer's patch. These results suggest that the bacteria sense and respond to the particular environment of the Peyer's patch, a critical site for the replication of Salmonella serovar Typhimurium

24. **Construction of lux bacteriophages and the determination of specific bacteria and their antibiotic sensitivities. Ulitzur, S., Kuhn, J. (2000). *Methods in Enzymology* 305:543-557.**

25. **Clay minerals protect bacteriophage PBS1 of *Bacillus subtilis* against inactivation and loss of transducing ability by UV radiation. Vettori, C.,**

Gallori, E., Stotzy, G. (2000). *Canadian Journal of Microbiology* 46:770-773. The effect of UV radiation on the survival of and transduction by phage PBS1 of Bacillus subtilis, free or adsorbed on the clay minerals montmorillonite (M) and kaolinite (K), was studied. After free or clay-associated phage (similar to 10(7) PFU.mL(-1)) was irradiated with UV light (254 nm) for 0, 1, 2, 5, 10, and 30 min and then allowed to infect *B. subtilis* FB300 (thiB4 metA29 argF4 Rfm(r)), the phage was titered, and Met(+) transductants were enumerated on selective media. After 1 min of irradiation, the titer of free and clay-associated phage decreased significantly (similar to 1.6 times for free phage, and similar to 4.9 and 6.8 times for M and K, respectively), whereas the transduction frequency increased significantly (similar to 3 times for free phage and similar to 1.4 and 2.2 times for M and K, respectively). The titer and transduction frequency of clay-associated phage remain essentially constant between 1 and 10 min of irradiation, whereas the titer of free phage decreased by similar to 1 order of magnitude after 5 min of irradiation. When free phage was irradiated for 10 min, the titer and transduction frequency decreased by similar to 2 and 0.5 orders of magnitude, respectively, whereas 30 min of irradiation was necessary to obtain comparable decreases with clay-associated phage. These results indicated that phages are protected to some extent from UV radiation when adsorbed on clay minerals.

26. **Bacterial carbon production in Lake Erie is influenced by viruses and solar radiation. Wilhelm, S. W., Smith, R. E. H. (2000). *Canadian Journal of Fisheries and Aquatic Sciences* 57:317-326.** Bacterial production is an integral recycling mechanism that facilitates carbon flow through aquatic food webs. Factors influencing bacterial activity therefore impact carbon flow. Although ecologists consider grazing and dissolved organic carbon flux to be the major regulators of bacterial activity, we explored two other important pressures. Virus-like particle abundance ranged from 3.7 to 37.9 x 10^{10} L-1 in samples collected during August 1997 and July 1998. Bacterial abundance during these periods ranged from 1.8 to 4.6 x 10^{9} L-1. Based on electron microscopic analysis, viruses in Lake Erie would have been responsible for 12.1 to 23.4 % of bacterial mortality and, in quasi-steady state conditions, a comparable loss of bacterial productivity. In the central basin, solar radiation was also demonstrated to regulate bacterial productivity. Ultraviolet radiation (UVR, 295-400 nm) was shown to inhibit bacterial productivity according to a cumulative exposure kinetic model, and biological weighting functions were derived to enable calculation of time- and depth-integrated photoinhibition. The daytime photoinhibitory loss of bacterial carbon production was estimated to be 14 to 30% over the upper 5 m, primarily due to UVR > 320 nm. Viruses and sunlight are therefore of comparable importance as regulators of bacterial activity in this system.

27. **Toward selection of internalizing antibodies from phage libraries. Becerril, B., Poul, M. A., Marks, J. D. (1999). *Biochemical & Biophysical Research Communications* 255:386-393.** Antibodies which bind cell surface receptors in a manner whereby they are endocytosed are useful molecules for the delivery of drugs, toxins, or DNA into the cytosol of mammalian cells for therapeutic applications. Traditionally, internalizing antibodies have been identified by screening hybridomas. For this work, we studied a human scFv (C6.5) which binds ErbB2 to determine the feasibility of directly selecting internalizing antibodies from phage libraries and to identify the most efficient display format. Using wild-type C6.5 scFv displayed monovalently on a phagemid, we demonstrate that anti-ErbB2 phage antibodies can undergo receptor-mediated endocytosis. Using affinity mutants and dimeric diabodies of C6.5 displayed as either single copies on a phagemid or multiple copies on phage, we define the role of affinity, valency, and display format on phage endocytosis and identify the factors that lead to the greatest enrichment for internalization. Phage displaying bivalent diabodies or multiple copies of scFv were more efficiently endocytosed than phage displaying monomeric scFv and recovery of infectious phage was increased by preincubation of cells with chloroquine. Measurement of phage recovery from within the cytosol as a function of applied phage titer indicates that it is possible to select for endocytosable antibodies, even at the low concentrations that would exist for a single phage antibody member in a library of 10(9).

28. **Vaginal bacterial phaginosis? Blackwell, A. L. (1999). *Sexually Transmitted Infections* 75:352-353.** The hypothesis that a sexually transmitted lactobacillus phage may specifically destroy the endogenous healthy lactobacillus vaginal flora and secondarily permit overgrowth of endogenous aerobic bacteria and *G. vaginalis* may explain why anaerobic vaginosis behaves epidemiologically as a sexually transmitted agent but recurrence rate is unaffected by antibacterial treatment of male partners. Unfortunately this hypothesis raises as many questions as answers. Are phages capable of destroying lactobacilli carried on the penis or are the lactobacillus phages derived from the woman's own gut flora and merely transferred into the vagina by sexually activity. Are there any phage resistant strains of lactobacilli which could be of therapeutic use? Is t here any possibility that a vaccine could be developed that could be given to women with recurrent anaerobic vaginosis, particularly perhaps, those who smoke or who have cervical intraepithelial neoplasia. Until the pathogenesis of anaerobic vaginosis is more fully understood, argument will undoubtedly remain concerning the best name for the conditions (?vaginal bacterial phaginosis) and treatment will be unsatisfactory. As a result some women will be burdened not only by the social consequences of recurrent genital malodour but may also be at risk of a plethora of complications. [see: http://sti.bmjjournals.com/cgi/reprint/75/5/352.pdf for full-text pdf]

29. **[An accelerated method for detecting coliphages in the drinking water]. Dmitrieva, R. A., Doskina, T. V., Nedachin, A. E., Sidorenko, S. G. (1999). *Gigiena i Sanitariia* 71-72.**

30. **Elimination of viruses, phages, bacteria and *Cryptosporidium* by a new generation Aquaguard point-of-use water treatment unit. Grabow, W. O., Clay, C. G., Dhaliwal, W., Vrey, M. A., Muller, E. E. (1999). *Zentralblatt fur Hygiene und Unweltmedizin* 202:399-410.** The elimination of human viruses, phages, bacteria and *Cryptosporidium* oocysts by a new generation commercial Aquaguard purifier for the domestic treatment of drinking water, has been evaluated. The unit basically consists of a candle prefilter, activated carbon filter and ultraviolet irradiation compartment. Drinking water seeded with selected laboratory test strains of resistant micro-organisms was passed through the unit. Similar tests were carried out with sewage-contaminated river water and secondary treated waste water containing naturally occurring organisms. Test procedures were based on internationally accepted principles for the evaluation of point-of-use water treatment units, including a standard test protocol of the United States Environmental Protection Agency. Reduction in numbers of seeded test organisms at several log levels higher than those expected in water for which the unit is intended, was determined by the cultivation of viable organisms. In the case of seeded viruses and Cryptosporidium parvum oocysts the qualitative absence of nucleic acid was determined by the reverse transcriptase polymerase chain reaction (RT-PCR). At the design flow rate of one litre per minute, numbers of polio, hepatitis A, adeno types 2 and 41, rota SA11, human rota and astro viruses, as well as somatic and MS2 coliphages, and *Escherichia coli*, *Streptococcus faecalis*, *Clostridium perfringens*, total coliform bacteria, enterococci, heterotrophic bacteria and *C. parvum* oocysts, were reduced by more than 99.999% in all waters tested. This efficiency conforms to specifications for such units. The quality of the treated water was well within microbiological limits of international specifications for drinking water

31. **Virus removal by filtration. Graf, E. G., Jander, E., West, A., Pora, H., Aranha-Creado, H. (1999). *Developments in Biological Standarization* 99:89-94.** Advances in membrane technology have allowed the expansion of the size-exclusion removal principle to viruses of concern in the processing of pharmaceutical drug products derived from biological fluids and cell-culture techniques. Direct flow- and cross-flow filters are complementary techniques for virus removal and may be used either independently or as an adjunct to other virus clearance methods. Representative virus titre reduction data for microfiltration and ultrafiltration membranes are presented along with a validation model using bacteriophages as challenge viruses. Non-destructive filter integrity tests before and after filtration and a stringent process validation regime are applied to enhance product safety

32. **[Cryostabilization of biological properties of plague phages]. Kadetov, V. V., Kudriakova, T. A., Terent'ev, A. N., Kachkina, G. V., Borodina, T. N., Saiamov, S. R. (1999). *Voprosy Virusologii* 44:136-139.** Conditions of cryostabilization of *Yersinia pestis* phages preserving their biological properties at very low temperature are studied.

33. **Alternative origins for nannobacteria-like objects in calcite. Kirkland, Brenda L., Folk, Robert L., Lynch, F. L., McLean, Robert J. C., Molineux, Ian J., Rahnis, Michael A. (1999). *Geology (Boulder)* 27:347-350.** More than 40 calcite-precipitation experiments were performed under sterile conditions

in order to investigate the origins of 25-300 nm spherical-, rod-, and ovoid-shaped objects that have been widely interpreted as evidence of nanometer-scale life (i.e., nannobacteria). Individual experiments included the addition of soluble organic compounds, common species of eubacteria, or phage-induced eubacterial lysates. These experiments indicate that many of the nanometer-scale objects have inorganic or nonnannobacterial origins. In the precipitation experiments, calcite formed euhedral crystals 50-800 nm in diameter and smaller (<50 nm) anhedral or rounded particles or protocrystals. The small anhedral or rounded solids resembled nannobacteria. The relative amount of anhedral or rounded calcite was greatest in experiments with a dissolved organic component. These controlled experiments are in accord with observations that rounded nanometer-scale objects are more common in minerals formed in organic-rich environments. Bacterial fragments occur as rounded to irregularly shaped particles that included cell-wall fragments, expulsed cytoplasm, and relict capsules that also closely resembled nannobacteria. Acid etching of the large euhedral crystals produced in the precipitation experiments also resulted in the formation of nanometer-scale features that resembled nannobacteria in natural carbonates. The shapes of the etching artifacts vary as a function of the strength of the acid and the duration of etching. Much caution is advisable in interpreting the origin of rounded features <50 nm.

34. **[The history of the discovery and study of *Brucella* bacteriophages]. Liapustina, L. V., Liamkin, L. I., Taran, I. F. (1999). *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* 123-124.**

35. **Bacteriophages in the evolution of pathogen-host interactions. Miao, E. A., Miller, S. I. (1999). *Proceedings of the National Academy of Sciences, USA* 96:9452-9454.** The term "emerging infectious diseases" has recently been popularized as a way to describe the introduction of new infectious agents to human populations (1). Bacterial infectious diseases can "emerge" by different methods. Emergence may involve the discovery that a disease of unknown etiology has a microbial etiology, such as peptic ulcers caused by infection with *Helicobacter pylori* bacteria (2). Infectious diseases can also emerge as a result of exposure of specific human populations to microorganisms that are new to those populations. Such strains could emerge as new epidemics or pandemics, as was the case with the spread of the bacterial cause of cholera, *Vibrio cholerae*, from Asia to South America (3). In addition to these well appreciated mechanisms, advances in the understanding of the molecular basis of microbial pathogenesis have led to the hypothesis that more virulent bacterial strains could emerge through recent acquisition of virulence factors. The current worldwide epidemic of the Gram-negative enterobacteriaciae *Salmonella* has significant public health implications (4) and may provide an important example to illustrate this principle. [see http://www.pnas.org/cgi/reprint/96/17/9452.pdf for full-text pdf]

36. **Model systems to study the parameters determining the success of phage antibody selections on complex antigens. Mutuberria, R., Hoogenboom, H. R., van, der Linden, de, Bruine AP, Roovers, R. C. (1999). *Journal of Immunological Methods* 231:65-81.** Phage antibody display technology offers a powerful tool for the isolation of specific antibodies to defined target antigens. Most selection strategies described to date have relied on the availability of purified and often recombinant antigen, providing the possibility to perform selections on a well-defined antigen source. However, when the target antigen cannot be purified (e.g., an integral membrane protein), or if the antigen is unknown (e.g., when searching for novel markers on cells or tissues), panning of phage antibody libraries has to be performed on complex antigen sources such as cell surfaces or tissue sections, or even by in vivo selection methods. This provides a series of technical and experimental challenges. One focus of our research is to select antibodies directed to novel cancer-induced antigens expressed by tumours and by the tumour vasculature. To understand the parameters governing selection on complex antigen sources and to assess the efficiency of these phage library selections, we have set up two model selection systems in which both tumour cells and vascular endothelial cells serve as target "antigen". We describe a model based on phage antibodies directed to the tumour antigen epithelial glycoprotein-2, to compare phage antibody selections on a range of different antigen sources including purified and recombinant antigen, whole live cells, tissue cryosections and in vivo grown solid tumours. Secondly, we describe a model based on a phage antibody directed against the endothelial cell inducible adhesion molecule E-selectin. We compare selections on cultured cell monolayers with selections on cell suspensions immobilised on columns, to determine which selection approach is most suitable for the identification of novel tumour endothelial cell markers. Our data provide insight into the efficiency and thus potency of different selection strategies and show that there are very large differences in the recovery and enrichment of binding phage between the different methods tested. Our results further demonstrate the feasibility of phage antibody selections on whole, intact cells and show that these may sometimes compare favourably to selections on purified antigen. Selections on endothelial cells immobilised on columns compare favourably with selections on cell-monolayers; the most favourable conditions for both selection procedures are described. The implications of our data for phage antibody selections on these different complex antigen sources using either non-immune or immune phage antibody repertoires are discussed. The use of model systems such as the ones described here will help to determine optimal experimental conditions for phage library selections on complex antigens and aid in developing more powerful selection procedures for target discovery

37. **Microbial gene transfer: an ecological perspective. Paul, J. H. (1999). *Journal of Molecular Microbiology and Biotechnology* 1:45-50.** Microbial gene transfer or microbial sex is a means of exchanging loci amongst prokaryotes and certain eukaryotes. Historically viewed as a laboratory artifact, recent evidence from natural populations as well as genome research has indicated that this process may be a major driving force in microbial evolution. Studies with natural populations have taken two approaches-either adding a defined donor with a traceable gene to an indigenous community, and detecting the target gene in the indigenous bacteria, or by adding a model recipient to capture genes being transferred from the ambient microbial flora. However, both approaches usually require some cultivation of the recipient, which may result in a dramatic underestimation of the ambient transfer frequency. Novel methods are just evolving to study *in situ* gene transfer processes, including the use of green fluorescent protein (GFP)-marked plasmids, which enable detection of transferrants by epifluorescence microscopy. A transduction-like mechanism of transfer from viral-like particles produced by marine bacteria and thermal spring bacteria to *Escherichia coli* has been documented recently, indicating that broad host range transduction may be occurring in aquatic environments. The sequencing of complete microbial genomes has shown that they are a mosaic of ancestral chromosomal genes interspersed with recently transferred operons that encode peripheral functions. Archaeal genomes indicate that the genes for replication, transcription, and translation are all eukaryotic in complexity, while the genes for intermediary metabolism are purely bacterial. And in eukaryotes, many ancestral eukaryotic genes have been replaced by bacterial genes believed derived from food sources. Collectively these results indicate that microbial sex can result in the dispersal of loci in contemporary microbial populations as well as having shaped the phylogenies of microbes from multiple, very early gene transfer events. [see http://www.jmmb.net/v1n1/07/07.html for full-text entry]

38. **Diversity of *Bacteroides fragilis* strains in their capacity to recover phages from human and animal wastes and from fecally polluted wastewater. Puig, A., Queralt, N., Jofre, J., Araujo, R. (1999). *Applied and Environmental Microbiology* 65:1772-1776.** Great differences in capability to detect bacteriophages from urban sewage of the area of Barcelona existed among 115 strains of *Bacteroides fragilis*. The capability of six of the strains to detect phages in a variety of feces and wastewater was studied. Strains HSP40 and RYC4023 detected similar numbers of phages in urban sewage and did not detect phages in animal feces. The other four strains detected phages in the feces of different animal species and in wastewater of both human and animal origin. Strain RYC2056 recovered consistently higher counts than the other strains and also detected counts ranging from 10^1 to approximately 10^3 phages per ml in urban sewage from different geographical areas. This strain detected bacteriophages in animal feces even though their relative concentration with respect to the other fecal indicators was significantly lower in wastewater polluted with animal feces than in urban sewage

39. **Resistance to DNA damage in natural viral communities from the Gulf of Mexico. Weinbauer, M. G., Wilhelm, S. W., Pledger, R. J., Mitchell, D. L., Suttle, C. A. (1999). *Aquatic Microbial Ecology* 17:111-120.** Using a highly specific radioimmunoassay, the sunlight-induced formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts ([6-4] PPs) in viral DNA was investigated for natural virus communities in offshore and coastal waters of the western Gulf of Mexico as well as for clonal viral isolates. Concentrations of (6-4) PPs were consistently lower than CPD concentrations, and ranged from 1.5 to 17.0% of total measured photodamage. The accumulation of photoproducts varied among the natural viral community,

the marine Vibrio phage PWH3a-P1 and the Synechococcus sp. DC2 (WH7803) cyanophage SYN-M3, which were deployed in situ from dawn until dark. Natural viral communities were more resistant to DNA damage than the cyanophage isolate SYN-M3, which was more resistant to damage than bacteriophage PWH3a-P1. Moreover, depth profiles revealed that photodamage in viral isolates deployed in the water column accumulated more rapidly at offshore stations than at coastal stations. In natural virus communities collected from offshore surface waters, photodamage accumulated during the solar day with maximum damage occurring between 15:00 and 18:00 h. Depth profiles obtained during calm seas showed that photodamageconcentrations were high in surface waters at the offshore stations and at 1 coastal station. Results at other coastal stations undergoing significant mixing demonstrated no photoproduct accumulations. Results demonstrate that natural virus communities were more tolerant to DNA damaging radiation than the laboratory isolates used in this study. Consequently, laboratory isolates can be poor proxies for UV impacts on natural viral communities.

40. **Biodistribution of filamentous phage-Fab in nude mice. Yip, Y. L., Hawkins, N. J., Smith, G., Ward, R. L. (1999). *Journal of Immunological Methods* 225:171-178.** In vivo panning of peptide libraries in mice has allowed the isolation of peptides which target the vasculature of specific organs. The application of this approach to phage displaying Fab fragments (phage-Fab) could lead to the isolation of antibodies which recognize novel tumor antigens. In this study, we have evaluated the biodistribution of phage-Fab in nude mice. Balb/c nude mice were injected intravenously with 10(9) TU of phage displaying the anti-colon cancer Fab c30.6. Blood samples were collected at nine time points over a period of 72 h and three groups of four mice were sacrificed at 4 min, 24 h and 72 h. Normal tissues (liver, colon, spleen, kidneys, lungs, skeletal muscle) and faeces were collected at these time points and the number of viable phage in each sample was determined. The distribution of phage in tissues was also examined by immunohistochemical analysis of paraffin-embedded tissues. Regression analysis of plasma kinetic data showed that the half-life and the volume of distribution of phage was 3.6 h and 1 ml, respectively. Phage uptake occurred predominantly in lungs, kidneys, spleen and liver. Relatively few phage were distributed to colon and muscle, and phage were eliminated from the circulation by 72 h. Immunohistochemical analysis showed phage to be mainly within the vasculature at 4 min, whereas notable phage extravasation was observed at 24 h and 72 h. In conclusion, this study provides information on the in vivo behavior of phage-Fab which will be useful in the design of in vivo panning strategies. By choosing appropriate time points for tissue collection, it may be possible to isolate novel Fabs against both intra- and extravascular targets

41. **Human enteric viruses in the water environment: a minireview. Bosch, A. (1998). *Int Microbiol* 1:191-196.** Water virology started around half a century ago, with scientists attempting to detect poliovirus in water samples. Since that time, other enteric viruses responsible for gastroenteritis and hepatitis, among a great variety of virus strains, have replaced enteroviruses as the main target for detection in the water environment. Technical molecular developments, polymerase-chain reaction (PCR) amplification being the method of choice, enable the detection of fastidious health-significant viruses. However, shortcomings of molecular procedures include their potential incompatibility with concentration methods, indispensable to reduce the water sample volume to assay for viruses, the inability to discern between infectious and non infectious material. On the other hand, these procedures are restrained to sophisticated laboratories and detection of alternative indicator organisms has been proposed. Bacterial indicators fail to give a reliable clue of the virological quality of water. Selected bacteriophage groups appear as a better choice for their use as virus indicators

42. **New cholera phages for *Vibrio cholerae* serovar O139 [letter]. Chakrabarti, A. K., Ghosh, A. N., Sarkar, B. L. (1998). *Journal of Infection* 36:131-132.**

43. **Removal of waterborne human enteric viruses and coliphages with oxidized coal. Cloete, T. E., Da, Silva E., Nel, L. H. (1998). *Current Microbiology* 37:23-27.** Human enteric viruses and coliphages have been detected in water that has undergone what is generally considered adequate treatment, including chlorination. Because small numbers of virus particles are needed for the initiation of a productive virus infection, the presence of any number of virus particles in water resources will always be of concern. In this investigation the ability of oxidized coal to remove viruses from water was investigated. The oxicoal product was found to be able to remove not only coliphages, but also various pathogenic human viruses from seeded water sources. Removal was dependent upon the type of virus, the period of exposure, and the concentration of oxidized coal

44. **Virus occurrence and transport in a school septic system and unconfined aquifer. DeBorde, Dan C., Ball, Patrick N., Lauerman, Bruce, Woessner, William W. (1998). *Ground Water* 36:825-834.** Federal efforts to establish reliable natural disinfection criteria for ground water supplies require the identification of appropriate indicator viruses to represent pathogenic viruses and an understanding of parameters affecting virus survival and transport in a variety of hydrogeologic settings. A high school septic system and the associated fecal waste-impacted unconfined sand and gravel aquifer were instrumented to: (1) evaluate if the concentrations of enterovirus and coliphage in this system were sufficient to allow their use as indicator viruses; (2) establish viral transport rates, transport distances, and concentrations in a highly conductive cold water aquifer. Enteroviruses were found in only two of eight assays of the septic tank effluent (0.26 and 4.4 virus/L) and were below detection in eight ground water samples. Male-specific and somatic coliphage were detectable in both the septic tank effluent (averaging 674,000 and 466,000 coliphage/L, respectively) and in the impacted underlying ground water, decreasing to detection limits beyond 38 m of the drainfield. Virus transport parameters in this aquifer were measured by seeding high numbers of MS2 and OX174 coliphage into the ground water and documenting their transport over 17.4 m. A portion of the seeded virus traveled at least as fast as the bromide tracer (1 to 2.9 m/d). Proposed natural disinfection criteria would not be met in this aquifer using standard 30.5 m setback distances. In addition, the virus sorption processes and long survival times resulted in presence of viable seed virus for more than nine months

45. **Evolutionary relationships among putative RNA-dependent RNA polymerases encoded by a mitochondrial virus-like RNA in the Dutch elm disease fungus, Ophiostoma novo-ulmi, by other viruses and virus-like RNAs and by the Arabidopsis mitochondrial genome. Hong, Y., Cole, T. E., Brasier, C. M., Buck, K. W. (1998). *Virology* 246:158-169.** The nucleotide sequence (2617 nucleotides) of virus-like double-stranded (ds) RNA 3a in a diseased isolate, Log1/3-8d2 (Ld), of the ascomycete fungus *Ophiostoma novo-ulmi* has been determined. One strand of the dsRNA contains an open reading frame (ORF) with the potential to encode a protein of 718 amino acids, and the complementary strand contains two smaller ORFs with the potential to encode proteins of 178 and 182 amino acids, respectively. The large ORF contains 12 UGA codons which code for tryptophan in ascomycete mitochondria and has a codon bias typical of mitochondrial genes, consistent with the localization of Ld dsRNAs within the mitochondria. The amino acid sequence contains motifs characteristic of RNA-dependent RNA polymerases (RdRps). This putative RdRp was shown to be related to putative RdRps of mitochondrial dsRNAs of another ascomycete and a basidiomycete fungus and also to a putative RdRp encoded by the mitochondrial genome of Arabidopsis thaliana. In multiple sequence alignments, the fungal mitochondrial dsRNA-encoded RdRp-like proteins formed a cluster, ancestrally related to the RdRps of the yeast 20S and 23S RNA replicons and of the positive-stranded RNA bacteriophages of the Leviviridae family, but distinct from RdRps of other families and genera of fungal RNA viruses and related plant and animal RNA viruses. Northern blot analysis with RNA 3a strand-specific probes indicated that nucleic acid extracts of Ld contain more single-stranded (positive-stranded) RNA than dsRNA, consistent with an evolutionary relationship between RNA 3a and positive-stranded RNA phages

46. **Fluorescent *Escherichia coli* C for enumeration of coliphages from environmental samples. Jothikumar, N., Cliver, D. O. (1998). *BioTechniques* 24:546-550.**

47. **Biologicheskiye sistemy kontrolya mutagennykh faktorov okruzhayushchey sredy. Pererva, T. P., Aleksandrov, Yu, Miryuta, A. Y., Miryuta, N. Y. (1998). *Dopovidi Natsional'noyi Akademiyi Nauk Ukrayini. Matematika, Prirodoznavstvo, Tekhnichni Nauki* 1998:188-192.** Comparative analysis of RNA-containing MS2 phage, DNA-containing lambda phage and Drosophila melanogaster as possible test-objects for study of environmental mutagenic loading is carried out. According to the obtained results, MS2 phage is the most sensible to the combined mutagenic action of studied soil specimens, Drosophila is completely insensible, while lambda phage is located on the intermediate position showing only an insignificant mutagenic response under

experimental conditions

48. **The origin and evolution of viruses (a review). Sinkovics, J., Horvath, J., Horak, A. (1998).** ***Acta Microbiologica et Immunologica Hungarica*** **45:349-390.** Viroids and prions might have existed early at the border of inanimate and living worlds. Most extant viruses can be characterized as derivatives of ancestors originating from episomal elements of prokaryotes (DNA phages) and later from eukaryotes. Retroviruses very likely originated from cellular retrotransposons. Retrograde evolution of some large viruses from obligatory intracellular bacteria is possible but the ontogenesis of extant bacteria does not include a viral form of existence (the filterable L forms are not viruses) and well-defined viruses do not regenerate back into vegetative bacterial forms. Biologists experimenting with the evolution of prokaryotic and eukaryotic ancient cells cannot ignore the earliest appearance of viruses within or outside the living matter. Viruses participated in and gave direction to the evolution and natural selection by coexisting with uni- and multicellular organisms for billions of years. The coevolution of viruses and their host cells is characterized by incessant attacks and counterattacks through gene rearrangements and mutations (induced in the virus by an immunological counterattack of the host or by transgression of species barriers by the virus) and recombinations. Recombinations occurred between viral and viral or viral and host genes. Acts of "molecular piracy" as practiced by ancient viruses endowed the virus with the expression of several host genes for the advantage of the virus in its replicative cycle and host-to-host spread. Probably the first immortalized and malignantly transformed cells were induced by viruses as viruses evolved anti-apoptotic measures. While infected cells resort to apoptotic death before the assembly of a new viral progeny, prominent are the anti-apoptotic measures viruses evolved in order to assure the completion of their full replicative cycle. Further, viruses may escape neutralization by host antibodies and may survive a counterattack by the host's T cells directed at virally infected cells of its own. Viruses may induce a form of tolerance and coexist with their host without inducing disease. Persistent and apparently or deceivingly apathogenic or even attenuated viral "quasi-species" populations may contain individual particles that regain virulence due to recombinations and/or gene rearrangements, especially when transgressing species barriers. Xenotropic viruses of animals may replicate in human cells and vice versa confounding experiments with xenotransplants or with use of veterinary viral vaccines for the treatment of human diseases

49. **Detection, quantification and morphological characterization of** ***Vibrio cholerae*** **indicator bacteriophages [Spanish]. Talledo, M. A., Gutiérrez, S., Merino, F., Rojas, N. (1998).** ***Rev. Peru. Biol.*** **5:90-97.**

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Acknowledgements

Thanks and happy 2001!

Contact Steve Abedon (microdude+@osu.edu) with suggestions, criticisms, comments, or anything else that might help make this a better site.