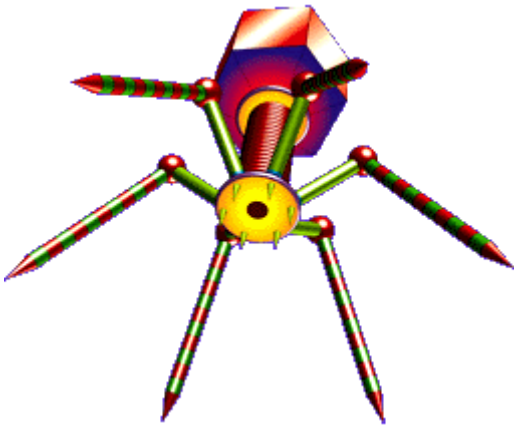


Bacteriophage Ecology Group News

- - - - - - - - - - - - - - - - - - -

@ www.phage.org

by Stephen T. Abedon (editor)

April 1, 2005 issue (#24)

Beg News, the Final *Quarterly* Issue

Stephen T. Abedon, The Ohio State University

Bacteriophage Ecology Group News (*BEG News*) 24

When I began BEG News six years ago, for a July 1, 1999 issue, I had hoped that I might keep up the pace of putting out one issue a quarter for a year or two. But one issue turned into a dozen and now to two dozen, dutifully put out each quarter by yours truly. Not always on time, mind you, but not so late as to matter. The core of the newsletter came to be an editorial and a list of new phage ecology references. Between that and entering all of the new "non-members" into the BEG database (particularly as subscribers to BEG News via BioMed Central), I've devoted something approaching one full workweek to getting each issue out.

The original intent was to do this once a quarter to inspire me to update phage.org on a regular basis. Indeed, early issues of BEG News documented those updates. Ultimately, however, the result has been that I've spent far more time putting together BEG News than working on the rest of phage.org. Perhaps as a consequence, www.phage.org is no longer the Google number one site for a "phage" search (though I suspect the real reason we're no longer number one is that they've rejiggered how they score sites). Please, everybody, for the sake of the Bacteriophage Ecology Group, place a link on your web sites that points to http://www.phage.org (rather than to http://www.mansfield.ohio-state.edu/~sabedon, which goes to the same place but is not the same thing). We're still the "phage ecology" and "bacteriophage ecololgy" number one Google hit, however, so all is not yet lost. ☺

I would like to thank Hans Ackermann for his endless support as well as Steve McQuinn for his tireless devotion to computer rendering of the phage T4 virion. I would like to thank those of you, in addition to Hans, who contributed editorials to BEG News: Ry Young, Jim Karam, and Andrew Kropinski (and, of course, Betty Kutter for this issue). Thanks also go to Matt Sullivan for his updates to the Cyanophage Literome, and to those many individuals who have provided me with quarterly phage images over the past six years.

The Bacteriophage Ecology Group will keep on going, even without a quarterly BEG News, still soliciting members—visit the BEG Bibliography for bibliography updates at www.phage.org/beg_bibliography.htm. Even BEG News may return from time to time, particularly if I receive enough submissions, editorials, or art from people to justify an issue. In other words, consider BEG News as "new and improved" rather than defunct, where publication is now driven by *content* rather than by calendar.

Thank you everybody for your ongoing support of phage.org and bacteriophage ecology.

Overview and History of Current and Recurring Phage-Related Meetings

Elizabeth "Betty" Kutter, The Evergreen State College

Bacteriophage Ecology Group News (*BEG News*) 24

In 1945, Max Delbrück greatly stimulated and redirected the course of phage research by organizing the first of a very long series of annual Phage Courses at Cold Spring Harbor, Long Island, drawing students and senior personnel from around the world into his project to use phage to develop an understanding of the organization and functioning of the gene and signaling the birth of molecular biology. The results were enormously successful. Phage research and funding subsequently reached its peak in about 1980, but then declined as the newly-developed phage-derived techniques enabled the explosion of molecular analyses of a variety of other organisms. Now, however, interest in phages is again exploding in new directions as we become aware of phage ecology and its worldwide roles, the possibilities of phage therapy, potentials phage offer for understanding microbial physiology and genomics, the application of phage in display technologies and in nanotechnology, and the uses of the rapidly-growing families of phage-encoded proteins in a variety of technologies**.**

There are 3 important, complementary phage-related meetings this summer, all of which grew out of the associated annual Cold Spring Harbor phage meeting and the changes that have happened in that meeting since it started over 50 years ago:

►**The XVIX Phage/Virus Assembly Conference** (June 7th – 12th) will be held in Winter Park, Colorado this year. Bob Edgar, Bill Wood and Fred Eiserling began this series of conferences in 1968 at a time when the Cold Spring Harbor meeting was primarily focusing on such phenomena as gene regulation and lysogeny; held different places each year, it has developed a good balance between phage and other viruses (www.phagevirusassembly.org).

►**The Molecular Genetics of Bacteria and Phages Meeting** (Aug. 2nd – 7th) now alternates between Cold Spring Harbor and Madison, Wisconsin, where it will be held this year. Its main focus is on microbial transcription mechanisms, regulatory phenomena, bacterial cell biology, genomics, and microbial pathogenesis, but it also will have a session on "bacteriophage development and host interactions" (www.union.wisc.edu/conferenceservices/phages/index.html).

►**The 16th Evergreen International Phage Biology Meeting** (Aug. 7th – 12th) will be held in Olympia, Washington. The only current meeting devoted exclusively to phages, it is a particularly good place to build collaborations. It started in 1975 as a west coast phage meeting, reflecting the fact that the Cold Spring Harbor meeting had evolved into a broader yet narrower Phage and Microbial Genetics meeting and had become very expensive, particularly for students. With the help of many – particularly Bruce Alberts, Chris Mathews, Gisela Mosig, Fumio Arisaka, Jan Drake, Eleanor Spicer, Peter Gauss, Steve Abedon, Wolfgang Rueger, Vadim Mesyanzhinov, and Jim Karam – it quickly grew into a worldwide meeting for T4 and other large lytic phages and was the birthplace of the T4 genome project and the 1983 and 1994 ASM Bacteriophage T4 books. With encouragement from Ian Molineux, Rich Calendar, Mike DuBow and others, it grew into a more general phage biology meeting, emphasizing phage therapy, phage ecology and genomics along with regulation, biochemistry and assembly. Planned sessions this year include **Phage Genomics, Phage Regulatory Systems; Molecular Mechanisms; Phage Ecology; The Real-world Phage Infection Process; Human Phage Therapy; Phages In agriculture and Food Safety; Dairy Phages; Innovative Applications of Phage and Their Products; Phage Structure and Function -- and a special tribute to Eduard Kellenberger and Wolfram Zillig**. We are in the process of selecting a pair of chair people for each session; about half the spots are still being decided, and suggestions are welcome. The session order and lengths will be determined by the number of related abstracts we receive For more info go to www.evergreen.edu/phage.

Much is also now happening in broader meetings to reflect the widespread resurgence of interest in phage biology and applications.

►**The American Society for Microbiology 105th General Meeting** (June 5th – 9th) in Atlanta Georgia, will have sessions on *Phage Therapy: New Life for an Old Idea*, *Getting your DNA Inside*, *Taking Over RNA Polymerase,* and *Phage Genomics and Beyond*. (www.asm.org/Meetings/index.asp?bid=697). Last August, their **"New Phage Biology Meeting"** pulled together all of the various areas of phage interest for a "Phage Summit" in Miami which drew 350 participants with 250 posters (!!).

►**The International Union of Microbiological Societies triennial meeting** (July 23rd – 28th) in San Francisco, "Microbes in a Changing World" will have sessions on *Bacteriophage Life Cycles* and *Phage Evolution and Genomics* (www.iums2005.org).

►**The British Society for General Microbiology 156th Meeting** (April 4rd – 7th) in Edinburgh, Scotland just had 2 full days of talks on various aspects of phage biology: *Phage Genomics and Evolution; Phage Ecology and Phage-Host Response; Phages and Virulence;* and *Phage Therapy* (www.socgenmicrobiol.org.uk/meetings/mtgpages/hw.cfm).

-- The following is an advertisement --

T-shirts with this design are available at www.thebacteriophages.com/sales.htm

Help Save the G. Eliava Bacteriophage Institute (in Tblisi, Republic of Georgia)

The George Eliava Institute for Bacteriophage, Microbiology and Virology (GEIBMV) in Tbilisi, Republic of Georgia, was founded in 1923 and has researched and developed bacteriophage medicines for over seventy years. These medicines formed a key element of the treatment of a wide range of bacterial infections during the Soviet era and the GEIBMV supplied the whole of the former USSR with bacteriophage therapeutics. The Institute survived the murder of Dr. Eliava, the first Head of the Institute, in 1937, and the civil war that followed the break-up of the Soviet Union in 1991.

Bacteriophage therapy is rapidly emerging as an important alternative to conventional chemotherapy for the treatment of bacterial infections at a time when antibiotic drug resistance threatens our continued ability to combat serious infections in our hospitals and in our communities, and at a time when the pharmaceutical industry is drastically scaling down its development of the new antibiotics that we so badly need. The GEIBMV has amassed a unique and extensive collection of medically important bacteriophages and the scientists and technicians within the Institute have unparalleled experience in research, development and clinical use of bacteriophage medicines.

All this is now under threat. The Georgian Academy of Sciences have revealed plans to merge the GEIBMV with five other Institutes in Georgia as a simple cost cutting exercise, and plan to disperse the resources and expertise that currently resides under one roof in Tbilisi. Such a move will spell the end of this unique microbiological institution, with all that entails for the future development of unconventional but effective anti-infective medicines. The proposal to merge the Institute has serious implications for the future, in particular:

- Loss of commercial funding opportunities
- Loss of key staff members
- Loss of external assistance in technical and commercial fields
- Disruption of collaboration agreements already in place
- Loss of new opportunities to develop commercial/joint ventures
- Potential repayment of some grants owing to changed management and ownership

Under this scenario the proposed move will result NOT in cost savings, but instead in additional costs and the lost opportunities for Georgia in biotechnology on the world stage. No decision should be taken about the future merging of the Eliava Institute until a proper study is undertaken of all the economic, technical and financial implications.

Help to ensure that this does not happen – sign this petition and you will send a message to the Georgian Academy of Sciences that the loss of GEIBMV would be a blow not only to science in Georgia but also will significantly impoverish the global fight against the ravages of infectious disease.

The Petition: We the undersigned hereby urge the Georgian Government to recognize the strategic importance of the G. Eliava Institute of Bacteriophage, Microbiology and Virology to the future of the people of Georgia, and it's extraordinary impact in the development of anti- infective biomedicine worldwide, by establishing the Institute as an independent public institution – the Eliava National Institute – within the Ministry of Education and Science of Georgia.

To sign the petition, please visit www.phage.org/GEIBMV_petition.pdf. Please print out this one-page document, fill it out as completely as you can, sign and date it, and then either scan the completed document to email to Nino Chanishvili (n_chanish.ibmv@caucasus.net) or send it by snail mail send it to Kazbegi street, 41, VERA region, 380079, Tbilisi, Georgia.

The Entrance to the G. Eliave Bacteriophage, Virology and Microbiology Institute.

Two views of Tbilisi: from above the city at Turtle Lake and a view of Metekhi, built in the 12th century.

Phage Art Show at ASM in Atlanta

Dear fellow microbiologists and phage enthusiasts,

We are excited to announce “The Art of Phage: An Exhibition”, which will be held at the ASM meeting in Atlanta, Georgia. The main art show will be held at the Division H, ,S, K and T Mixer on Tuesday, June 7 at the Omni Hotel. Slide shows will be presented in selected sessions and colloquia of Division M and Division H.

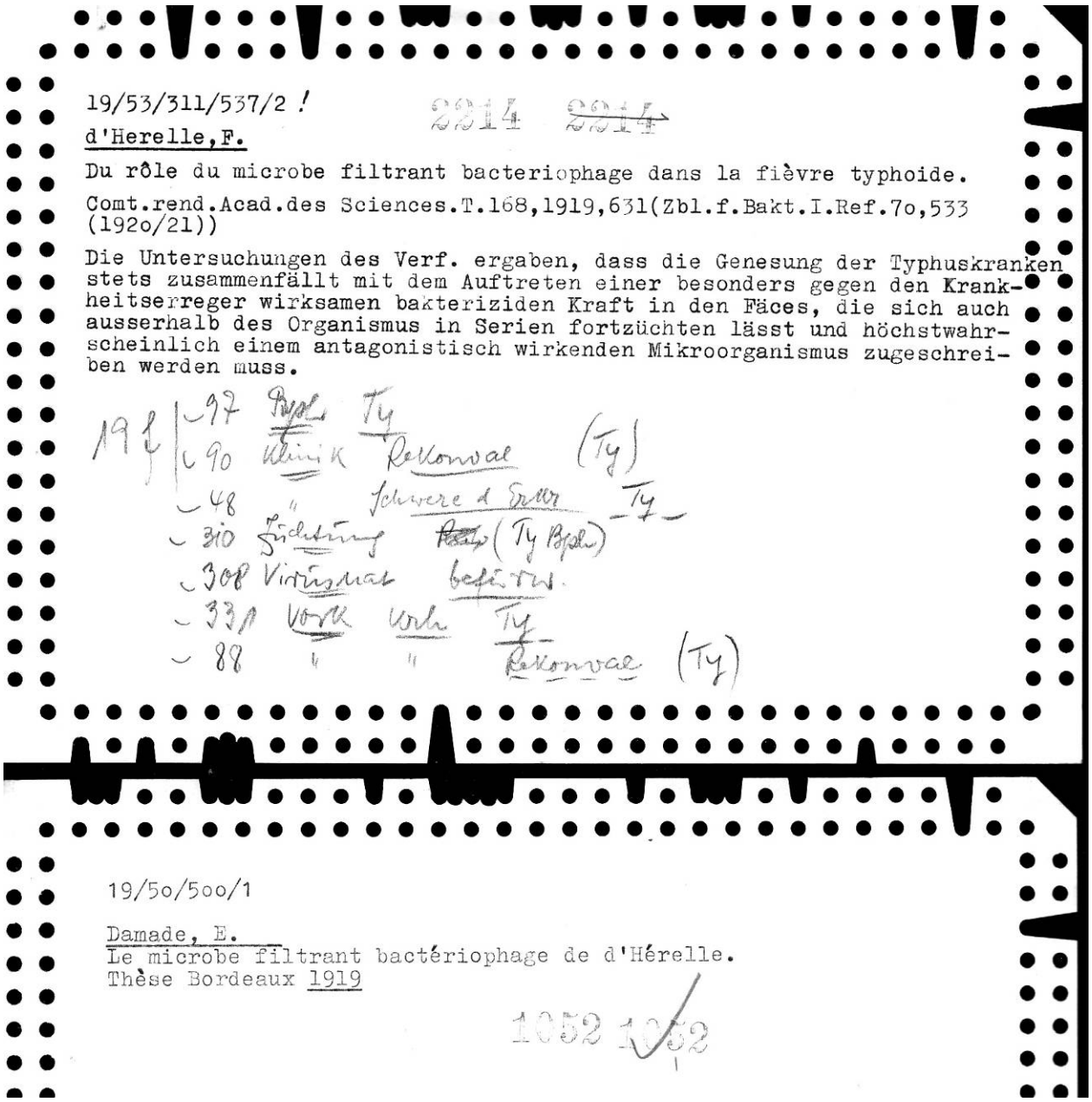
Pieces of art will be for sale and any inquires can be communicated with Neilan Kuntz. We look forward to sharing the vision and creativity of thse bacteriophage-inspired artists. Artist information, art gallery and automated slide show of some of the selected art can also be seen at the following link: http://phage.sdsu.edu/imagery/gallery/images/artshow.php.

Sincerely,

Dr. Forest Rohwer (San Diego State University)
Dr. Anca Segall (San Diego State University)
Neilan Kuntz (Polymerlinks.org)
Dr. Stephen Abedon (Ohio State University)

Supported by the **NSF BioComplexity Program** and **San Diego State University**

Raettig Punch Cards!



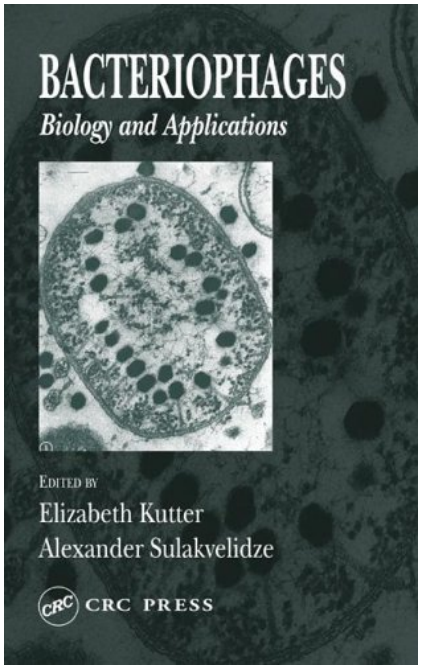
Shown is a piece of phage history. Hansjürgen Raettig (1911-1997) assembled and published in 1958, and again in 1967, the definitive phage bibliographies, containing a total of approximately 11,405 references. To assemble and index these references he employed early computer punch cards, such as the one shown above.

Thank you to Hans Ackermann, who acquired and scanned the card you are viewing, and to Dr. H. Gelderblom who provided the cards. Click the following links to view full-sized scans of this card and a second (or here and here to view reduced, grayscale versions).

New BEG Members

go to www.phage.org/beg_join.htm for joining information

name (home page links)	status	address/research interests
Anna Ivanova	PI	Department of Physics, Tbilisi State University, 3, Chavchavadze str., 0128, Tbilisi, Georgia
	interests:	Physico-chemical properties of bacteriophages and their receptors (for example, thermal and hydrodynamical properties); also phage DNA ejection in a model system consisting of phages, bacterial membrane fragments, and receptors. (contents | BEG members | top of page)
Jeffrey B. Jones	PI	University of Florida, Plant Pathology Dept., Gainesville, FL 32611
	interests:	Integrated approach to controlling bacterial diseases of plants. We are using phage therapy for this IPM approach. (contents | BEG members | top of page)
Leonard Peruski	PI	Associate Professor, Microbiology and Immunology, Indiana University School of Medicine, Northwest Center, 3400 Broadway, Gary, Indiana 46408-1197
	interests:	Evolution of bacteriophage that infect the Bacillus cereus genetic group, with special emphasis on lytic and temperate phage of B. anthracis. (contents | BEG members | top of page)
Manan Sharma	PI	USDA-ARS, ANRI, Food Technology and Safety Lab, Bldg. 201, BARC-East, 10300 Baltimore Ave., Beltsville, MD 20705
	interests:	Food safety, specifically post-harvest interventions that may be applied to reduce microbial pathogens and spoilage organisms. (contents | BEG members | top of page)
Juan E. Suárez	PI	Area de Microbiologia, Facultad de Medicina, Julian Claveria 6, E-33006 Oviedo, Spain Telephone: +34 985103559
	interests:	We work with phages that infect lactic acid bacteria, mainly those active on industrial starters but also on those affecting probiotic bacteria. We are starting a new line on phage therapeutics, taking advantage of our location in a Faculty of Medicine that has an adjunct hospital. (contents | BEG members | top of page)



Bacteriophages: Biology and Applications

Elizabeth Kutter & Alexander Sulakvelidze (eds)

CRC Press, Boca Raton, Florida

© 2004

ISBN: 0849313368

510 pages

Reprint of Forward from Kutter and Sulakvelidze, 2004

Bruce Alberts, President, National Academy of Sciences; Washington, DC

Bacteriophage Ecology Group News (*BEG News*) 24

It is a privilege for me to have this opportunity to provide a brief foreword to *Bacteriophages: Biology and Applications* by Elizabeth Kutter and Alexander Sulakvelidze. I was one of many who first became fascinated with the romance of science by reading the book *Arrowsmith* as a teenager. In that novel written by Sinclair Lewis in 1925, an attempt to develop phage therapies against bacterial diseases played a central role. But by the early 1950s, when I read the book, the widespread success of newly introduced antibiotics had seemed to make this alternative approach to the selective killing of bacteria unnecessary.

Instead, a small set of bacteriophages had begun to attract attention as "model organisms" – prime systems for probing the basic chemistry of life. These phages were attractive to scientists, because they were much easier to study with the then-available tools than were more complex life forms such as bacterial or human cells. They had relatively small genomes and multiplied rapidly, making them unusually amenable to genetic analyses that aimed at obtaining multiple mutants in each bacteriophage gene. To enable the essential genes for viral multiplication to be genetically identified, screening techniques were developed that focused on *conditional lethal* mutations – for example, through the identification of "temperature-sensitive" phage mutants that would grow at low but not high temperatures. Moreover, because large amounts of infected cells were easy and inexpensive to obtain, biochemical approaches could be readily employed, so that the products of the genes identified by genetic screens could be isolated and characterized in cell-free systems.

The model organism approach worked better than anyone had had a right to expect, in part because the mechanisms that are used to control gene expression and to recombine and replicate DNA genomes turned out to be much more highly conserved across life forms than anyone had suspected. Much of the work was concentrated on several viruses that infect the bacterium *E. coli* – most notably the bacteriophages lambda, T4 and T7. The findings made in multiple laboratories could thereby be combined, yielding results that were immensely important in developing the field of molecular biology, as reviewed in the early chapters of this book.

To give a personal example, for 30 years beginning in 1965, my own laboratory would exploit the combined genetic and biochemical advantages of the T4 virus for study of fundamental DNA replication mechanisms. In the end, the "protein machine" mechanisms revealed at the replication fork through bacteriophage studies turned out to be highly similar to those used to move the replication forks of higher organisms, including that of humans (Alberts 2003).

In the 1960s and 1970s, many advances were made in a wide range of laboratories studying both bacteriophages and the bacterial cells themselves. The new knowledge of biological mechanisms that resulted soon allowed the development of more powerful research tools (such as DNA cloning). With these new tools, researchers could begin to unravel the molecular mechanisms in more complex cells and organisms. As a result, by the 1980s most of the action and excitement in molecular biology had moved away from simpler organisms to investigations of mammalian cells.

For several unrelated reasons, we may have come full circle over the course of the last 80 years. First of all, there is an urgent need for new types of antibacterial therapies. We now live in an evermore crowded, more interconnected world in which resistant strains of microorganisms spread with amazing rapidity. Modern science has increased our ability to design countermeasures to these diseases of humans and animals; the standard countermeasures have been new drug and vaccine developments. But producing a new drug is an enormously expensive endeavor. In addition, market failures have discouraged the development of new vaccines in the private sector. As a result, the world now faces a serious challenge in dealing with a host of microbial threats that were once thought to be defeated rather easily by antibiotics (Institute of Medicine, 2003). As described in Chapters 12 to 14, there is therefore every reason to reintroduce bacteriophage therapies as an additional tool in the war against bacterial diseases.

A second feature of modern biology that is reawakening interest in bacteriophages is our new ability to obtain the DNA sequences of large number of organisms inexpensively. From this DNA sequence information, we can determine the relatedness of organisms and attempt to retrace the past history of life on

the Earth. The sequencing of bacteriophages is only just beginning. Not only are there immense numbers of novel proteins yet to be discovered among what could be 100 million different bacteriophages in the environment, the vast majority not yet known (the genomes of only about 400 have thus far been completely sequenced), but it is now suspected that some of the lytic phages carry genes that trace back in evolutionary history to the common ancestor of eukaryotic and prokaryotic cells (see Chapter 5). In summary, bacteriophages represent a huge untapped genetic reservoir that can be productively mined -- both by those interested in proteomics and by those who are trying to decipher the mysterious nature of the early cells that predated the split between the three families of cells that are alive today: the archaea, the bacteria, and eukaryotes.

Now that we have access to the complete molecular anatomy of a cell, a third reason for a new focus on bacteriophages stems from the realization – sobering to scientists like myself -- that biological systems are so complex that they can not be understood without new methods of analyzing and conceptualizing them. Thus, for example, the nearly 500 different protein molecules that are encoded by the genome of the simplest known living cell, the small bacterium *Mycoplasma genitalium*, interact with each other and with substrates in an enormous number of ways. Even if we had a complete catalog of all of these interactions and their rate constants, information we are far from achieving today, we could not claim to understand this cell in any deep sense – that is, in the sense of being able to explain how it is able to grow and reproduce itself as a chemical system. Living systems are made possible by a huge web of networked chemical reactions, and we presently lack the tools to decipher what is most significant within such complexity. This realization, new to most molecular biologists, raises the question of whether it might be productive to focus once again on one or a few bacterial viruses that could serve as model organisms – far simpler than any free-living cell -- for developing new types of complexity analyses. If so, which viruses should be targeted and through what types of experimental strategies?

Finally, the increasingly large role that science and technology will play in driving societal changes in the 21st century argues strongly for a new type of science education in our schools. Beginning with 5 year olds, what is needed is an education that allows students to explore the world around them using evidence and logic, so that they leave school learning to solve problems the way that scientists do. They also need to understand what science is and why it represents a special way of knowing about the natural world, if they are to respect its judgments concerning the many important issues that they will need to decide in their lifetimes – such as whether they should avoid exposures to substances that could adversely affect their health in the future, or whether their nation should make sacrifices to reduce the release of greenhouse gases into the atmosphere.

The National Science Education Standards call for a revolutionary change in science teaching, with an emphasis on teaching science as inquiry (National Research Council, 1996). As the ultimate step in such an education effort, it should be possible for a select group of students to participate in a real scientific investigation in their upper years of high school. It is thus encouraging to find high school students appearing as coauthors of a major publication from the University of Pittsburgh, in which a diverse set of novel bacteriophages that infect mycobacteria have been identified and sequenced (Pedulla, et al. 2003).

The National Academy of Sciences has just published the results of an unusual workshop in which 25 leading scientists outside the field were exposed to the biology of the smallpox virus and challenged with the task of suggesting new approaches to antiviral therapies (Harrison, et al. 2004). As this exercise made clear, we badly need a new infusion of talent and energy into the field of virology, where there is an enormous opportunity for scientific breakthroughs whose results will be of great practical benefit to human health (Alberts and Fineberg 2004). What better way to recruit outstanding young people into such fields than to expose them as teenagers to a scientific exploration of the wonderfully rich and diverse world of bacteriophages?

I would like to end by congratulating both the coauthors and the many contributors to this volume for their dogged persistence in sticking to bacteriophage research over many decades. They have survived their years in the shadows, and now we can all appreciate the strong platform their work has established for the many exciting years of research ahead.

REFERENCES

1. Alberts, B. M., 2003, DNA replication and recombination. Nature 421: 431-435.

2. Alberts, B. M. and Fineberg, H. V., 2004 Harnessing new science is vital for biodefense and global health. Proc Natl Acad Sci U S A 101: 11177.

3. Harrison, S. C., Alberts, B. M., Ehrenfeld, E., Enquist, L., Fineberg, H. V., Mcknight, S. L., Moss, B., et al., 2004 Discovery of antivirals against smallpox. Proc Natl Acad Sci U S A 101: 11178-11192.

4. Institute of Medicine, 2003. Microbial Threats to Health: Emergence, Detection, and Response. Mark S. Smolinski, Margaret A. Hamburg, and Joshua Lederberg, Editors. Natl. Acad. Press, Washington, D. C.

5. National Research Council, 1996. National Science Education Standards. Natl. Acad. Press, Washington, D. C.

6. Pedulla, M. L., Ford, M. E., Houtz, J. M., Karthikeyan, T., Wadsworth, C., Lewis, J. A., Jacobs-Sera, D., et al., 2003 Origins of highly mosaic mycobacteriophage genomes. Cell 113: 171-182.

Bruce Alberts, President, National Academy of Sciences; Washington, DC 20037, Professor of Biochemistry and Biophysics, University of California, San Francisco; San Francisco, CA 94143

New Bacteriophage-Ecology References

Anonymous 2004. **Renaissance phage.** Nature Reviews Microbiology 2:922. **Abstract:** In today's culture of spin, 'renaissance' is a term that is often applied undeservingly to particular areas of science. In the case of current phage research, however, its use is easily justified.

Allwood, P. B., Y. S. Malik, S. Maherchandani, K. Vought, L. A. Johnson, C. Braymen, C. W. Hedberg, and S. M. Goyal. 2004. **Occurrence of *Escherichia coli*, noroviruses, and F-specific coliphages in fresh market-ready produce.** J. Food Prot.67:2387-2390. **Abstract:** Forty samples of fresh produce collected from retail food establishments were examined to determine the occurrence of *Escherichia coli*, F-specific coliphages, and noroviruses. An additional six samples were collected from a restaurant undergoing investigation for a norovirus outbreak. Nineteen (48%) of the retail samples and all outbreak samples were preprocessed (cut, shredded, chopped, or peeled) at or before the point of purchase. Reverse transcription-PCR, with the use of primers JV 12 and JV 13, failed to detect norovirus RNA in any of the samples. All six outbreak samples and 13 (33%) retail samples were positive for F-specific coliphages (odds ratio undefined, P = 0.003). Processed retail samples appeared more likely to contain F-specific coliphages than unprocessed samples (odds ratio 3.8; 95% confidence interval 0.8 to 20.0). Only two (5.0%) retail samples were positive for *E. coli*; outbreak samples were not tested for *E. coli*. The results of this preliminary survey suggest that F-specific coliphages could be useful conservative indicators of fecal contamination of produce and its associated virological risks. Large-scale surveys should be conducted to confirm these findings.

Avery, S. M., L. D. Walters, and M. L. Hutchison. 2005. **Fate of *Escherichia coli* O157 and detection of stx phage during fermentation of maize, an animal feedstuff.** Lett. Appl. Microbiol. 40:99-105. **Abstract:** AIMS: The fate of inoculated *Escherichia coli* O157, stx phages and the physico-chemical properties of maize were studied during laboratory-scale fermentation by naturally occurring lactic acid bacteria. METHODS AND RESULTS: Before fermentation, chopped maize was inoculated with 6.2 log(10) CFU g^{-1} of a five-isolate mixture of *E. coli* O157. After fermentation, the silage contained 70.6 g kg^{-1} dry matter (DM) lactic acid and 12.8 g kg^{-1} DM acetic acid and was pH 4.0. Levels of E. coli O157 fell rapidly, and none of the five isolates could be recovered from the fermenting maize after 8 days. Using a resuscitation step did not consistently enhance recovery of *E. coli* O157. Stx phages were not isolated from the fermenting maize at any time. Pulsed-field gel electrophoresis (PFGE) genotyping showed that *E. coli* O157 2975 and 64a/01 survived better than the other three isolates studied. *Escherichia coli* O157 isolate 1474/00 was particularly sensitive to the laboratory procedures used to harvest the inocula and contaminate the maize. Some colonies recovered during the fermentation had one to three band alterations compared with the initial PFGE genotypes. SIGNIFICANCE AND IMPACT OF THE STUDY: None of the five different *E. coli* O157 genotypes survived maize fermentation. Fermentation of maize produces an animal feedstuff that is unlikely to contain *E. coli* O157 or stx phages.

Bailey, S., M. R. J. Clokie, A. Millard, and N. H. Mann. 2004. **Cyanophage infection and photoinhibition in marine cyanobacteria.** Res. Microbiol. 155:720-725. **Abstract:** Members of two cyanobacterial genera, *Synechococcus* and *Prochlorococcus*, are dominant within the prokaryotic component of the picophytoplankton and contribute significantly to global photosynthetic productivity. These organisms are known to be susceptible to infection by bacteriophages (viruses that infect bacteria) and it is believed that phage infection in the oceans has exerted selective pressures on the evolution of both phage and host and continues to influence community structure. Understanding of the processes of host-phage interaction within the marine environment is limited; however, new insights have arisen from sequence analysis of the genome of the bacteriophage S-PM2, which infects *Synechococcus* strains. The phage was found to encode homologs of the key photosystem II reaction center core polypeptides, D1 and D2. These reaction center polypeptides are known to be rapidly turned over in uninfected cells in a repair cycle that helps to protect oxygenic phototrophs against photoinhibition. This finding suggests that bacteriophages infecting marine cyanobacteria may play an active role in protecting their hosts against photoinhibition, thereby ensuring an energy supply for replication by preventing the deleterious effects on host cell integrity seen during acute photoinhibition.

Balogh, B., Jones, J. B., Momol, M. T., Olson, S. M., Obradovic, A., Jackson, L. E. 2003. **Improved Efficacy of Newly Formulated Bacteriophages for Management of Bacterial Spot on Tomato.** Plant Disease 87:949-954. **Abstract:** Bacteriophages are currently used as an alternative method for controlling bacterial spot disease on tomato incited by *Xanthomonas campestris* pv. *vesicatoria*. However, the efficacy of phage is greatly reduced due to its short residual activity on plant foliage. Three formulations that significantly increased phage longevity on the plant surface were tested in field and greenhouse trials: (i) PCF, 0.5% pregelatinized corn flour (PCF) + 0.5% sucrose; (ii) Casecrete, 0.5% Casecrete NH-400 + 0.5% sucrose + 0.25% PCF; and (iii) skim milk, 0.75% powdered skim milk + 0.5% sucrose. In greenhouse experiments, the nonformulated, PCF-, Casecrete-, and skim milk- formulated phage mixtures reduced disease severity on plants compared with the control by 1, 30, 51, and 62%, respectively. In three consecutive field trials, nonformulated phage caused 15, 20, and 9% reduction in disease on treated plants compared with untreated control plants, whereas plants treated with PCF- and Casecrete-formulated phage had 27, 32, and 12% and 30, 43, and 24% disease reduction, respectively. Plants receiving copper-mancozeb treatments were included in two field trials and had a 20% decrease in disease in the first trial and a 13% increase in the second one. Skim milk-formulated phage was tested only once and caused an 18% disease reduction. PCF-formulated phage was more effective when applied in the evening than in the morning, reducing disease on plants by 27 and 13%, respectively. The Casecrete formulated phage populations were over 1,000-fold higher than the nonformulated phage populations 36 h after phage application.

Blanch, A. R., L. Belanche-Munoz, X. Bonjoch, J. Ebdon, C. Gantzer, F. Lucena, J. Ottoson, C. Kourtis, A. Iversen, I. Kuhn, L. Moce, M. Muniesa, J. Schwartzbrod, S. Skraber, G. Papageorgiou, H. D. Taylor, J. Wallis, and J. Jofre. 2004. **Tracking the origin of faecal pollution in surface water: an ongoing project within the European Union research programme.** Journal of water and health 2:249-260. **Abstract:** The objectives of this study are to generate knowledge about methods to track the sources of faecal pollution in surface waters, with the aim of having one or a few easy procedures applicable to different geographic areas in Europe. For this, a first field study using already proposed methods (genotypes of F-specific RNA bacteriophages, bacteriophages infecting *Bacteroides fragilis*, phenotypes of faecal coliforms and enterococci, and sterols) has been done in five areas representing a wide array of conditions in Europe. The present faecal indicators (faecal coliforms, enterococci, sulfite reducing clostridia and somatic coliphages) have also been included in this first field study. At the same time some emerging methods have been settled or adapted to water samples and assayed in a limited number of samples. The results of this first field study indicate that no single parameter alone is able to discriminate the sources, human or non-human, of faecal pollution, but that a 'basket' of 4 or 5 parameters, which includes one of the present faecal indicators, will do so. In addition, numerical analysis of the data shows that this 'basket' will allow the successful building of predictive models. Both the statistical analyses and the studied predictive models indicate that genotype II of F-specific RNA bacteriophages, the coprostanol and the ratio coprostanol: coprostanol+epicoprostanol are, out of the studied parameters, those with a greater discriminating power. Either because unsuccessful adaptation of the methods to water samples or because the preliminary assays in water samples indicated low discriminating capability, only three (sorbitol-fermenting bifidobacteria, some species of bifidobacteria detected by PCR with specific primers and phages infecting Bacteroides tethaiotaomicron) of the newly assayed methods have been considered for a second field study, which is currently underway. Expectations are that these new tools will minimize the number of parameters in the 'basket', or at least minimize the difficulty in assaying them.

Borchardt, M. A., N. L. Haas, and R. J. Hunt. 2004. **Vulnerability of drinking-water wells in La Crosse, Wisconsin, to enteric-virus contamination from surface water contributions.** Appl. Environ. Microbiol. 70:5937-5946. **Abstract:** Human enteric viruses can contaminate municipal drinking-water wells, but few studies have examined the routes by which viruses enter these wells. In the present study, the objective was to monitor the municipal wells of La Crosse, Wisconsin, for enteric viruses and determine whether the amount of Mississippi River water infiltrating the wells was related to the frequency of virus detection. From March 2001 to February 2002, one river water site and four wells predicted by hydrogeological modeling to have variable degrees of surface water contributions were sampled monthly for enteric viruses, microbial indicators of sanitary quality, and oxygen and hydrogen isotopes. $^{18}O/^{16}O$ and $^{2}H/^{1}H$ ratios were used to determine the level of surface water contributions. All samples were collected prior to chlorination at the wellhead. By reverse transcription-PCR (RT-PCR), 24 of 48 municipal well water samples (50%) were

positive for enteric viruses, including enteroviruses, rotavirus, hepatitis A virus (HAV), and noroviruses. Of 12 river water samples, 10 (83%) were virus positive by RT-PCR. Viable enteroviruses were not detected by cell culture in the well samples, although three well samples were positive for culturable HAV. Enteroviruses detected in the wells by RT-PCR were identified as several serotypes of echoviruses and group A and group B coxsackieviruses. None of the well water samples was positive for indicators of sanitary quality, namely male-specific and somatic coliphages, total coliform bacteria, *Escherichia coli*, and fecal enterococci. Contrary to expectations, viruses were found in all wells regardless of the level of surface water contributions. This result suggests that there were other unidentified sources, in addition to surface water, responsible for the contamination.

Bordenstein, S. R. and J. J. Wernegreen. 2004. **Bacteriophage Flux in Endosymbionts (*Wolbachia*): Infection Frequency, Lateral Transfer, and Recombination Rates.** Mol. Biol. Evol. 21:1981-1991. **Abstract:** The highly specialized genomes of bacterial endosymbionts typically lack one of the major contributors of genomic flux in the free-living microbial world-bacteriophages. This study yields three results that show bacteriophages have, to the contrary, been influential in the genome evolution of the most prevalent bacterial endosymbiont of invertebrates, *Wolbachia*. First, we show that bacteriophage WO is more widespread in *Wolbachia* than previously recognized, ccurring in at least 89% (35/39) of the sampled genomes. Second, we show through several phylogenetic approaches that bacteriophage WO underwent recent lateral transfers between *Wolbachia* bacteria that coinfect host cells in the dipteran *Drosophila simulans* and the hymenopteran *Nasonia vitripennis*. These two cases, along with a previous report in the lepidopteran *Ephestia cautella*, support a general mechanism for genetic exchange in endosymbionts-the "intracellular arena" hypothesis-in which genetic material moves horizontally between bacteria that coinfect the same intracellular environment. Third, we show recombination in this bacteriophage; in the region encoding a putative capsid protein, the recombination rate is faster than that of any known recombining genes in the endosymbiont genome. The combination of these three lines of genetic evidence indicates that this bacteriophage is a widespread source of genomic instability in the intracellular bacterium *Wolbachia* and potentially the invertebrate host. More generally, it is the first bacteriophage implicated in frequent lateral transfer between the genomes of bacterial endosymbionts. Gene transfer by bacteriophages could drive significant evolutionary change in the genomes of intracellular bacteria that are typically considered highly stable and prone to genomic degradation.

Brion, G. M., N. B. O'Banion, and G. L. Marchin. 2004. **Comparison of bacteriophages for use in iodine inactivation: batch and continuous flow studies.** Journal of water and health 2:261-266. **Abstract:** Inactivation rates in batch studies for four commonly used surrogate bacteriophages were measured in stable aqueous iodine solutions for the purpose of determining which was the most suited to evaluate iodine disinfection efficacy in batch and continuous flow conditions. Two types of group Leviviridae bacteriophages were used, Type I (MS2) and Type II (GA), along with group Microviridae, ΦX174, and group Tectiviridae, PRD1. Inactivation was compared at iodine doses of 1.0-1.5 mg l2/l. MS2 was the most susceptible to iodine inactivation of the four phages tested. Inactivation of naked, icosahedral bacteriophages, MS2 and ΦX174 demonstrated removals to below detection limits (>99. 99%) in less than 10 min. Lipid-containing PRD1 and F+ssRNA GA bacteriophages demonstrated the greatest iodine resistance in batch experiments with an average of 1.82 logs of inactivation (98.5%) after 60 min and 1.05 logs of inactivation (91.1%) after 30 min respectively. Similarly, in continuous flow studies through pentaiodide quaternary ammonium strong base resin, MS2, GA and ΦX174 were more strongly inactivated than PRD1. The lipid component of PRD1 is thought to enhance resistance to iodine over non-lipid-containing bacteriophages by protecting easily oxidized groups on the protein capsid, but further research is needed before proving this hypothesis. The results from this research may provide a surrogate standard for more rigorous and developed research into the mode of iodine disinfection and its inactivation kinetics.

Canchaya, C., G. Fournous, and H. Brussow. 2004. **The impact of prophages on bacterial chromosomes.** Mol. Microbiol. 53:9-18. **Abstract:** Prophages were automatically localized in sequenced bacterial genomes by a simple semantic script leading to the identification of 190 prophages in 115 investigated genomes. The distribution of prophages with respect to presence or absence in a given bacterial species, the location and orientation of the prophages on the replichore was not homogeneous. In bacterial pathogens, prophages are particularly prominent. They frequently encoded virulence genes and were major

contributors to the genetic individuality of the strains. However, some commensal and free-living bacteria also showed prominent prophage contributions to the bacterial genomes. Lysogens containing multiple sequence-related prophages can experience rearrangements of the bacterial genome across prophages, leading to prophages with new gene constellations. Transfer RNA genes are the preferred chromosomal integration sites, and a number of prophages also carry tRNA genes. Prophage integration into protein coding sequences can lead to either gene disruption or new proteins. The phage repressor, immunity and lysogenic conversion genes are frequently transcribed from the prophage. The expression of the latter is sometimes integrated into control circuits linking prophages, the lysogenic bacterium and its animal host. Prophages are apparently as easily acquired as they are lost from the bacterial chromosome. Fixation of prophage genes seems to be restricted to those with functions that have been co-opted by the bacterial host.

Chibani-Chennoufi, S., J. Sidoti, A. Bruttin, M. L. Dillmann, E. Kutter, F. Qadri, S. A. Sarker, and H. Brüssow. 2004. **Isolation of *Escherichia coli* bacteriophages from the stool of pediatric diarrhea patients in Bangladesh.** J. Bacteriol. 186:8287-8294. **Abstract:** A 3-week coliphage survey was conducted in stool samples from 140 Bangladeshi children hospitalized with severe diarrhea. On the *Escherichia coli* indicator strain K803, all but one phage isolate had 170-kb genomes and the morphology of T4 phage. In spot tests, the individual T4-like phages infected up to 27 out of 40 diarrhea-associated *E. coli*, representing 22 O serotypes and various virulence factors; only five of them were not infected by any of these new phages. A combination of diagnostic PCR based on g32 (DNA binding) and g23 (major capsid protein) and Southern hybridization revealed that half were T-even phages sensu strictu, while the other half were pseudo-T-even or even more distantly related T4-like phages that failed to cross-hybridize with T4 or between each other. Nineteen percent of the acute stool samples yielded T4-like phages, and the prevalence was lower in convalescent stool samples. T4-like phages were also isolated from environmental and sewage water, but with low frequency and low titers. On the enteropathogenic *E. coli* strain O127:K63, 14% of the patients yielded phage, all of which were members of the phage family Siphoviridae with 50-kb genomes, showing the morphology of Jersey- and beta-4 like phages and narrow lytic patterns on *E. coli* O serotypes. Three siphovirus types could be differentiated by lack of cross-hybridization. Only a few stool samples were positive on both indicator strains. Phages with closely related restriction patterns and, in the case of T4-like phages, identical g23 gene sequences were isolated from different patients, suggesting epidemiological links between the patients.

Dabrowska, K., A. Opolski, J. Wietrzyk, K. Switala-Jelen, J. Godlewska, J. Boratynski, D. Syper, B. Weber-Dabrowska, and A. Gorski. 2004. **Anticancer activity of bacteriophage T4 and its mutant HAP1 in mouse experimental tumour models.** Anticancer research 24:3991-3995. **Abstract:** BACKGROUND: Previously, we have shown the ability of the bacteriophage T4 and its substrain HAP1 (selected for a higher affinity to melanoma cells) to reveal antimetastatic activity in a mouse melanoma model. Here, we investigated the potential phage anticancer activity in primary tumour models. MATERIALS AND METHODS: Mice were inoculated subcutaneously with B16 or LLC cells (collected from in vitro culture). Bacteriophages T4 and HAP1 were injected intraperitoneally daily (8×10^8pfu/mouse, except the experiment concerning the dose-dependence). RESULTS: Treatment with purified preparations of bacteriophage T4 resulted in significant reduction of tumour size, the effect being dose-dependent. HAP1 was more effective than T4 and its activity was also dose-dependent. Parallel experiments with non-purified bacteriophage lysates resulted in significant stimulation of tumour growth. CONCLUSION: These data suggest that purified bacteriophages may inhibit tumour growth, a phenomenon with potentially important clinical implications in oncology.

Danovaro, R., E. Manini, and A. Dell'Anno. 2002. **Higher abundance of bacteria than of viruses in deep mediterranean sediments.** Appl. Environ. Microbiol. 68:1468-1472. **Abstract:** The interactions between viral abundance and bacterial density, biomass, and production were investigated along a longitudinal transect consisting of nine deep-sea stations encompassing the entire Mediterranean basin. The numbers of viruses were very low (range, 3.6×10^7 to 12.0×10^7 viruses g^{-1}) and decreased eastward. The virus-to-bacterium ratio was always < 1.0, indicating that the deep-sea sediments of the Mediterranean Sea are the first example of a marine ecosystem not numerically dominated by viruses. The lowest virus numbers were found where the lowest bacterial metabolism and turnover rates and the largest cell size were observed, suggesting that bacterial doubling time might play an important role in benthic virus development.

Daubin, V. and H. Ochman. 2004. **Start-up entities in the origin of new genes.** Cur. Opin. Gen. Devel. 14:616-619. **Abstract:** The remarkable diversity in the contents of genomes raises questions about how new genes and new functions originate. Recent evidence indicates that parasitism, particularly the molecular interactions between phage and their bacterial hosts, is a likely mechanism for generating new genes. This invention of such novel functions seems to be founded on a strategy that secures the short-term survival of parasitic elements and thereby contributes to the renovation of gene repertoires in their host.

Debattista, J. 2004. **Phage therapy: where East meets West.** Exp. Rev. Anti-Infect. Ther. 2:815-819.

Dennehy, P. P. and P. E. Turner. 2004. **Reduced fecundity is the cost of cheating in RNA virus ϕ6.** Proc. R. oc. Lond. B Biol. Sci. 271:2275-2282. **Abstract:** Co-infection by multiple viruses affords opportunities for the evolution of cheating strategies to use intracellular resources. Cheating may be costly, however, when viruses infect cells alone. We previously allowed the RNA bacteriophage ϕ6 to evolve for 250 generations in replicated environments allowing coinfection of Pseudomonas phaseolicola bacteria. Derived genotypes showed great capacity to compete during co-infection, but suffered reduced performance in solo infections. Thus, the evolved viruses appear to be cheaters that sacrifice between-host fitness for within-host fitness. It is unknown, however, which stage of the lytic growth cycle is linked to the cost of cheating. Here, we examine the cost through burst assays, where lytic infection can be separated into three discrete phases (analogous to phage life history): dispersal stage, latent period (juvenile stage), and burst (adult stage). We compared growth of a representative cheater and its ancestor in environments where the cost occurs. The cost of cheating was shown to be reduced fecundity, because cheaters feature a significantly smaller burst size (progeny produced per infected cell) when infecting on their own. Interestingly, latent period (average burst time) of the evolved virus was much longer than that of the ancestor, indicating the cost does not follow a life history trade-off between timing of reproduction and lifetime fecundity. Our data suggest that interference competition allows high fitness of derived cheaters in mixed infections, and we discuss preferential encapsidation as one possible mechanism.

Fischer, C. R., M. Yoichi, H. Unno, and Y. Tanji. 2005. **The coexistence of *Escherichia coli* serotype O157:H7 and its specific bacteriophage in continuous culture.** FEMS Microbiol. Lett. 241:171-177. **Abstract:** For the development of phage therapy, systematic understanding mechanisms of bacteriophage resistance will be required. We describe a new strain of *Escherichia coli* O157:H7, named MuL, which stably co-exists with the O157:H7-specific lytic bacteriophage PP01. Chemostat cultures of *E. coli* O157:H7 infected with PP01 showed unchanging cell concentration, but phage concentrations which increased by 10^8 PFU mL^{-1}. However, the latent period, burst size, and growth rate of MuL were the same as in a PP01-susceptible strain. The binding rate of PP01 to the cell surface was diminished 8.5-fold in MuL. By observation of the binding of fluorescently labeled O157:H7-specific phage to individual MuL cells, we found that clonal MuL cultures were heterogeneous in their ability to bind bacteriophage. 15% of the MuL population was completely resistant to PP01 infection. MuL also co-existed with bacteriophages unrelated to PP01. Broad-range phage resistance by clonal heterogeneity represents a new class of bacteria-phage interactions.

Froissart, R., C. O. Wilke, R. Montville, S. K. Remold, L. Chao, and P. E. Turner. 2004. **Co-infection weakens selection against epistatic mutations in RNA viruses.** Genetics 168:9-19. **Abstract:** Co-infection may be beneficial in large populations of viruses because it permits sexual exchange between viruses that is useful in combating the mutational load. This advantage of sex should be especially substantial when mutations interact through negative epistasis. In contrast, co-infection may be detrimental because it allows virus complementation, where inferior genotypes profit from superior virus products available within the cell. The RNA bacteriophage phi6 features a genome divided into three segments. Co-infection by multiple phi6 genotypes produces hybrids containing reassorted mixtures of the parental segments. We imposed a mutational load on phi6 populations by mixing the wild-type virus with three single mutants, each harboring a deleterious mutation on a different one of the three virus segments. We then contrasted the speed at which these epistatic mutations were removed from virus populations in the presence and absence of co-infection. If sex is a stronger force, we predicted that the load should be purged faster in the presence of co-infection. In contrast, if complementation is more important we hypothesized that mutations would be eliminated faster in the absence of co-infection. We found that the load was purged

faster in the absence of co-infection, which suggests that the disadvantages of complementation can outweigh the benefits of sex, even in the presence of negative epistasis. We discuss our results in light of virus disease management and the evolutionary advantage of haploidy in biological populations.

Gamage, S. D., A. K. Patton, J. F. Hanson, and A. A. Weiss. 2004. **Diversity and host range of Shiga toxin-encoding phage. Infect.** Immun. 72:7131-7139. **Abstract:** Shiga toxin 2 (Stx2) from the foodborne pathogen *Escherichia coli* O157:H7 is encoded on a temperate bacteriophage. Toxin-encoding phages from C600::933W and from six clinical *E. coli* O157:H7 isolates were characterized for PCR polymorphisms, phage morphology, toxin production, and lytic and lysogenic infection profiles on O157 and non-O157 serotype *E. coli*. The phages were found to be highly variable, and even phages isolated from strains with identical pulsed-field gel electrophoresis profiles differed. Examination of cross-plaquing and lysogeny profiles further substantiated that each phage is distinct; reciprocal patterns of susceptibility and resistance were not observed and it was not possible to define immunity groups. The interaction between Shiga toxin-encoding phage and intestinal *E. coli* was examined. Lytic infection was assessed by examining Shiga toxin production following overnight incubation with phage. While not common, lytic infection was observed, with a more-than-1,000-fold increase in Stx2 seen in one case, demonstrating that commensal *E. coli* cells can amplify Shiga toxin if they are susceptible to infection by the Shiga toxin-encoding phages. Antibiotic-resistant derivatives of the Stx2-encoding phages were used to examine lysogeny. Different phages were found to lysogenize different strains of intestinal *E. coli*. Lysogeny was found to occur more commonly than lytic infection. The presence of a diverse population of Shiga toxin-encoding phages may increase the pathogenic fitness of *E. coli* O157:H7

Glud, R. N. and M. Middelboe. 2004. **Virus and bacteria dynamics of a coastal sediment: Implication for benthic carbon cycling.** Limnol. Oceanogr. 49:2073-2081. **Abstract:** We measured microbial heterotrophic activity, bacteria, and virus-like particle (VLP) abundance in homogenized, undiluted, and anoxic enclosures of sediment collected at a coastal station. The bacterial growth rate and VLP net production increased along with the respiratory activity in response to temperature. This suggests that VLPs represent a dynamic component of benthic microbial communities and that the net production of viriobenthos is regulated by the metabolic activity of bacteria. The abundance, net production, and decay rate of VLPs were significantly higher than those encountered in most pelagic systems. However, the rates were lower than the very few available potential rates (three studies) of viriobenthic activity, which all were obtained applying different slurry approaches. Our measurements support the general observation that virus abundance and production correlate with the trophic status of the environment and show that microbial activity can regulate the viriobenthic production in undiluted, homogenized marine sediments. The virus-induced bacterial mortality corresponded to similar to 20% of bacterial net production and similar to 2% h^{-1} of the total bacterial population. This is moderate compared with the results of most pelagic studies, and the associated leakage of lysates (dissolved organic carbon) only amounted to 4-8% of the produced dissolved inorganic carbon. Despite high standing stocks and relatively high turnover rates, VLP-induced bacterial lysis represented only a minor shunt in the benthic carbon cycle at the investigated site.

Goh, S., B. J. Chang, and T. V. Riley. 2005. **Effect of phage infection on toxin production by *Clostridium difficile*.** J. Med. Microbiol. 54:129-135. **Abstract:** Infection with *Clostridium difficile* and subsequent production of toxins A and B may result in *C. difficile*-associated diarrhoea and pseudomembranous colitis in hospital patients. The effect of four temperate phages, obtained by induction of clinical *C. difficile* isolates, on toxin production by *C. difficile* was determined. None of these phages converted a lysogenized non-toxigenic C. difficile strain to toxin production. One of the accessory toxin genes, tcdE, was detected in three phages, ϕC2, ϕC6 and ϕC8; however, the non-repeating regions of tcdA and tcdB encoding the enzymic domains were not carried on phage DNA. Phage infection of toxigenic strains increased toxin B production in four of six lysogens, although the level of tcdB transcription as determined by real-time RT-PCR was not significantly altered. However, levels of toxin A transcription in two lysogens were significantly altered without any corresponding differences in toxin A production.

Goodridge, L. D. 2004. **Bacteriophage biocontrol of plant pathogens: fact or fiction?** Trends in Biotechnology 22:384-385. **Abstract:** Bacterial resistance due to the misuse of antibiotics has become a global issue and alternative methods are being developed that might decrease the use of antimicrobials in

agricultural settings. Bacteriophage therapy represents a novel way to control the growth of plant-based bacterial pathogens. Although this method shows promise, a recent paper by Gill and Abedon has shown that the complex bacteriophage-host interactions in the plant environment must be investigated further.

Gorski, A. and B. Weber-Dabrowska. 2005. **The potential role of endogenous bacteriophages in controlling invading pathogens.** Cell Mol. Life Sci. 62:511-519. **Abstract:** Bacteriophages (phages) are omnipresent in our environment, and recent studies highlight their potential impact on the microbial world. Phages can also be present in mammalian organisms, including man (intestines, oral cavity, urine, sputum and serum). Data are available which suggest that those endogenous phages could play an important role in eliminating bacteria and regulating the body ecosystem. Furthermore, our most recent findings suggest that phages can exert immunosuppressive action in the gut, helping control local inflammatory and autoimmune reactions, and demonstrate anticancer activity. We hypothesize that phages could act in concert with the immune system in immunosurveillance against bacteria, viruses and cancer.

Griffith, J. F., S. B. Weisberg, and C. D. McGee. 2003. **Evaluation of microbial source tracking methods using mixed fecal sources in aqueous test samples.** Journal of water and health 1:141-151. **Abstract:** Microbiological source tracking (MST) methods are increasingly being used to identify fecal contamination sources in surface waters, but these methods have been subjected to limited comparative testing. In this study, 22 researchers employing 12 different methods were provided sets of identically prepared blind water samples. Each sample contained one to three of five possible fecal sources (human, dog, cattle, seagull or sewage). Researchers were also provided with portions of the fecal material used to inoculate the blind water samples for use as library material. No MST method that was tested predicted the source material in the blind samples perfectly. Host-specific PCR performed best at differentiating between human and non-human sources, but primers are not yet available for differentiating between all of the non-human sources. Virus and F+ coliphage methods reliably identified sewage, but were unable to identify fecal contamination from individual humans. Library-based isolate methods correctly identified the dominant source in most samples, but also had frequent false positives in which fecal sources not in the samples were incorrectly identified as being present. Among the library-based methods, genotypic methods generally performed better than phenotypic methods.

Hagens, S., A. Habel, U. von Ahsen, A. von Gabain, and U. Bläsi. 2004. **Therapy of experimental pseudomonas infections with a nonreplicating genetically modified phage.** Antimicrob. Agents Chemother. 48:3817-3822. **Abstract:** Bacteriophage therapy of bacterial infections has received renewed attention owing to the increasing prevalence of antibiotic-resistant pathogens. A side effect of many antibiotics as well as of phage therapy with lytic phage is the release of cell wall components, e.g., endotoxins of gram-negative bacteria, which mediate the general pathological aspects of septicemia. Here we explored an alternative strategy by using genetically engineered nonreplicating, nonlytic phage to combat an experimental *Pseudomonas aeruginosa* infection. An export protein gene of the *P. aeruginosa* filamentous phage Pf3 was replaced with a restriction endonuclease gene. This rendered the Pf3 variant (Pf3R) nonreplicative and concomitantly prevented the release of the therapeutic agent from the target cell. The Pf3R phage efficiently killed a wild-type host in vitro, while endotoxin release was kept to a minimum. Treatment of *P. aeruginosa* infections of mice with Pf3R or with a replicating lytic phage resulted in comparable survival rates upon challenge with a minimal lethal dose of 3. However, the survival rate after phage therapy with Pf3R was significantly higher than that with the lytic phage upon challenge with a minimal lethal dose of 5. This higher survival rate correlated with a reduced inflammatory response elicited by Pf3R treatment relative to that with the lytic phage. Therefore, this study suggests that the increased survival rate of Pf3R-treated mice could result from reduced endotoxin release. Thus, the use of a nonreplicating modified phage for the delivery of genes encoding proteins toxic to bacterial pathogens may open up a new avenue in antimicrobial therapy.

Herold, S., H. Karch, and H. Schmidt. 2004. **Shiga toxin-encoding bacteriophages—genomes in motion.** Int. J. Med. Microbiol. 294:115-121. **Abstract:** Shiga toxins (Stx) represent a group of bacterial toxins that are involved in human and animal disease. Stx are mainly produced by *Escherichia coli* isolated from human and non-human sources, *Shigella dysenteriae* type 1, and sporadically, by *Citrobacter freundii*, *Enterobacter cloacae* and *Shigella flexneri*. The genes encoding Stx are encoded in the genome of heterogeneous

lambdoid prophages (Stx-converting bacteriophages; Stx-phages). They are located in a similar position in the late region of the prophage genome and stx is under control of phage genes. Therefore, induction of Stx-converting prophages triggers increased production of Stx. Following induction, Stx-phages can infect other bacteria in vivo and in vitro. Stx-phages may be considered to represent highly mobile genetic elements that play an important role in the expression of Stx, in horizontal gene transfer, and hence in genome diversification.

Hewson, I. and J. A. Fuhrman. 2003. **Viriobenthos production and virioplankton sorptive scavenging.** Microb. Ecol. 46:337-347. **Abstract:** Virus production in oxic surface sediments and virioplankton sorption to suspended particles was estimated across three stations in the Southern California region (33. 4°N, 118.6°W). Viriobenthos production was estimated using a sterile sediment and filtered porewater dilution technique that targeted production from both attached bacteria and bacteria living free in the porewater, and attached bacteria alone. Potential virus production rates by bacteria free in the porewater ranged from 1.7 to 4.6 x 10^8 VLP cm^{-3} h^{-1}, while attached bacteria had slower potential production rates of between 0. 4 and 1.1 x 10^8 VLP cm^{-3} h^{-1}, suggesting turnover rates of viruses in sediments (1-5 h) which are significantly higher than those of virioplankton (similar to24-48 h). Virioplankton adsorbed to small (<150 mum) suspended sediments at stations with high ambient suspended solid concentrations. Virioplankton scavenging rates combined with published sedimentation rates demonstrate that this mechanism of virus arrival could only account for 0.01% of daily benthic virus production. Calculated mortality rates of benthic bacteria (4-14% h^{-1}) suggest viruses may play an important role in sediment carbon cycling.

Hewson, I., G. A. Vargo, and J. A. Fuhrman. 2003. **Bacterial diversity in shallow oligotrophic marine benthos and overlying waters: Effects of virus infection, containment, and nutrient enrichment.** Microb. Ecol. 46:322-336. **Abstract:** Little is known of the factors shaping sediment bacterial communities, despite their high abundance and reports of high diversity. Two factors hypothesized to shape bacterial communities in the water column are nutrient (resource) availability and virus infection. The role these factors play in benthic bacterial diversity was assessed in oligotrophic carbonate-based sediments of Florida Bay (USA). Sediment-water mesocosm enclosures were made from 1-m diameter clear polycarbonate cylinders which were pushed into sediments to 201 cm sediment depth enclosing similar to 80 L of water. Mesocosms were amended each day for 14 d with 10 muM NH4+ and 1 muM PO43-. In a second experiment, viruses from a benthic flocculent layer were concentrated and added back to flocculent layer samples which were collected near the mesocosm enclosures. Photosynthesis by microalgae in virus-amended incubations was monitored by pulse-amplitude modulated (PAM) fluorescence. In both experiments, bacterial diversity was estimated using automated rRNA intergenic spacer analysis (ARISA), a high-resolution fingerprinting approach. Initial sediment bacterial operational taxonomic unit (OTU) richness (236 +/- 3) was higher than in the water column (148 +/- 9), where an OTU was detectable when its amplified DNA represented >0.09% of the total amplified DNA. Effects on bacterial diversity and operational taxonomic unit (OTU) richness in nutrient-amended mesocosms may have been masked by the effects of containment, which stimulated OTU richness in the water column, but depressed OTU richness and diversity in sediments. Nutrient addition significantly elevated virus abundance and the ratio of viruses to bacteria ($p < 0.05$ for both) in the sediments, concomitant with elevated bacterial diversity. However, water column bacterial diversity (in unamended controls) was not affected by nutrient amendments, which may be due to rapid nutrient uptake by sediment organisms or adsorption of P to carbonate sediments. Addition of live viruses to benthic flocculent layer samples increased bacterial OTU diversity and richness compared with heat-killed controls; however, cluster analyses showed that the community structure in the virus-amended mesocosms varied greatly between replicates. Despite the strong effects upon eubacterial communities, photosynthesis of co-occurring protists and cyanobacteria was not significantly altered by the presence of virus concentrates. This study supports the hypothesis that nutrient availability plays a key role in shaping sediment bacterial communities, and also that viruses may regulate the abundance of the dominant competitors and allow less dominant organisms to maintain or increase their abundance in a community due to decreased competition for resources.

Hewson, I., S. R. Govil, D. G. Capone, E. J. Carpenter, and J. A. Fuhrman. 2004. **Evidence of *Trichodesmium* viral lysis and potential significance for biogeochemical cycling in the oligotrophic ocean.** Aquat. Microb. Ecol. 36:1-8. **Abstract:** The planktonic cyanobacterium *Trichodesmium* spp. is a

globally important diazotroph, fixing at least 80 Tg N yr^{-1} in tropical waters. Despite its biogeochemical importance, the mechanisms of *Trichodesmium* mortality, and the means by which its fixed N enters upper levels of the food web, are poorly understood. Potential virus-like particle (VLP) production by *Trichodesmium* spp. was observed in both culture (IMS101) and field samples from the tropical North Pacific Ocean, in oceanic waters around the Hawaiian Islands. VLP observed by TEM in IMS101 lysate were approximately 56 nm wide and untailed, and VLP with similar morphology were observed in tissue treated with mitomycin C. A most-probable number cultivation technique (utilizing bacterized cultures of *Trichodesmium*) detected moderate abundances of *Trichodesmium*-infecting cyanophage (605 to 9750 ml^{-1} infecting only 1 cultured strain) in 0.2 mum-filtered seawater samples from surface, subsurface and deep chlorophyll maximum samples. Estimation of mortality from virus production was not possible due to rapid VLP release in the first 6 h of dilution incubations. Rather, an indirect approach using burst size (determined from mitomycin C treatment of washed trichomes resuspended in virus-free seawater), decay rate of VLP (from latter part of virus production incubations) and average cyanophage titer was used to estimate mortality. These conservative calculations suggested that 0. 3 to 6.5 % d^{-1} (mean = 1.65 +/- 0. 99 % d^{-1}) of trichomes could potentially be lysed by viruses, representing the release of approximately 3 to 64 % fixed N d^{-1}. These estimates are based upon a steady-state maintenance of observed VLP abundance, which in nature could be from lysogeny, pseudolysogeny, carrier state, or successive lytic infection. Viral lysis therefore may represent a significant novel mechanism of N release from *Trichodesmium* spp., even in non-bloom conditions.

Hodgson, C. J., J. Perkins, and J. C. Labadz. 2004. **The use of microbial tracers to monitor seasonal variations in effluent retention in a constructed wetland.** Water Res. 38:3833-3844. **Abstract:** Effluent retention in a constructed wetland was determined using both microbial and chemical tracers. Seasonal variation in effluent retention was the main focus of the study. The biotracers used in the study were the coliphage MS2, a bacteriophage of *Enterobacter cloacae* and antibiotic resistant endospores of *Bacillus globigii*. Two separate tracer runs were conducted, Winter high flow (January 2002) and Summer low flow (June 2002). The three biotracers were evaluated simultaneously on both occasions, with the commonly used chemical tracer, rhodamine WT, a bright red fluorescent dye, being evaluated during the final experiment. The Winter tracer run was conducted during a typical Winter storm, with a mean effluent discharge of 4.1 ls^{-1}. Tracer recovery was 98% MS2, 91% *Ent. cloacae* phage and 2% endospore. Effluent retention was estimated at between 2 and 4 h at 90% phage tracer recovery. The Summer tracer run was conducted at a typical site operating discharge rate of 0.8 ls^{-1}. Tracer recovery was 23% MS2, 36% *Ent. cloacae* phage, 8% rhodamine and 14% for the endospores. Effluent retention was estimated at between 11 and 18 h at 90% of phage tracer recovery. Initial results are encouraging and indicate bacteriophage to have further potential as tracing agents in wetlands.

Hudson, J. A., C. Billington, G. Carey-Smith, and G. Greening. 2005. **Bacteriophages as biocontrol agents in food.** J. Food Prot. 68:426-437. **Abstract:** Bacteriophages possess attributes that appear to be attractive to those searching for novel ways to control foodborne pathogens and spoilage organisms. These phages have a history of safe use, can be highly host specific, and replicate in the presence of a host. Campylobacter, Salmonella, and Listeria monocytogenes and various spoilage organisms have responded to phage control on some foods. However, the use of phages as biocontrol agents is complicated by factors such as an apparent requirement for a threshold level of host before replication can proceed and by suboptimal performance, at best, at temperatures beneath the optimum for the host. This review is a summary of the information on these issues and includes brief descriptions of alternative phage-based strategies for control of foodborne pathogens.

Huff, W. E., G. R. Huff, N. C. Rath, J. M. Balog, and A. M. Donoghue. 2004. **Therapeutic efficacy of bacteriophage and Baytril (enrofloxacin) individually and in combination to treat colibacillosis in broilers.** Poult Sci 83:1944-1947. **Abstract:** A study was conducted to evaluate the therapeutic efficacy of bacteriophage and the antibiotic enrofloxacin individually and in combination to treat colibacillosis. The experimental design was a 2 x 2 x 2 factorial with 8 treatments and 4 replicate pens of 10 birds. The treatments were 1) control, 2) unchallenged birds treated with bacteriophage, 3) enrofloxacin, or 4) the combination; 5) birds challenged with *Escherichia coli*, and birds challenged with *E. coli* and treated with 6) bacteriophage, 7) enrofloxacin, or 8) the combination of bacteriophage and enrofloxacin. Birds in the *E. coli*

challenged treatments were challenged at 7 d of age by injecting 10^4 cfu of *E. coli* into the thoracic air sac. The antibiotic treatment was initiated immediately after the birds were challenged and consisted of 50 ppm enrofloxacin in the drinking water for 7 consecutive days. The bacteriophage treatment consisted of a single intramuscular injection of 2 different bacteriophage (10^9 pfu) administered immediately after the *E. coli* challenge. Mortality in the birds challenged with *E. coli* and untreated was 68%, and the bacteriophage and enrofloxacin treatments significantly decreased mortality to 15 and 3%, respectively. There was total protection in birds that received both the bacteriophage and enrofloxacin representing a significant synergy. The decrease in mortality with enrofloxacin (3%) was significantly better than the decrease in mortality with bacteriophage (15%). Airsacculitis lesion scores and lesion incidence in surviving birds were significantly less in the enrofloxacin treatment compared with the bacteriophage treatment. Both bacteriophage and enrofloxacin provided effective treatments of colibacillosis, and the synergy between these 2 treatments suggests that bacteriophage combined with antibiotic treatment has significant value

Johannessen, G. S., C. E. James, H. E. Allison, D. L. Smith, J. R. Saunders, and A. J. McCarthy. 2005. **Survival of a Shiga toxin-encoding bacteriophage in a compost model.** FEMS Microbiol. Lett. 245:369-375. **Abstract:** Bacteriophages that carry the Shiga toxin gene (*stx*) represent an additional hazard in cattle manure-based fertilizers in that their survival could lead to toxigenic conversion of *Escherichia coli* and other bacteria post-composting. A Stx-phage in which the Shiga toxin (*stx*2) gene was inactivated by insertion of a chloramphenicol resistance gene was used in combination with a rifampicin-resistant *E. coli* host where RecA is constitutively activated so that all infectious phage particles could be enumerated by plaque assay. PCR-based confirmation methods and the additional application of a host enrichment protocol ensured that very low numbers of surviving bacteriophage could be detected and unequivocally identified. Stx-bacteriophage numbers declined rapidly over the first 48h and none could be detected after 3 days. The host enrichment method was applied after 6 days and no bacteriophages were recovered. While addition of fresh *E. coli* cells at intervals after the compost temperature had reduced below 40°C demonstrated that *E. coli* growth could be supported in the compost, Stx-phages or their lysogens were never detected. Here, we demonstrate that composting animal manure for 40 days during which a temperature of >60°C is maintained for at least 5 days is effective at removing both *E. coli* and a model infectious *Stx*-encoding bacteriophage.

Keller, R., R. F. Passamani-Franca, F. Passamani, L. Vaz, S. T. Cassini, N. Sherrer, K. Rubim, T. D. Sant'Ana, and R. F. Goncalves. 2004. **Pathogen removal efficiency from UASB + BF effluent using conventional and UV post-treatment systems.** Water Sci. Technol. 50:1-6. **Abstract:** The aim of this study was to verify the efficiency of removal of microorganisms in effluents of a Wastewater Treatment Plant (WWTP) comprising an association of a UASB reactor followed by three submerged aerated biofilters (BAF) and one tertiary filter. The WWTP designed to treat domestic wastewater from a population of 1,000 inhabitants showed high removal efficiency for organic matter and suspended solids. Helminth eggs were also efficiently removed from the tertiary effluent and were found in the sludge from the UASB reactor; however, removal of bacteria in this system was very low. To enhance the efficiency of the system, the effluent from tertiary filters was submitted to UV disinfection in a real scale reactor. Our results showed that UV irradiation was very effective at lowering the concentrations of *E. coli*, thermotolerant coliforms and coliphages to acceptable levels for agricultural reuse. *Salmonella* spp. and helminth eggs were seeded into the tertiary effluent before passing through the UV reactor. *Salmonella* was not found in the final effluent, but helminth eggs were not completely inactivated by UV irradiation and viable eggs were detected after 28 d of incubation.

Lisle, J. T. and J. C. Priscu. 2004. **The occurrence of lysogenic bacteria and microbial aggregates in the lakes of the McMurdo Dry Valleys, Antarctica.** Microb. Ecol. 47:427-439. **Abstract:** The McMurdo Dry Valleys of Antarctica form the coldest and driest ecosystem on Earth. Within this region there are a number of perennially ice-covered (3-6 m thick) lakes that support active microbial assemblages and have a paucity of metazoans. These lakes receive limited allochthonous input of carbon and nutrients, and primary productivity is limited to only 6 months per year owing to an absence of sunlight during the austral winters. In an effort to establish the role that bacteria and their associated viruses play in carbon and nutrient cycling in these lakes, indigenous bacteria, free bacteriophage, and lysogen abundances were determined. Total bacterial abundances (TDC) ranged from 3.80×10^4 to 2.58×10^7 cells mL^{-1} and virus-like particle (VLP) abundances ranged from 2.26×10^5 to 5.56×10^7 VLP mL^{-1}. VLP abundances were significantly correlated (P

< 0.05) with TDC, bacterial productivity (TdR), chlorophyll a (Chl a), and soluble reactive phosphorus (SRP). Lysogenic bacteria, determined by induction with mitomycin C, made up between 2.0% and 62.5% of the total population of bacteria when using significant decreases and increases in TDC and VLP abundances, respectively, and 89.5% when using increases in VLP abundances as the sole criterion for a successful induction event. The contribution of viruses released from induced lysogens contributed <0.015% to the total viral production rate. Carbohydrate and protein based organic aggregates were abundant within the water column of the lakes and were heavily colonized by bacteria and VLPs. Alkaline phosphatase activity was detected within the matrix of the aggregates, implying phosphorus deficiency and consortial nutrient exchanges among microorganisms

Lisle, J. T., J. J. Smith, D. D. Edwards, and G. A. McFeters. 2004. **Occurrence of microbial indicators and *Clostridium perfringens* in wastewater, water column samples, sediments, drinking water, and Weddell seal feces collected at McMurdo Station, Antarctica.** Appl. Environ. Microbiol. 70:7269-7276. **Abstract:** McMurdo Station, Antarctica, has discharged untreated sewage into McMurdo Sound for decades. Previous studies delineated the impacted area, which included the drinking water intake, by using total coliform and *Clostridium perfringens* concentrations. The estimation of risk to humans in contact with the impacted and potable waters may be greater than presumed, as these microbial indicators may not be the most appropriate for this environment. To address these concerns, concentrations of these and additional indicators (fecal coliforms, *Escherichia coli*, enterococci, coliphage, and enteroviruses) in the untreated wastewater, water column, and sediments of the impacted area and drinking water treatment facility and distribution system at McMurdo Station were determined. Fecal samples from Weddell seals in this area were also collected and analyzed for indicators. All drinking water samples were negative for indicators except for a single total coliform-positive sample. Total coliforms were present in water column samples at higher concentrations than other indicators. Fecal coliform and enterococcus concentrations were similar to each other and greater than those of other indicators in sediment samples closer to the discharge site. *C. perfringens* concentrations were higher in sediments at greater distances from the discharge site. Seal fecal samples contained concentrations of fecal coliforms, *E. coli*, enterococci, and *C. perfringens* similar to those found in untreated sewage. All samples were negative for enteroviruses. A wastewater treatment facility at McMurdo Station has started operation, and these data provide a baseline data set for monitoring the recovery of the impacted area. The contribution of seal feces to indicator concentrations in this area should be considered.

Lopez, R. 2004. ***Streptococcus pneumoniae* and its bacteriophages: one long argument.** Int. Microbiol. 7:163-171. **Abstract:** Infectious diseases currently kill more than 15 million people annually, and the WHO estimates that every year 1.6 million people die from pneumococcal diseases. *Streptococcus pneumoniae* (pneumococcus), a bacterium with a long biological pedigree, best illustrates the rapid evolution of antibiotic resistance, which has led to major public health concern. This article discusses the molecular basis of the two main virulence factors of pneumococcus, the capsule and cell-wall hydrolases, as well as new approaches to developing medicinal weapons for preventing pneumococcal infections. In addition, current knowledge regarding pneumococcal phages as potential contributors to virulence and the use of lytic enzymes encoded by these phages as therapeutic tools is reviewed.

Lucena, F., A. E. Duran, A. Moron, E. Calderon, C. Campos, C. Gantzer, S. Skraber, and J. Jofre. 2004. **Reduction of bacterial indicators and bacteriophages infecting faecal bacteria in primary and secondary wastewater treatments.** J. Appl. Microbiol. 97 :1069-1076. **Abstract:** AIMS: To compare the suitability of various bacterial and viral indicators to assess the removal of faecal micro-organisms by primary and secondary wastewater treatment processes. METHODS AND RESULTS: The numbers of several bacterial indicators [faecal coliforms (FC), enterococci (ENT) and sulphite-reducing clostridia (SRC)] and bacteriophages (somatic coliphages, F-specific RNA phages and bacteriophages infecting *Bacteroides fragilis* strain RYC2056) were determined in incoming raw sewage and effluents from various primary and secondary wastewater treatment processes in several geographical areas. Reductions in the numbers of indicators were calculated as $\log_{10}$ reductions. Processes based on removal and mild disinfection, showed no significant differences in the elimination of any of the indicators tested or between geographical areas. In contrast, treatment processes that include strong microbial inactivation, such as lime-aided flocculation and lagooning, showed significant differences between the $\log_{10}$ reductions of the various micro-organisms

studied, FC showing the highest reduction and spores of SRC and phages infecting *B. fragilis* the lowest. CONCLUSIONS: The microbial elimination performance of treatment processes based principally on removal and mild disinfection can be evaluated with a single indicator. In contrast, processes with additional disinfecting capabilities require more than one indicator for accurate evaluation of the treatment; bacteriophages are good candidates for use as second indicators. SIGNIFICANCE AND IMPACT OF THE STUDY: Bacteriophages provide additional information for the evaluation of microbial elimination in some treatment plants. The easy, fast and cheap methods available for phage determination are feasible both in industrialized and developing countries.

Luther, K. and R. Fujioka. 2004. **Usefulness of monitoring tropical streams for male-specific RNA coliphages.** Journal of water and health 2:171-181. **Abstract:** The objective of this study was to evaluate the usefulness of monitoring streams in Hawaii for FRNA coliphages as a reliable indicator of sewage contamination. This study was undertaken as a result of our previous findings that monitoring streams in Hawaii for traditional faecal indicator bacteria (faecal coliform, *Escherichia coli*, enterococci) was not useful in determining when streams are contaminated with sewage, because environmental (soil) sources rather than sewage accounted for the high concentrations of faecal bacteria in streams. Two perennial streams, sewage and soil samples were monitored for traditional faecal indicator bacteria (faecal coliform, *E. coli*, enterococci) and FRNA coliphages. The results showed that sewage treatment processes and disinfection drastically reduced the concentrations of traditional faecal indicator bacteria but FRNA coliphages were still present in significant concentrations in the treated sewage effluents. These results indicate that monitoring sewage effluents and environmental waters for only traditional faecal indicator bacteria may not be adequately protective of human health effects. Ambient concentrations of traditional faecal indicator bacteria in soil and streams of Hawaii were consistently high but consistently low for FRNA coliphages, indicating that monitoring streams of Hawaii for FRNA coliphages can be used to determine when streams are contaminated with sewage.

Madera, C., C. Monjardin, and J. E. Suarez. 2004. **Milk contamination and resistance to processing conditions determine the fate of *Lactococcus lactis* bacteriophages in dairies.** Appl. Environ. Microbiol. 70:7365-7371. **Abstract:** Milk contamination by phages, the susceptibility of the phages to pasteurization, and the high levels of resistance to phage infection of starter strains condition the evolution dynamics of phage populations in dairy environments. Approximately 10% (83 of 900) of raw milk samples contained phages of the quasi-species c2 (72%), 936 (24%), and P335 (4%). However, 936 phages were isolated from 20 of 24 (85%) whey samples, while c2 was detected in only one (4%) of these samples. This switch may have been due to the higher susceptibility of c2 to pasteurization (936-like phages were found to be approximately 35 times more resistant than c2 strains to treatment of contaminated milk in a plate heat exchanger at 72 degrees C for 15 s). The restriction patterns of 936-like phages isolated from milk and whey were different, indicating that survival to pasteurization does not result in direct contamination of the dairy environment. The main alternative source of phages (commercial bacterial starters) does not appear to significantly contribute to phage contamination. Twenty-four strains isolated from nine starter formulations were generally resistant to phage infection, and very small progeny were generated upon induction of the lytic cycle of resident prophages. Thus, we postulate that a continuous supply of contaminated milk, followed by pasteurization, creates a factory environment rich in diverse 936 phage strains. This equilibrium would be broken if a particular starter strain turned out to be susceptible to infection by one of these 936-like phages, which, as a consequence, became prevalent.

McAlister, M., H. Aranha, and R. Larson. 2004. **Use of bacteriophages as surrogates for mammalian viruses.** Developments in biologicals 118:89-98. **Abstract:** The threat of viral contamination is common to all processes using biological products of animal or human origin. Therefore, demonstration of virus clearance (i. e. validation of virus removal and/or inactivation steps) is of utmost importance to the biopharmaceutical industries. Ultimately, virus clearance studies should show that any virus removal/inactivation stage incorporated into the manufacturing process not only removes or inactivates known viruses that may be conceivably present (e.g. from cell banks and source materials), but also other viruses that may be introduced adventitiously (e.g. by addition of supplements downstream of the manufacturing process). In this paper, we outline the shared properties of mammalian viruses and similar sized bacteriophages, and factors that may influence the virus clearance process. We also present test data

from filtration studies, showing similar titre reductions for both types of virus. We propose that well-characterised bacteriophage, such as PP7 and PR772 can be used as models for mammalian viruses if the virus removal mechanism is based on size exclusion.

Mendez, J., A. Audicana, M. Cancer, A. Isern, J. Llaneza, B. Moreno, M. Navarro, M. L. Tarancon, F. Valero, F. Ribas, J. Jofre, and F. Lucena. 2004. **Assessment of drinking water quality using indicator bacteria and bacteriophages.** Journal of water and health 2:201-214. **Abstract:** Bacterial indicators and bacteriophages suggested as potential indicators of water quality were determined by public laboratories in water from springs, household water wells, and rural and metropolitan water supplies in north-eastern Spain. Indicator bacteria were detected more frequently than bacteriophages in springs, household water wells and rural water supplies. In contrast, positive bacteriophage detections were more numerous than those of bacteria in metropolitan water supplies. Most of the metropolitan water supply samples containing indicators had concentrations of chlorine below 0.1 mg l^{-1}, their indicator loads resembling more closely those of rural water supplies than any other samples taken from metropolitan water supplies. The number of samples from metropolitan water supplies containing more than 0.1 mg l^{-1} of chlorine that contained phages clearly outnumbered those containing indicator bacteria. Some association was observed between rainfall and the presence of indicators. Sediments from service reservoirs and water from dead ends in the distribution network of one of the metropolitan water supplies were also tested. Bacterial indicators and phages were detected in a higher percentage than in samples of tap water from the same network. Additionally, indicator bacteria were detected more frequently than bacteriophages in sediments of service reservoirs and water from dead end samples. We conclude that naturally occurring indicator bacteria and bacteriophages respond differently to chlorination and behave differently in drinking water distribution networks. Moreover, this study has shown that testing for the three groups of phages in routine laboratories is easy to implement and feasible without the requirement for additional material resources for the laboratories.

Mi, B., C. Eaton, J. H. Kim, C. K. Colvin, J. C. Lozier, and B. J. Marinas. 2004. **Removal of biological and non-biological viral surrogates by spiral-wound reverse osmosis membrane elements with intact and compromised integrity.** Water Res. 38:3821-3832. **Abstract:** The removal of bacteriophage MS2 and fluorescent-dyed polystyrene microspheres with intact and purposely compromised spiral-wound RO membrane elements was investigated. MS2 rejection with intact membrane elements was >99. 9995%. A model developed for data evaluation revealed that the advective passage of MS2 through imperfections of intact membrane elements was $<2 \times 10^{-5}$% of the overall product water flow produced. The advective passage of MS2 and microspheres through a pinhole induced in one of the elements was 0.05-0.1% of the overall product water flow. Prolonged testing of both intact and compromised elements resulted in increased MS2 rejection corresponding to advective MS2 passage through membrane imperfections of $<3 \times 10^{-7}$% of the overall product water flow. The permeate flow rate obtained with an element with a larger pinhole was 5-13% greater than that of the intact element, and the corresponding rejection of MS2 and microspheres was similar to that observed for sodium chloride. The use of a cracked o-ring in the connection of the permeate tube to the element vessel end-cup resulted in advective passage of MS2 through the crack of <0.0001% of the overall permeate flow.

Middelboe, M., R. N. Glud, and K. Finster. 2003. **Distribution of viruses and bacteria in relation to diagenetic activity in an estuarine sediment.** Limnol. Oceanogr. 48:1447-1456. **Abstract:** The distribution of viruses and bacteria was investigated in relation to bacterial sulfate reduction and total respiration (production of dissolved inorganic carbon, [DIC]) in a coastal sediment. Viral and bacterial abundance ranged from about 0.5×10^8 to 8×10^8 viruses cm^{-3} and 0.1×10^8 to 4×10^8 bacteria cm^{-3} in the upper 16 cm of the sediment and showed large and systematic changes within scales of a few centimeters. In general, viral abundance was highest in the sediment surface (0-1 em); however subsurface peaks at 3-5 cm depth associated with increased diagenetic activity were also observed. The virus-bacterium ratio ranged from 1. 4 to 7.8 and increased significantly with depth in the upper 6 cm ($P < 0.001$). Viral abundance showed significant positive correlation with both bacterial abundance and activity (P much less than 0.001), suggesting that the distribution and abundance of viruses were closely coupled to the activity of the bacterial community and that viruses are produced by bacteria within the sediment. The significant coupling between viral abundance and sulfate reduction rate and DIC production is the first indication of viral production associated with diagenetic active bacteria in marine sediments. This coupling between viral abundance and

bacterial activity and the distinct pattern of vertical distribution show that viruses are a dynamic component of the benthic community. The morphological analysis indicated that interstitial viral communities were dominated by long (>1 mum) filamentous forms with a helical symmetry. Several types of these filamentous forms were observed, as well as a variety of tailed forms with icosahedral symmetry. Filamentous forms are rarely found in the water column, which suggests that they are adapted to the benthic environment and specific to the interstitial hosts.

Middelboe, M., L. Riemann, G. L. Steward, V. Hansen, and O. Nybroe. 2003. **Virus-induced transfer of organic carbon between marine bacteria in a model community.** Aquat. Microb. Ecol. 33:1-10. **Abstract:** Viral lysis results in the transformation of living cells into dissolved and colloidal organic matter referred to as lysate. When viruses are included in food web models it is generally assumed that lysates are readily metabolized by bacteria in the community. We hypothesized that the production of lysate by viruses could also influence microbial community composition by mediating the diversification of carbon sources. To test this hypothesis, we established simple model communities containing various combinations of 2 marine bacteria (Cellulophaga sp. and Photobacterium sp.) and 2 viruses (one specific to each bacterial type) grown in a seawater-based medium with lactose as the sole carbon source. This medium supported vigorous growth of Cellulophaga sp. but not of Photobacterium sp. In control experiments, where Photobacterium sp. was cultured with either Cellulophaga sp. or Cellulophaga-specific virus, the biomass of Photobacteriurn sp. increased by 50% or less. In contrast, the Photobacterium sp. biomass significantly increased by 8-fold ($p < 0.001$, $n = 3$) in co-cultures with the Cellulophaga sp. virus-host pair. These data indicate that the substrate supporting growth by Photobacteriurn sp. was primarily Cellulophaga lysate and not material introduced with the host and virus inocula nor material secreted by Cellulophaga during normal growth. Estimates of the trophic transfer suggested that 28% of the Cellulophaga sp. lysate was converted into new bacterial biomass, which indicated that at least 62% of the lysate was metabolized by Photo bacterium sp. Our results from this simple marine model community illustrate that the activity of a virus-host system can effect the transfer of organic material from one bacterial type to another whose growth would otherwise be limited by a lack of suitable substrates.

Miedzybrodzki, R., W. Fortuna, B. Weber-Dabrowska, and A. Gorski. 2005. **Bacterial viruses against viruses pathogenic for man?** Virus Research 110:1-8. **Abstract:** In this review, we discuss possible models of bacteriophage-virus interactions. The first is based on the mechanism by which phages may interact indirectly with viruses. Its essence is that bacteriophage-derived nucleic acid may inhibit pathogenic virus infection. It seems that this phenomenon can be partly explained on the basis of interferon induction. We also discuss a study by Borecky's group (conducted over two decades ago) which provided some clinical data on the effectiveness of the application of native bacteriophage RNA in the treatment of viral infections. The second interaction model is based on the direct competition of bacteriophages and viruses for cellular receptors for viral cell-entry. The use of bacteriophages as inducers or displayers of antibodies with antiviral action is considered as the third model. In this part of the article, we also discuss other data and hypotheses on conceivable interactions between bacterial and animal viruses. ¶ As our current supply of antiviral drugs is quite limited, using natural agents such as bacteriophages as a weapon against pathogenic viruses could be an attractive and cost-efficient alternative, and further studies are urgently needed to test this possibility.

Mooijman, K. A., Z. Ghameshlou, M. Bahar, J. Jofre, and A. H. Havelaar. 2005. **Enumeration of bacteriophages in water by different laboratories of the European Union in two interlaboratory comparison studies.** J. Virol. Meth. **Abstract:** As part of a European project on bacteriophages in bathing waters two interlaboratory comparison studies were carried out (May 1997 and March 1998). During these studies phage reference materials as well as naturally polluted standard samples were analysed in 16 European laboratories. Three groups of bacteriophages were tested using standardised methods: somatic coliphages, F-specific RNA-phages and phages of *Bacteroides fragilis*. Many of the participating laboratories applied one or more of the phage methods for the first time, after a one-week training session in a central laboratory. Nevertheless, the values of repeatability (r=1. 35-1. 38 calculated on log10-scale) and reproducibility (R=1.52-2.04 calculated on log10-scale) when analysing phage reference materials were close to the theoretical optimum for a Poisson distribution. When analysing the naturally polluted samples more variation in results within and between laboratories was found (r=1.63-2. 34; R=3.10-5.72), in comparison with the results obtained with the pure phage reference materials.

Müller-Merbach, M., T. Rauscher, and J. Hinrichs. 2005. **Inactivation of bacteriophages by thermal and high-pressure treatment.** Int. Dairy J. **Abstract:** Dairy companies commonly experience fermentation failures due to bacteriophages that are spread mainly by milk, whey or air. Heat or high-pressure treatment may potentially reduce the phage titre, but further knowledge about the inactivation kinetics is desirable. Inactivation experiments were carried out with the commonly occurring lactococcal phages P001 and P008. Phage suspensions in calcium-enriched M17-broth were heated at 55-80 °C, or high-pressure treated at up to 600 MPa. Kinetic analysis showed that the order of inactivation reaction was above 1; thus, inactivation kinetics were approximated by a non-linear regression model. The Arrhenius parameters, rate constant, *k*(*p, T*), and activation energy, *E*A (for heat treatments), and the volume of activation, □*V*# (for pressure treatments) were calculated. Both measured and calculated results indicate that phage P008 was the more heat- and pressure-resistant of the two. By combining the results from heat and pressure inactivations, a pressure-temperature diagram for phage P008 was established.

Nicolle, P. 1979. [**About of the therapy with the bacteriophages**]. Bull. Acad. Med. Paris 163:58-60.

Obradovic, A., Jones, J. B., Momol, M. T., Balogh, B., Olson, S. M. 2004. **Management of tomato bacterial spot in the field by foliar applications of bacteriophages and SAR inducers.** Plant Disease 88:736-740. **Abstract:** Various combinations of the harpin protein, acibenzolar-*S*-methyl, and bacteriophages were compared for controlling tomato bacterial spot in field experiments. Harpin protein and acibenzolar- *S*-methyl were applied every 14 days beginning twice before transplanting and then an additional four applications throughout the season. Formulated bacteriophages were applied prior to inoculation followed by twice a week at dusk. A standard bactericide treatment, consisting of copper hydroxide plus mancozeb, was applied once prior to inoculation and then every 7 days, while untreated plants served as an untreated control. Experiments were conducted in north and central Florida fields during fall 2001, spring 2002, and fall 2002. In three consecutive seasons, acibenzolar-*S*-methyl applied in combination with bacteriophage or bacteriophage and harpin significantly reduced bacterial spot compared with the other treatments. However, it did not significantly affect the total yield compared with the standard or untreated control. Application of host-specific bacteriophages was effective against the bacterial spot pathogen in all three experiments, providing better disease control than copper-mancozeb or untreated control. When results of the disease severity assessments or harvested yield from the bacteriophage-treated plots were grouped and compared with the results of the corresponding nonbacteriophage group, the former provided significantly better disease control and yield of total marketable fruit.

Olson, M. R., R. P. Axler, and R. E. Hicks. 2004. **Effects of freezing and storage temperature on MS2 viability.** J. Virol. Meth. 122:147-152. **Abstract:** Monitoring human enteric virus levels in domestic wastewater effluent is crucial to protecting human health. Occasionally, during intensive sampling, wastewater samples must be stored for later viral analysis. Little data exist regarding how enteric viruses survive during storage at different temperatures in secondary treated wastewater. During a field-scale study assessing pathogen removal performance by various onsite treatment technologies, the MS2 bacteriophage, an indicator of enteric viruses, was inoculated into septic tank (STE), sand filter, peat filter and constructed wetland (CW) effluents to determine virus decay at various storage temperatures. Virus stored at temperatures ≥10°C and at -20°C decayed nearly twice as fast as those stored at 4°C or -80°C. Decreased water quality decreased viral decay rates at 4°C and -80°C, with slowest decay occurring in STE and the fastest in sterile PBS and low pH peat effluent. In CW effluent after 8 days, less MS2 was inactivated when stored at 4°C (20%) compared to -80°C (58%); however, during extended storage (approximately 300 days), less MS2 was inactivated at -80°C (75%) compared to 4°C (93%). We recommend that viruses in wastewater be stored in the dark at 4°C unless storage for >40 days is necessary.

Øvreås, L., D. Bourne, R. -A. Sandaa, E. O. Casamayer, S. Bnlloch, V. Goddard, G. Smerdon, M. Heldal, and T. F. Thingstad. 2003. **Response of bacterial and viral communities to nutrient manipulations in seawater mesocosms.** Aquat. Microb. Ecol. 31:109-121. **Abstract:** Changes in natural bacterial and viral assemblages were studied in seawater mesocosms manipulated with inorganic (nitrate + phosphate) and inorganic + organic (glucose) nutrient additions. As inferred from the gel band patterns obtained by DGGE, only moderate changes within the bacterial community took place when mineral nutrients were added alone.

Supplementing the mineral nutrients with glucose in excess of what the bacteria could consume led, however, to major changes in band patterns. Based on fluorescence in situ hybridisation (FISH), the major bacterial response was identified as an increase in the population of gamma-Proteobacteria with a smaller response in alpha-Proteobacteria. Sequencing of bands from the DGGE gels indicated that glucose + mineral nutrients led to a Vibrio-dominated bacterial community. A specific FISH probe was designed from a band sequence affiliated to Vibrio splendidus, and linked a large-celled bacterial morphotype to the DGGE-gel bands dominating in glucose-amended mesocosms. A similar difference in the response of the viral populations among treatments was demonstrated using pulsed field gel electrophoresis (PFGE). The number of bands on DGGE gels and PFGE gels were similar (mean ratio 0. 98). We suggest an interpretation of these results where coexistence of nutrient-competing bacterial hosts is controlled by viral lysis. We also suggest that the success of large bacteria in glucose-replete treatments was not based on superior glucose-utilisation abilities, but rather on an advantage in competition for limiting mineral nutrients derived from the combination of a large cell surface with a low cellular content of the limiting element, possible for cells with large C-rich inclusion bodies.

Pantucek, R., J. Doskar, V. Ruzicková, P. Kaspárek, E. Orácová, V. Kvardová, and S. Rosypal. 2004. **Identification of bacteriophage types and their carriage in *Staphylococcus aureus*.** Archives in Virology 149:1689-1703. **Abstract:** Conserved genomic sequences distinctive of *Staphylococcus aureus* phage types 3A, 11, 77, 187 and Twort, representative of phage serogroups A, B, F, L and D, were identified and characterized. PCR primers designed for the above sequences were used for development of a multiplex PCR assay which enabled us not only to classify all phages of the International Typing Set plus 16 additional phages, but also to detect prophages in *S. aureus* genomes. One to four different prophages were unambiguously detected in experimentally lysogenized *S. aureus* strains, and substantial variation in prophage content was found in 176 *S. aureus* clinical strains of different provenance. In addition, by using a comparative genomics approach, all the prophages in the *S. aureus* genomes sequenced to date could be revealed and classified.

Paul, J. H. and M. B. Sullivan. 2005. **Marine phage genomics: what have we learned?** Curr. Opin. Biotechnol. **Abstract:** Marine phages are the most abundant and diverse form of life on the planet, and their genomes have been described as the largest untapped reservoir of genomic information. To date, however, the complete genome sequences of only 17 marine phage are known. Nevertheless, these genomes have revealed some interesting features, including the presence of photosynthetic genes in cyanophage and common patterns of genomic organization. Intriguing findings are also being made from studies of the uncultivated marine viral community genome ('metavirome'). The greatest challenge in interpreting the biology of these phages, and for making comparisons with their terrestrial counterparts, is the high proportion of unidentifiable open reading frames (60%). Future studies are likely to focus on sequencing more marine phage genomes from disparate hosts and diverse environments and on further basic studies of the biology of existing marine phages.

Payne, M., J. Oakey, and L. Owens . 2004. **The ability of two different *Vibrio* spp. bacteriophages to infect *Vibrio harveyi*, *Vibrio cholerae* and *Vibrio mimicus*.** J. Appl. Microbiol. 97:663-672. **Abstract:** AIMS: To determine the host range of the *Vibrio harveyi* myovirus-like bacteriophage (VHML) and the cholera toxin conversion bacteriophage (CTX Phi) within a range of *Vibrio cholerae* and *V. mimicus* and *V. harveyi*, *V. cholerae* and *V. mimicus* isolates respectively. METHODS AND RESULTS: Three *V. harveyi*, eight *V. cholerae* and five *V. mimicus* isolates were incubated with VHML and CTX Phi. Polymerase chain reaction (PCR) was used to determine the presence of VHML and CTX Phi in infected isolates. We demonstrated that it was possible to infect one isolate of *V. cholerae* (isolate ACM #2773/ATCC #14035) with VHML. This isolate successfully incorporated VHML into its genome as evident by positive PCR amplification of the sequence coding part of the tail sheath of VHML. Attempts to infect all other *V. cholerae* and *V. mimicus* isolates with VHML were unsuccessful. Attempts to infect *V. cholerae* non-01, *V. harveyi* and *V. mimicus* isolates with CTX Phi were unsuccessful. CONCLUSIONS: Bacteriophage infection is limited by bacteriophage-exclusion systems operating within bacterial strains and these systems appear to be highly selective. One system may allow the co-existence of one bacteriophage while excluding another. VHML appears to have a narrow host range which may be related to a common receptor protein in such strains. The lack of the vibrio pathogenicity island bacteriophage (VPI Phi) in the isolates used in this study may

explain why infections with CTX Phi were unsuccessful. SIGNIFICANCE AND IMPACT OF THE STUDY: The current study has demonstrated that *Vibrio* spp. bacteriophages may infect other *Vibrio* spp.

Sachs, J. L. and J. J. Bull. 2005. **Experimental evolution of conflict mediation between genomes.** Proc. Natl. Acad. Sci. USA 102:390-395. **Abstract:** Transitions to new levels of biological complexity often require cooperation among component individuals, but individual selection among those components may favor a selfishness that thwarts the evolution of cooperation. Biological systems with elements of cooperation and conflict are especially challenging to understand because the very direction of evolution is indeterminate and cannot be predicted without knowing which types of selfish mutations and interactions can arise. Here, we investigated the evolution of two bacteriophages (f1 and IKe) experimentally forced to obey a life cycle with elements of cooperation and conflict, whose outcome could have ranged from extinction of the population (due to selection of selfish elements) to extreme cooperation. Our results show the de novo evolution of a conflict mediation system that facilitates cooperation. Specifically, the two phages evolved to copackage their genomes into one protein coat, ensuring cotransmission with each other and virtually eliminating conflict. Thereafter, IKe evolved such extreme genome reduction that it lost the ability to make its own virions independent of f1. Our results parallel a variety of conflict mediation mechanisms existing in nature: evolution of reduced genomes in symbionts, cotransmission of partners, and obligate coexistence between cooperating species.

Sano, E., S. Carlson, L. Wegley, and F. Rohwer. 2004. **Movement of viruses between biomes.** Appl. Environ. Microbiol. 70:5842-5846. **Abstract:** Viruses are abundant in all known ecosystems. In the present study, we tested the possibility that viruses from one biome can successfully propagate in another. Viral concentrates were prepared from different near-shore marine sites, lake water, marine sediments, and soil. The concentrates were added to microcosms containing dissolved organic matter as a food source (after filtration to allow 100-kDa particles to pass through) and a 3% (vol/vol) microbial inoculum from a marine water sample (after filtration through a 0. 45-μm-pore-size filter). Virus-like particle abundances were then monitored using direct counting. Viral populations from lake water, marine sediments, and soil were able to replicate when they were incubated with the marine microbes, showing that viruses can move between different ecosystems and propagate. These results imply that viruses can laterally transfer DNA between microbes in different biomes.

Schwalbach, M. S., I. Hewson, and J. A. Fuhrman. 2004. **Viral effects on bacterial community composition in marine plankton microcosms.** Aquat. Microb. Ecol. 34:117-127. **Abstract:** Recent theory suggests that viruses influence bacterial community composition by killing the winners of resource competition. We tested aspects of this theory by growing natural marine bacteria communities in seawater microcosms that had either significantly reduced or increased virus abundances and monitoring changes in the bacterial communities. Bacterial community composition was assayed by 2 whole-community fingerprinting techniques, terminal restriction fragment length polymorphisms (TRFLP) and automated ribosomal intergenic spacer analysis (ARISA), at the beginning and end of experiments to examine the effect of changes in viral abundance on bacterial community composition. Our results suggest that changes in viral abundances have mixed effects on microbial community fingerprints. Modest, but statistically significant, changes in community fingerprints were seen when most viruses were removed by filtration and bacteria subsequently grown over 5 d compared to growth at normal virus density. There were no significant differences between community fingerprints from microcosms grown with a normal versus 3-fold density of viruses over 2 d (possibly because of slow growth rates); however, significant changes occurred over time, regardless of virus abundance, suggesting that manipulation and containment alone had a strong influence on community fingerprints, which may have masked some virus effects. Also, moderate natural variation in composition between replicate microcosms made it difficult to distinguish statistically significant virus effects. Given that relatively few significant changes were apparent in community fingerprints between virus treatments at the end of our experiments, it is possible that current models of virus infection and the possible roles of viruses in controlling community composition need re-evaluation. The persistence of high viral abundance and apparent high turnover, in concert with our results, suggests that viruses and their hosts may have a more stable coexistence than is now currently thought. However, it is possible that the modest effects of viral infection observed in this short-term study could be significantly amplified over longer time-scales of

weeks or months, resulting in viruses having a more substantial influence on bacterial community composition.

Serwer, P., S. J. Hayes, S. Zaman, K. Lieman, M. Rolando, and S. C. Hardies. 2004. **Improved isolation of undersampled bacteriophages: finding of distant terminase genes.** Virology 329:412-424. **Abstract:** Isolation and characterization of new environmental bacteriophages are performed for (1) analyzing microbial evolution and ecology and (2) delivering biological therapy. The sampling of environmental bacteriophages appears, however, to be limited by the procedure (usually liquid enrichment culture) used to propagate them. An alternative, less competitive procedure is developed here for the purpose of isolating new bacteriophages. This procedure involves extraction directly into and then propagation in a dilute agarose gel. Adaptations of this procedure are used to avoid repeated isolation of the same bacteriophage. Some newly isolated bacteriophages grow so poorly that they appear inaccessible to liquid enrichment culture. Four comparatively high titer bacteriophages were isolated and characterized by a genomic sequence survey. Some had genomes with extremely distant relationships to those of other bacteriophages, based on a tree built from the large terminase genes. These methods find novel genomes by rapidly isolating and screening diverse bacteriophages.

Short, C. M. and C. A. Suttle. 2005. **Nearly identical bacteriophage structural gene sequences are widely distributed in both marine and freshwater environments.** Appl. Environ. Microbiol. 71:480-486. **Abstract:** Primers were designed to amplify a 592-bp region within a conserved structural gene (g20) found in some cyanophages. The goal was to use this gene as a proxy to infer genetic richness in natural cyanophage communities and to determine if sequences were more similar in similar environments. Gene products were amplified from samples from the Gulf of Mexico, the Arctic, Southern, and Northeast and Southeast Pacific Oceans, an Arctic cyanobacterial mat, a catfish production pond, lakes in Canada and Germany, and a depth of ca. 3,246 m in the Chuckchi Sea. Amplicons were separated by denaturing gradient gel electrophoresis, and selected bands were sequenced. Phylogenetic analysis revealed four previously unknown groups of g20 clusters, two of which were entirely found in freshwater. Also, sequences with >99% identities were recovered from environments that differed greatly in temperature and salinity. For example, nearly identical sequences were recovered from the Gulf of Mexico, the Southern Pacific Ocean, an Arctic freshwater cyanobacterial mat, and Lake Constance, Germany. These results imply that closely related hosts and the viruses infecting them are distributed widely across environments or that horizontal gene exchange occurs among phage communities from very different environments. Moreover, the amplification of g20 products from deep in the cyanobacterium-sparse Chuckchi Sea suggests that this primer set targets bacteriophages other than those infecting cyanobacteria.

Sickbert-Bennett, E. E., D. J. Weber, M. F. Gergen-Teague, M. D. Sobsey, G. P. Samsa, and W. A. Rutala. 2005. **Comparative efficacy of hand hygiene agents in the reduction of bacteria and viruses.** Am. J. Infect. Contr. 33:67-77. **Abstract:** BACKGROUND: Health care-associated infections most commonly result from person-to-person transmission via the hands of health care workers. METHODS: We studied the efficacy of hand hygiene agents (n = 14) following 10-second applications to reduce the level of challenge organisms (*Serratia marcescens* and MS2 bacteriophage) from the hands of healthy volunteers using the ASTM-E-1174-94 test method. RESULTS: The highest log 10 reductions of *S marcescens* were achieved with agents containing chlorhexidine gluconate (CHG), triclosan, benzethonium chloride, and the controls, tap water alone and nonantimicrobial soap and water (episode 1 of hand hygiene, 1.60-2.01; episode 10, 1.60-3.63). Handwipes but not alcohol-based handrubs were significantly inferior from these agents after a single episode of hand hygiene, but both groups were significantly inferior after 10 episodes. After a single episode of hand hygiene, alcohol/silver iodide, CHG, triclosan, and benzethonium chloride were similar to the controls in reduction of MS2, but, in general, handwipes and alcohol-based handrubs showed significantly lower efficacy. After 10 episodes, only benzethonium chloride (1. 33) performed as well as the controls (1.59-1.89) in the reduction of MS2. CONCLUSIONS: Antimicrobial handwashing agents were the most efficacious in bacterial removal, whereas waterless agents showed variable efficacy. Alcohol-based handrubs compared with other products demonstrated better efficacy after a single episode of hand hygiene than after 10 episodes. Effective hand hygiene for high levels of viral contamination with a nonenveloped virus was best achieved by physical removal with a nonantimicrobial soap or tap water alone.

Sillankorva, S., R. Oliveira, M. J. Vieira, I. Sutherland, and J. Azeredo. 2004. ***Pseudomonas fluorescens* infection by bacteriophage PhiS1: the influence of temperature, host growth phase and media.** FEMS Microbiol. Lett. 241 :13-20. **Abstract:** The influence of host growth temperature, phase and media, together with the effect of infection temperature on bacteriophage PhiS1 infection of *Pseudomonas fluorescens* were examined. The rates of cell lysis and phage release were determined and showed that the efficacy of phage infection was optimal with host cells grown and infected at 26°C. The host physiological state also affected these rates. Infection was dependent on the presence of cell wall proteins with molecular weights of 17.5+/-1 and 99+/-5 kDa.

Simboli, N., H. Takiff, R. McNerney, B. Lopez, A. Martin, J. C. Palomino, L. Barrera, and V. Ritacco. 2005. **In-house phage amplification assay is a sound alternative for detecting rifampin-resistant *Mycobacterium tuberculosis* in low-resource settings.** Antimicrob. Agents Chemother. 49:425-427. **Abstract:** An in-house mycobacteriophage amplification assay for detecting rifampin-resistant *Mycobacterium tuberculosis* showed 100% sensitivity, 97.7% specificity, and 95.2% predictive value for resistance in a test of 129 isolates from a hot spot area of multidrug-resistant *M. tuberculosis*. The applicability of the test was demonstrated in the routine work flow of a low-resource reference laboratory.

Tanner, B. D., S. Kuwahara, C. P. Gerba, and K. A. Reynolds. 2004. **Evaluation of electrochemically generated ozone for the disinfection of water and wastewater.** Water Sci. Technol. 50:19-25. **Abstract:** Effective wastewater treatment is critical to public health and well-being. This is especially true in developing countries, where disinfection of wastewater is frequently inadequate. People who live in these areas may benefit from wastewater disinfection using ozone. This study evaluated the ability of a new electrochemical process of ozone generation, which produced ozone continuously at high pressure and concentration by the electrolysis of water, to disinfect tap water and secondarily treated wastewater. Inactivation of *Klebsiella terrigena*, *Escherichia coli*, MS2 bacteriophage and poliovirus 1 was evaluated first in reverse osmosis (RO) treated water. Inactivation of *K. terrigena* (6-log), *E. coli* (6-log), MS2 (6-log) and poliovirus 1 (>3-log) was observed after 1 min of ozonation in a 1 L batch reactor. Experiments were then performed to assess the microbiological impact of disinfection using ozone on secondarily treated municipal wastewater. The effect of ozonation on wastewater was determined for total and faecal coliforms, bacteriophages and heterotrophic plate count (HPC) bacteria. Electrochemical ozone generators provided an effective, rapid and low-cost method of wastewater disinfection. Based on the results of this research, electrochemically generated ozone would be well suited to remote, small-scale, disinfection operations and may provide a feasible means of wastewater disinfection in developing countries.

Thyrhaug, R., A. Larsen, T. F. Thingstad, and G. Bratbak. 2003. **Stable coexistence in marine algal host-virus systems.** Mar. Ecol. Prog. Ser. 254:27-35. **Abstract:** All microalgal host-virus systems isolated to date are lytic: the viruses lyse the infected hosts within hours after infection. Moreover, current models of phytoplankton host-virus interactions predict rapid extinction of both host and virus. Nevertheless, marine phytoplankton and their respective viruses do coexist in marine ecosystems. To investigate this apparent paradox we performed a series of experiments which show that phytoplankton populations always recover after virus-induced lysis and that endemic viral infections may promote survival of the host population. We hypothesize that phenotypic plasticity of algal susceptibility to viral infection makes such coexistence of host and virus possible.

Tucker, C. P. and M. W. Heuzenroeder. 2004. **ST64B is a defective bacteriophage in *Salmonella enterica* serovar Typhimurium DT64 that encodes a functional immunity region capable of mediating phage-type conversion.** Int. J. Med. Microbiol. 294:59-63. **Abstract:** The *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S. Typhimurium*) defective bacteriophage ST64B has a putative immunity (immC) region consisting of cI, cro and cII-like genes. Since ST64B is widespread in *S. Typhimurium*, studies were undertaken to determine whether this region might be functional and influence phage typing results. Cloning of ST64B immC-like genes and their subsequent expression in *S. Typhimurium* DTs showed that this region is able to mediate phage-type conversion in DTs 41 and 44. This confirms the functionality of the immC region and the patterns of lysis produced by phage typing are consistent with the predicted mechanism of action of the encoded protein products.

Vieu, J. F., F. Guillermet, R. Minck, and P. Nicolle. 1979. [**New trends in therapeutic use of bacteriophages**]. Bull. Acad. Med. Paris 163:61-66.

Vinje, J., S. J. G. Oudejans, J. R. Stewart, M. D. Sobsey, and S. C. Long. 2004. **Molecular detection and genotyping of male-specific coliphages by reverse transcription-PCR and reverse line blot hybridization.** Appl. Environ. Microbiol. 70:5996-6004. **Abstract:** In recent years, there has been increased interest in the use of male-specific or F+ coliphages as indicators of microbial inputs to source waters. Sero- or genotyping of these coliphages can also be used for microbial source tracking (MST). Among the male-specific coliphages, the F+ RNA (FRNA) viruses are well studied, while little is known about the F+ DNA (FDNA) viruses. We have developed a reverse line blot hybridization (RLB) assay which allows for the simultaneous detection and genotyping of both FRNA as well as FDNA coliphages. These assays included a novel generic duplex reverse transcription-PCR (RT-PCR) assay for FRNA viruses as well as a generic PCR for FDNA viruses. The RT-PCR assays were validated by using 190 field and prototype strains. Subsequent DNA sequencing and phylogenetic analyses of RT-PCR products revealed the classification of six different FRNA clusters, including the well-established subgroups I through IV, and three different FDNA clusters, including one (CH) not previously described. Within the leviviruses, a potentially new subgroup (called JS) including strains having more than 40% nucleotide sequence diversity with the known levivirus subgroups (MS2 and GA) was identified. We designed subgroup-specific oligonucleotides that were able to genotype all nine (six FRNA, three FDNA) different clusters. Application of the method to a panel of 351 enriched phage samples from animal feces and wastewater, including known prototype strains (MS2, GA, Qβ, M11, FI, and SP for FRNA and M13, f1, and fd for FDNA), resulted in successful genotyping of 348 (99%) of the samples. In summary, we developed a novel method for standardized genotyping of F+ coliphages as a useful tool for large-scale MST studies

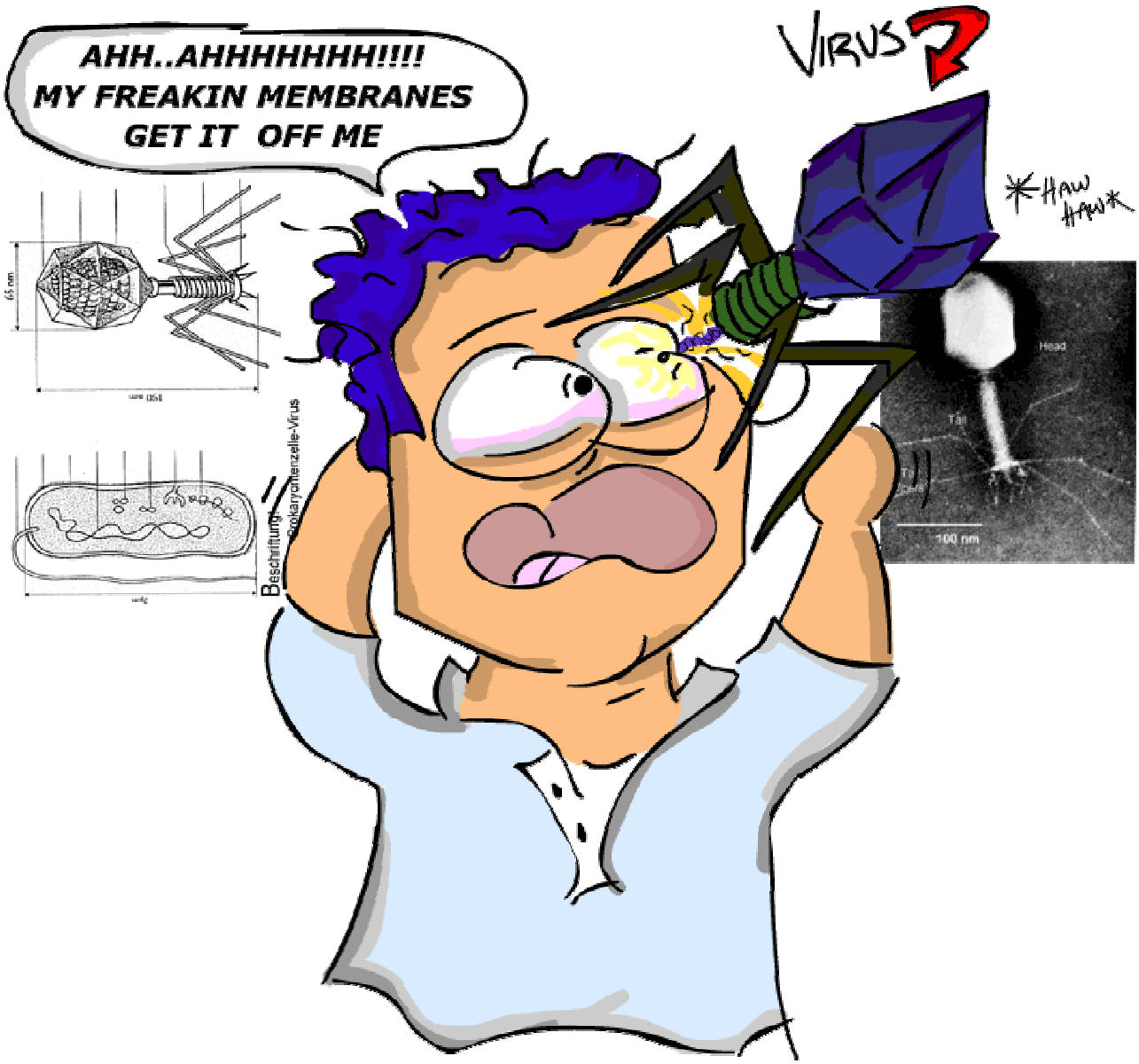
Webb, J. S., M. Lau, and S. Kjelleberg. 2004. **Bacteriophage and phenotypic variation in *Pseudomonas aeruginosa* biofilm development.** J. Bacteriol. 186:8066-8073. **Abstract:** A current question in biofilm research is whether biofilm-specific genetic processes can lead to differentiation in physiology and function among biofilm cells. In *Pseudomonas aeruginosa*, phenotypic variants which exhibit a small-colony phenotype on agar media and a markedly accelerated pattern of biofilm development compared to that of the parental strain are often isolated from biofilms. We grew *P. aeruginosa* biofilms in glass flow cell reactors and observed that the emergence of small-colony variants (SCVs) in the effluent runoff from the biofilms correlated with the emergence of plaque-forming Pf1-like filamentous phage (designated Pf4) from the biofilm. Because several recent studies have shown that bacteriophage genes are among the most highly upregulated groups of genes during biofilm development, we investigated whether Pf4 plays a role in SCV formation during *P. aeruginosa* biofilm development. We carried out immunoelectron microscopy using anti-Pf4 antibodies and observed that SCV cells, but not parental-type cells, exhibited high densities of Pf4 filaments on the cell surface and that these filaments were often tightly interwoven into complex latticeworks surrounding the cells. Moreover, infection of *P. aeruginosa* planktonic cultures with Pf4 caused the emergence of SCVs within the culture. These SCVs exhibited enhanced attachment, accelerated biofilm development, and large regions of dead and lysed cells inside microcolonies in a manner identical to that of SCVs obtained from biofilms. We concluded that Pf4 can mediate phenotypic variation in *P. aeruginosa* biofilms. We also performed partial sequencing and analysis of the Pf4 replicative form and identified a number of open reading frames not previously recognized in the genome of *P. aeruginosa*, including a putative postsegregational killing operon.

Wick, L. M., W. Qi, D. W. Lacher, and T. S. Whittam. 2005. **Evolution of genomic content in the stepwise emergence of *Escherichia coli* O157:H7.** J. Bacteriol. 187:1783-1791. **Abstract:** Genome comparisons have demonstrated that dramatic genetic change often underlies the emergence of new bacterial pathogens. Evolutionary analysis of *Escherichia coli* O157:H7, a pathogen that has emerged as a worldwide public health threat in the past two decades, has posited that this toxin-producing pathogen evolved in a series of steps from O55:H7, a recent ancestor of a nontoxigenic pathogenic clone associated with infantile diarrhea. We used comparative genomic hybridization with 50-mer oligonucleotide microarrays containing probes from both pathogenic and nonpathogenic genomes to infer when genes were acquired and lost. Many ancillary virulence genes identified in the O157 genome were already present in an O55:H7-like progenitor, with 27 of 33 genomic islands of >5 kb and specific for O157:H7 (O islands) that were acquired intact before the split

from this immediate ancestor. Most (85%) of variably absent or present genes are part of prophages or phage-like elements. Divergence in gene content among these closely related strains was approximately 140 times greater than divergence at the nucleotide sequence level. A >100-kb region around the O-antigen gene cluster contained highly divergent sequences and also appears to be duplicated in its entirety in one lineage, suggesting that the whole region was cotransferred in the antigenic shift from O55 to O157. The beta-glucuronidase-positive O157 variants, although phylogenetically closest to the Sakai strain, were divergent for multiple adherence factors. These observations suggest that, in addition to gains and losses of phage elements, O157:H7 genomes are rapidly diverging and radiating into new niches as the pathogen disseminates.

Withey, S., E. Cartmell, L. M. Avery, and T. Stephenson. 2005. **Bacteriophages—potential for application in wastewater treatment processes.** Science of Total Environment 339:1-18. **Abstract:** Bacteriophages are viruses that infect and lyse bacteria. Interest in the ability of phages to control bacterial populations has extended from medical applications into the fields of agriculture, aquaculture and the food industry. Here, the potential application of phage techniques in wastewater treatment systems to improve effluent and sludge emissions into the environment is discussed. Phage-mediated bacterial mortality has the potential to influence treatment performance by controlling the abundance of key functional groups. Phage treatments have the potential to control environmental wastewater process problems such as: foaming in activated sludge plants; sludge dewaterability and digestibility; pathogenic bacteria; and to reduce competition between nuisance bacteria and functionally important microbial populations. Successful application of phage therapy to wastewater treatment does though require a fuller understanding of wastewater microbial community dynamics and interactions. Strategies to counter host specificity and host cell resistance must also be developed, as should safety considerations regarding pathogen emergence through transduction.

Xu, J., R. W. Hendrix, and R. L. Duda. 2004. **Conserved translational frameshift in dsDNA bacteriophage tail assembly genes.** Molecular cell 16:11-21. **Abstract:** A programmed translational frameshift similar to frameshifts in retroviral gag-pol genes and bacterial insertion elements was found to be strongly conserved in tail assembly genes of dsDNA phages and to be independent of sequence similarities. In bacteriophage lambda, this frameshift controls production of two proteins with overlapping sequences, gpG and gpGT, that are required for tail assembly. We developed bioinformatic approaches to identify analogous -1 frameshifting sites and experimentally confirmed our predictions for five additional phages. Clear evidence was also found for an unusual but analogous -2 frameshift in phage Mu. Frameshifting sites could be identified for most phages with contractile or noncontractile tails whose length is controlled by a tape measure protein. Phages from a broad spectrum of hosts spanning Eubacteria and Archaea appear to conserve this frameshift as a fundamental component of their tail assembly mechanisms, supporting the idea that their tail genes share a common, distant ancestry.



Cartoon used with permission, courtesy of Daniel Chin, http://argh.tekkom.net