© Phage *et al.*

Bacteriophage Ecology Group (BEG) News

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

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October 1, 2003 issue (volume 18)

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Editorial

Phage T1: A Lambdoid Phage with Attitude?

by Andrew M. Kropinski

Coliphage T1, one of the original seven T phages suggested by Max Delbrück (3, 4) for concentrated study by the bacteriophage community, has been sequenced. This phage, which the International Council for the Taxonomy of Viruses (ICTV) has treated as a species within the "T1-like viruses" genus (family Siphoviridae), possesses a polyhedral head approximately 60 nm in diameter with a characteristically long (150 nm) flexible noncontractile tail. Other phages which may be part of this genus are: UC-1 (11), D20, Hi, 102, 103, 150, 168, and 174 (7). To this we can now add the TolC-specific phage TLS - previously known as U3 in many laboratories (5).

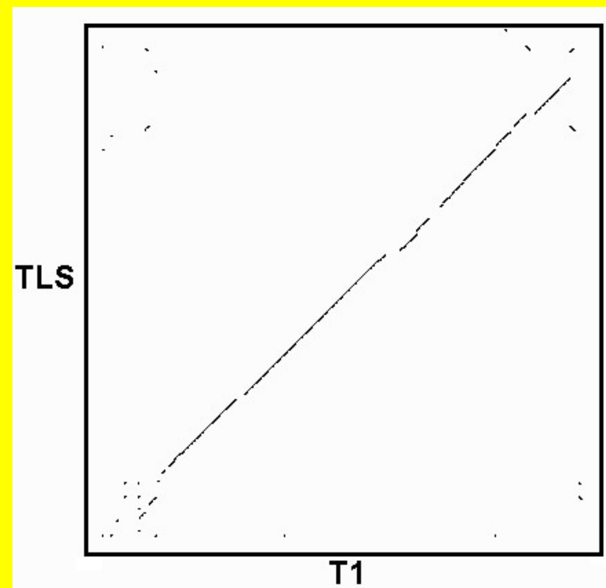
Initially made famous by its use as the selective agent in the famous fluctuation test conducted by Salvador Luria and Max Delbrück (12)[A], T1 gained notoriety because of its resistance to desiccation and its virulence. Unsubstantiated horror stories exist about its effect on industrial laboratories employing fermentations involving *Escherichia coli*[B]. Furthermore, while its potential impact was long appreciated by the phage community, the increase in molecular studies by biologists/biochemists unaware of its virulence has often resulted in unwanted infections. Today many biotech firms market T1-resistant competent cells (i.e. strains carrying a *tonA* marker; e.g. Cambio, Epicentre, Invitrogen).

Unfortunately research on this interesting and important virus largely languished after the mid 1980s, and prior to the current project the only T1 sequence data to be found in GenBank is for two genes one of which encodes a DNA N-6-adenine-methyltransferase (dam) (19). [We have found that this sequence (GenBank Accession No. BAA94133) contains an internal inframe deletion]. T1 sequence data has also inadvertently ended up in GenBank. A sequence reported to encode a European squid (Loligo forbesi) neurofilament-like protein (X66695) is, in fact, T1 sequence. The sequence of T1 has now been completed (18) revealing many of the secrets of this interesting virus. In addition, Drs. Gregory German and Rajeev Misra (Department of Microbiology, Arizona State University) have completed the sequence of phage TLS (6). In the following paragraphs I will briefly summarize some of the common properties of these two viruses.

The Phage T1 Genome

Previous studies on T1 DNA indicated that the genome size was in the order of 48.5 kb with a terminal redundancy of approximately 2800 bp (13). Sequencing has actually shown that the T1 genome size is 50.7 kb with terminal repeats of 1.9 kb. Phage TLS is about the same size (50.9 kb) but possesses shorter (1 kb) terminal repeats. While these two phages differ somewhat in their overall base composition: T1 is 45.6 mol%G+C while TLS is 42.7 G+C their genomes

show considerable overall sequence similarity as illustrated by the following Dotplot. Major differences in sequence and genes occur at the ends of the two genomes.

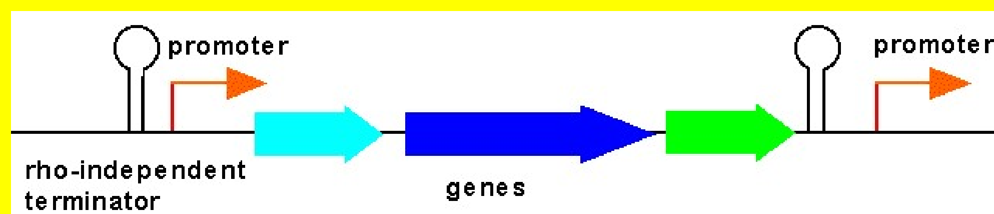


It has long been known that T1 DNA is insensitive to *Eco*BI [TGA(N8)TGCT] and *Eco*KI [AAC(N6)GTGC] type I restriction endonucleases. The reason for this has been revealed to be a complete lack of these site in the DNA. Phage TLS DNA has 13 *Eco*BI sites and a single *Eco*KI site. While it is unknown how this phage responds to these restriction endonucleases, German and Misra have evidence that TLS encodes a protein which inhibits type I restriction enzymes.

The T1 genome harbours 77 ORFs while that of TLS has 86. As suggested by the Dotplot results and confirmed by protein alignments many of the genes are similar. One significant difference is the finding that TLS encodes both a Dam and a Dcm (N-5-cytosine methyltransferase) methylase.

The work of Bourque and Christensen (2), employing host temperature-sensitive DNA replication mutants, showed that DNA polymerase III, DNA primase (DnaG) and clamp-loading protein (DnaX) were required for T1 replication, while replisome-organizer protein DnaA, helicase-loading protein DnaC and replicative DNA helicase DnaB were not. Sequencing has revealed the T1/TLS encode their own helicases, primases and single-stranded DNA-binding proteins. The origin for replication occurs, as it does in Salmonella phage P22, within the helicase gene. In addition, both phages contain RecE and Erf homologs which are part, in the case of T1, of a general recombination system termed "grn."

In coliphage early transcription involves host holo-RNA polymerase recognition of promoters which contain variants of the canonical hexamers (-35 TTGACA; -10 TATAAT) separated by 15-19 bp (15). While T1 contains many incidences of this type of promoter sequence its molecular approach to transcription is unusual, particularly within the morphogenesis genes. The late region is divided up into a series of transcriptional modules (transcriptons; Figure below) containing RpoD-dependent promoters and perhaps enhancers and is flanked by rho-independent terminators. The latter differ from those of coliphage T4 by lacking a UUCG or GNRA loop sequence (16).



Both T1 and TLS possess numerous 21 nt direct repeats located in the intergenic regions or overlapping the translational terminators of the preceding genes. While their high AT content is reminiscent of UP-elements in *E. coli* (10), their position suggests that they may function in a manner equivalent to eukaryotic enhancers. This transcriptional model differs fundamentally from that displayed by coliphage HK022 (Q-mediated transcriptional read-through) (8) or T7 (multiple phage RNA polymerases-specific promoters) and may account for the short latent period of 13 minutes observed with coliphage T1 (1, 3, 17).

Excluding the genes for the terminase subunits phage T1 has 23 genes which are most probably involved in morphogenesis. SDS-PAGE analysis has shown that the T1 virion is composed of 13-15 structural proteins (14, 20, 21) while TLS preparations contains fewer structural proteins. As part of the analysis of coliphage T1, Dr. Nancy Martin (Queen's University) analyzed the T1 proteome by two-dimensional gel electrophoresis/mass spectrometry. [She would be most interested in discussing potential collaborative phage proteomic projects with interested members of the phage community]. Packaging occurs in a headful manner from pac sites which have been localized in TLS to a 60 bp region which contains six tandem repeats of GATT(T/r) [G. German, personal communication (6)]. The analogous packaging site in T1 contains five adjacent repeats of ATATA.

With a couple of exceptions T1/TLS proteins display low sequence similarity to other phage proteins in the databases. The exceptions are the lysis proteins which possess 40% amino acid identity with lysozymes of *Escherichia coli* prophage CP-933K, and *Salmonella typhimurium* PS119 and PS34; and, the tail assembly genes. The latter, T1 genes 38 to 31, are homologous to N15 genes 16 to 23. In addition, both phages code for Cor homologs! Within this cluster are four proteins encoded by linked genes which have been implicated in tail cone assembly (9). The latter are related to similar genes in other members of the *Siphoviridae* infecting, or carried by, members of the class gamma-Proteobacteria including *Burkholderia thailandensis* phage phiE125 (22), and coliphages HK97, HK022, N15 and phi80. All of the latter phages

are classified as lambda-like viruses at NCBI Taxonomy Browser (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/) suggesting that, at a higher phylogenetic level, phages T1 and TLS might be said to be part of the order lambda within the Siphoviridae.

While many of the mysteries of T1 have been revealed through analysis of its genome sequence there are still many unanswered questions. Research contemplated or in progress will analyze of the temporal expression of the T1 genome, its regulation, and the role of the 21 nt direct repeats. How the host genome is degraded remains a mystery, and in light of the number of proteins potentially involved in morphogenesis the latter deserves further experimentation. Lastly, we have the universal phage genome question: what is the function of the 53% of the ORFs which failed to result in a BLAST hit?

For those who would like a preview look at the annotated T1 sequence data please visit: http://microimm.queensu.ca/Phage/.

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[A]At the time known as phage α. See pp. 482 and 483 of Abedon (2000) for a brief history of the original T set of coliphages.

[B]Knowledge of phage T1�s desiccation resistance likely forms the basis of the famous "Phage in a Letter" urban legend, which apparently has since morphed into "Phage M13 in a letter." M13 is also a desiccation-resistant phage, but one which few have rejected from their laboratories perhaps because M13 is relatively avirulent and otherwise popular as a platform for protein display. See: http://www.panix.com/~iayork/phage.shtml or http://www.urbanlegends.com/science/phage.html for popular discussion of the "Phage in a Letter" urban legend.

Editorial Archive

- BEG: What we are, Where we are, Where we're going by Stephen T. Abedon
- When Grown *In Vitro*, do Parasites of Multicellular Organisms (MOPs) become Unicellular Organism Parasites (UOPs)? by Stephen T. Abedon
- Bacteriophages as Model Systems by Stephen T. Abedon
- 2000 and Sun: A Phage Odyssey by Stephen T. Abedon

- Lytic, Lysogenic, Temperate, Chronic, Virulent, Quoi? by Stephen T. Abedon
- Which Ecology are You? by Stephen T. Abedon
- *Science* NetWatch October 13, 2000
- The Best of Times, the Worst of Times by Ry Young
- Naming Bacteriophages by Hans-Wolfgang Ackermann and Stephen T. Abedon
- The Bacteriophage Rise by Stephen T. Abedon
- Mathematics for Microbiologists by Stephen T. Abedon
- Shipping Phages by Hans-Wolfgang Ackermann
- Calling a Phage a "Phage" by Stephen T. Abedon
- Phage or Phages by Hans-Wolfgang Ackermann
- The Phage Manifesto by Ry Young
- The Félix d'Hérelle Phage Center Changes Hands by Hans-Wolfgang Ackermann
- Phage T4 Meets Microbial Diversity by Jim D. Karam
- Phage T1: A lambdoid phage with attitude? by Andrew Kropinski

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

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New BEG Members

Please welcome our newest members

name (home page links)	status	e-mail	address
Naomi Hoyle	---	naomisulinger @hotmail.com	Lab 1, The Evergreen State College, Olympia, WA 98505
	interests:	My main interests are in phages for people. I am currently working with *Pseudomonas* phages. I plan to become a Naturopathic Doctor and incorporate phages into my medical practice. (contents | BEG members | top of page)	
Stefan Miller	PI	stefan.miller @profos.de	PROFOS AG, Josef-Engert Str. 9 D-93053 Regensburg, Germany
	interests:	Biotechnological application of bacteriophages and bacteriophage-proteins.(contents | BEG members | top of page)	
Matthew Robison	---	tex12011 @hotmail.com	3138 Overhulse Rd NW I81, Olympia WA. 98502
	interests:	(contents | BEG members | top of page)	

The BEG members page can be found at www.phage.org/beg_members.htm. There are two ways of "joining" BEG. One, the "traditional" way, is to have your name listed on the web page and on the list server. The second, the "non-traditional" way, is to have your name only listed on the list server. The latter I refer to as "non-members" on that list. Members, e.g., individuals listed on the BEG members list page, should be limited to individuals who are actively involved in science (research, instruction, outreach, industry) and who can serve as a phage ecology resource to interested individuals. If you have an interest in phage ecology but no real expertise in the area, then you should join as a non-member. To join as a member, please contact BEG using the following link: microdude+@osu.edu . Include:

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

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

Meetings

Please send photos, etc. from meetings, etc. for inclusion in this section.

Evergreen International Phage Meeting

Click here for images from this year□s meeting (July, 2003).

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

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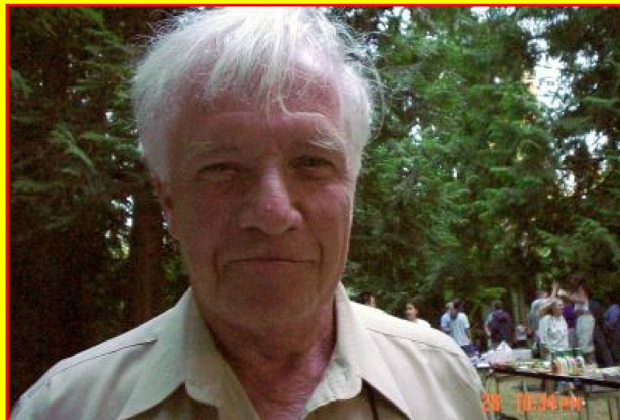
Submissions

Some Images of BEG Members

Steve Abedon



Hans Ackermann

Jason Gill

Naomi Hoyle

Michael McShan

David Prangishvili

Frederic Ropiquet

For more images, particularly from the 2003 Evergreen meeting, see http://www.phage.org/images/evergreen_2003_images/.

Submissions Archive

- On an Invisible Microbe Antagonistic to the Dysentery Bacillus by Felix d'Herelle
- Obituary: Hansjürgen Raettig - Collector of Bacteriophage References (October 12, 1911 - December 1, 1997)
- Some Quotations
- Bacteriophages: A Model System for Human Viruses
- How Big is 10^{30}?
- Selling Phage Candy
- A List of Phage Names
- An Expanded Overview of Phage Ecology
- Rendering Phage Heads
- The Contractile-Tail Sheath, In Three Dimensions
- Eye On The Needle: Phage T4 Puncturing Point May Answer Penetrating Questions
- Pioneering genetic researcher Gisela Mosig dies
- Updated Eiserling T4 Virion
- Some Recent Phage and Phage-Related U.S. Patents (1976-present)
- Some Images of BEG Members

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

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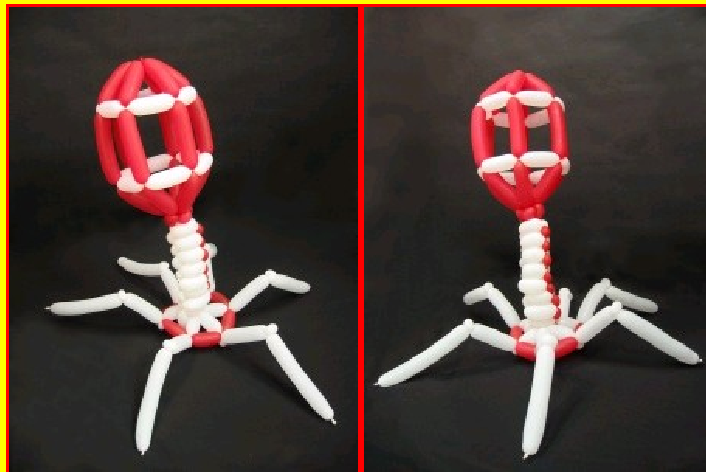
Phage Images



Click on images for larger view

Left to right: Jan Craven, phage T4, Larry Goodridge, Celeste O'Neil (the artist)

Phage Image Archive

- BEG Phage Images Page
- The Face of the Phage
- Bacteriophage T2
- SSV1-Type Phage
- Saline Lake Bacteriophage
- Coliphage LG1
- Bacteriophage HK97
- Phage T4 (art)
- Phage T4 on the pedestal outside of Barker Hall at Berkeley
- Electron micrograph of phage P22
- Thin section of T4 phages hitting a microcolony of *E. coli* K-12
- T4 phage v1
- T4 Tail Model
- Gingerbread phage
- T4 adsorbing en mass
- Lysis of *E.coli* O157
- Homologous Recombination - 2000 by Jake McKinlay
- X-Ray Structure of Bacteriophage HK97 by William R. Wikoff
- Balloon Phage T4 by Celeste O'Neil and Larry Goodridge

Please send any phage images that you would like to present in this section to "Phage Images," *The Bacteriophage Ecology Group*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Alternatively, you may scan the images yourself and send them as an attachment to microdude+@osu.edu . Please save all scans in gif or jpg formats and preferably with an image size (in terms of width, height, and kbytes) that will readily fit on a standard web page. No copyrighted material without permission, please!

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New Publications

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

1. Immunity profiles of wild-type and recombinant Shiga-Like toxin-encoding bacteriophages and characterization of novel double lysogens. Allison, H. E., Sergeant, M. J., James, C. E., Saunders, J. R., Smith, D. L., Sharp, R. J., Marks, T. S., McCarthy, A. J. (2003). *Infection and Immunity* 71:3409-3418. [PRESS FOR ABSTRACT]

2. Isolation and characterization of *Campylobacter* bacteriophages from retail poultry. Atterbury, R. J., Connerton, P. L., Dodd, C. E. R., Rees, C. E. D., Connerton, I. F. (2003). *Applied and Environmental Microbiology* 69:4511-4518. [PRESS FOR ABSTRACT]

3. Metagenomic analysis of an uncultured viral community from human feces. Breitbart, M., Hewson, I., Felts, B., Mahaffy, J. M., Nulton, J., Salamon, P., Rohwer, F. (2003). *Journal of Bacteriology* 185:6220-6223. [PRESS FOR ABSTRACT]

4. In vivo lysogenic conversion of Tox(-) Streptococcus pyogenes to Tox(+) with Lysogenic Streptococci or free phage. Broudy, T. B., Fischetti, V. A. (2003). *Infection and Immunity* 71:3782-3786.

5. The future of bacteriophage biology. Campbell, A. (2003). *Nature reviews Genetics* 4:471-477.

6. Microbiological aspects of an urban river used for unrestricted irrigation in the semi-arid region of north-east Brazil. Ceballos, B. S. O., Soares, N. E., Moraes, M. R., Catao, R. M. R., Konig, A. (2003). *Water Science and Technology* 47:51-57.

7. Comparative electrochemical inactivation of bacteria and bacteriophage. Drees, K. P., Abbaszadegan, M., Maier, R. M. (2003). *Water Research* 37:2291-2300. [PRESS FOR ABSTRACT]

8. Application of a novel immunomagnetic separation-bacteriophage assay for the detection of *Salmonella enteritidis* and *Escherichia coli* O157:H7 in food. Favrin, S. J., Jassim, S. A., Griffiths, M. W. (2003). *International Journal of*

Food Microbiology 85:63-71. [PRESS FOR ABSTRACT]

9. Evaluation of potential indicators of viral contamination in shellfish and their applicability to diverse geographical areas. Formiga-Cruz, M., Allard, A. K., Conden-Hansson, A. C., Henshilwood, K., Hernroth, B. E., Jofre, J., Lees, D. N., Lucena, F., Papapetropoulou, M., Rangdale, R. E., Tsibouxi, A., Vantarakis, A., Girones, R. (2003). *Applied and Environmental Microbiology* 69:1556-1563.

10. Improvement of phage defence in *Lactococcus lactis* by introduction of the plasmid encoded restriction and modification system LlaAI. Gabs, S., Josephsen, J. (2003). *Letters in Applied Microbiology* 36:332-336. [PRESS FOR ABSTRACT]

11. Bacteriophages as viral indicators for radiation processing of water: a chemical approach. Gehringer, P., Eschweiler, H., Leth, H., Pribil, W., Pfleger, S., Cabaj, A., Haider, T., Sommer, R. (2003). *Applied Radiation and Isotopes* 58:651-656. [PRESS FOR ABSTRACT]

12. Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. Goode, D., Allen, V. M., Barrow, P. A. (2003). *Applied and Environmental Microbiology* 69:5032-5036. [PRESS FOR ABSTRACT]

13. Morphological, host range, and genetic characterization of two coliphages. Goodridge, L., Gallaccio, A., Griffiths, M. W. (2003). *Applied and Environmental Microbiology* 69:5364-5371. [PRESS FOR ABSTRACT]

14. The complete sequence of marine bacteriophage VpV262 infecting *Vibrio parahaemolyticus* indicates that an ancestral component of a T7 viral supergroup is widespread in the marine environment. Hardies, S. C., Comeau, A. M., Serwer, P., Suttle, C. A. (2003). *Virology* 310:359-371. [PRESS FOR ABSTRACT]

15. Can an arbitrary sequence evolve towards acquiring a biological function? Hayashi, Y., Sakata, H., Makino, Y., Urabe, I., Yomo, T. (2003). *Journal of Molecular Evolution* 56:162-168. [PRESS FOR ABSTRACT]

16. Evaluation of biotracers to monitor effluent retention time in constructed wetlands. Hodgson, C. J., Perkins, J., Labadz, J. C. (2003). *Letters in Applied Microbiology* 36:362-371. [PRESS FOR ABSTRACT]

17. Evaluation of aerosol spray and intramuscular injection of bacteriophage to treat an *Escherichia coli* respiratory infection. Huff, W. E., Huff, G. R., Rath, N. C., Balog, J. M., Donoghue, A. M. (2003). *Poultry science* 82:1108-1112. [PRESS FOR ABSTRACT]

18. [Bacteriophage therapy]. Huovinen, P. (2003). *Duodecim; laaketieteellinen aikakauskirja* 119:581-583. [no abstract]

19. Efficient release of overproduced gene products from *Escherichia coli* BL21(DE3) by lytic infection with newly isolated bacteriophages. Iida, Yuichiro, Matsuda, Yoshinori, Saito, Ryuichiro, Nakasato, Masanori, Nonomura, Teruo, Kakutani, Koji, Tosa, Yukio, Mayama, Shigeyuki, Toyoda, H. (2003). *Bioscience Biotechnology and Biochemistry* 67:198-202. [PRESS FOR ABSTRACT]

20. The vertical distribution and diversity of marine bacteriophage at a station off Southern California. Jiang, S., Fu, W., Chu, W., Fuhrman, J. A. (2003). *Microbial Ecology* 45:399-410. [PRESS FOR ABSTRACT]

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41. Bacteriophages and *Clostridium* spores as indicator organisms for removal of pathogens by passage through saturated dune sand. Schijven, J. F., de Bruin, H. A. M., Hassanizadeh, S. M., Roda Husman, A. M. (2003). *Water Research* 37:2186-2194. [PRESS FOR ABSTRACT]

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55. Viral and bacterial production in the North Water polynya: In situ measurements, batch culture experiments, and characterization and distribution of a virus-host system. Middelboe, M., Nielson, T. G., Bjørnsen, P. K. (2002). *Deep-Sea Research II* 49:5063-5079. [PRESS FOR ABSTRACT]

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57. When phage, plasmids, and transposons collide: genomic islands, and conjugative- and mobilizable-transposons as a mosaic continuum. Osborn, A. M., Boltner, D. (2002). *Plasmid* 48:202-212. [PRESS FOR ABSTRACT]

58. Evidence for a phage proliferation threshold? Payne, R. J. H., Jansen, V. A. A. (2002). *Journal of Virology* 76:13123. [PRESS FOR ABSTRACT]

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63. Rapid detection of phylloplane bacterium *Enterobacter cloacae* based on chitinase gene transformation and lytic infection by specific bacteriophages. Takikawa, Y., Mori, H., Otsu, Y., Matsuda, Y., Nonomura, T., Kakutani, K., Tosa, Y., Mayama, S., Toyoda, H. (2002). *Journal of Applied Microbiology* 93:1042-1050. [PRESS FOR ABSTRACT]

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70. Viral density and virus-to-bacterium ratio in deep-sea sediments of the Eastern Mediterranean. Danovaro, R., Serresi, M. (2000). *Applied and Environmental Microbiology* 66:1857-1861. [PRESS FOR ABSTRACT]

71. Sensitivity of *Burkholderia cepacia* complex and *Pseudomonas aeruginosa* to transducing bacteriophages. Nzula, S., Vandamme, P., Govan, J. R. W. (2000). *FEMS Immunology and Medical Microbiology* 28:307-312. [PRESS FOR ABSTRACT]

72. Genome size distributions indicate variability and similarities among marine viral assemblages from diverse environments. Steward, G., Montiel, J., Azam, F. (2000). *Limnology and Oceanography* 45:1697-1706. [no abstract]

73. Significance of algal viruses and ecology of *Phaeocystis* hostvirus interactions. Bratbak, G., Heldal, M. (1999). in Bell, C. R., Brylinsky, M., Johnson-Green, P. (eds.) *Microbial Biosystems: New Frontiers*. Atlantic Canada Society for Microbial Ecology, Halifax, Canada. [PRESS FOR ABSTRACT]

74. Flagellar determinants of bacterial sensitivity to c-phage. Samuel, A. D., Pitta, T. P., Ryu, W. S., Danese, P. N., Leung, E. C., Berg, H. C. (1999). *Proceedings of the National Academy of Sciences, USA* 96:9863-9866.

75. Origin and evolution of viruses. Holland, J., Domingo, E. (1998). *Virus Genes* 16:13-21. [PRESS FOR ABSTRACT]

76. Evolution of Viral DNA-Dependent DNA Polymerases. Knopf, C. W. (1998). *Virus Genes* 16:47-58. [PRESS FOR ABSTRACT]

New Publications with Abstracts

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

1. **Immunity profiles of wild-type and recombinant Shiga-Like toxin-encoding bacteriophages and characterization of novel double lysogens. Allison, H. E., Sergeant, M. J., James, C. E., Saunders, J. R., Smith, D. L., Sharp, R. J., Marks, T. S., McCarthy, A. J. (2003). *Infection and Immunity* 71:3409-3418.** Pathogenicity of Shiga-like toxin (stx)-producing *Escherichia coli* (STEC), notably serotype 0157, the causative agent of hemorrhagic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura, is based partly on the presence of genes (stx_1 and/or stx_2) that are known to be carried on temperate lambdoid bacteriophages. Stx phages were isolated from different STEC strains and found to have genome sizes in the range of 48 to 62 kb and to carry either stx_1 or stx_2 genes. Restriction fragment length polymorphism patterns and sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein profiles were relatively uninformative, but the phages could be differentiated according to their immunity profiles. Furthermore, these were sufficiently sensitive to enable the identification and differentiation of two different phages, both carrying the genes for Stx_2 and originating from the same STEC host strain. The immunity profiles of the different Stx phages did not conform to the model established for bacteriophage lambda, in that the pattern of individual Stx phage infection of various lysogens was neither expected nor predicted. Unexpected differences were also observed among Stx phages in their relative lytic productivity within a single host. Two antibiotic resistance markers were used to tag a recombinant phage in which the stx genes were inactivated, enabling the first reported observation of the simultaneous infection of a single host with two genetically identical Stx phages. The data demonstrate that, although Stx phages are members of the lambdoid family, their replication and infection control strategies are not necessarily identical to the archetypical bacteriophage l, and this could be responsible for the widespread occurrence of *stx* genes across a diverse range of *E. coli* serotypes.*coli*.serotypes.

2. **Isolation and characterization of *Campylobacter* bacteriophages from retail poultry. Atterbury, R. J., Connerton, P. L., Dodd, C. E. R., Rees, C. E. D., Connerton, I. F. (2003). *Applied and Environmental Microbiology* 69:4511-4518.** The ability of phages to survive processing is an important aspect of their potential use in the biocontrol of *Campylobacter* in poultry production. To this end, we have developed a procedure to recover *Campylobacter* bacteriophages from chilled and frozen retail poultry and have validated the sensitivity of the method by using a characterized *Campylobacter* phage (i.e., NCTC 12674). By using this method, we have shown that *Campylobacter* phages can survive on retail chicken under commercial storage conditions. Retail chicken portions purchased in the United Kingdom were screened for the presence of endogenous *Campylobacter* phages. Thirty-four *Campylobacter* bacteriophages were isolated from 300 chilled retail chicken portions, but none could be recovered from 150 frozen chicken portions. The phage isolates were characterized according to their lytic profiles, morphology, and genome size. The free-range products were significantly more likely to harbor phages ($P < 0.001$ by single-factor analysis of variance) than were standard or economy products. This study demonstrates that *Campylobacter* bacteriophages, along with their hosts, can survive commercial poultry processing procedures and that the phages exhibited a wide range of recovery rates from chicken skin stored at 4°C.

3. **Metagenomic analysis of an uncultured viral community from human feces. Breitbart, M., Hewson, I., Felts, B., Mahaffy, J. M., Nulton, J., Salamon, P., Rohwer, F. (2003). *Journal of Bacteriology* 185:6220-6223.** Here we present the first metagenomic analyses of an uncultured viral community from human feces, using partial shotgun sequencing. Most of the sequences were unrelated to anything previously reported. The recognizable viruses were mostly siphophages, and the community contained an estimated 1,200 viral genotypes.

4. **In vivo lysogenic conversion of Tox(-) Streptococcus pyogenes to Tox(+) with Lysogenic Streptococci or free phage. Broudy, T. B., Fischetti, V. A. (2003). *Infection and Immunity* 71:3782-3786.** Temperate bacteriophage can transfer toxin-encoding genes between bacteria, often resulting in acquired pathogenicity. However, little is known regarding the effects of the eukaryotic host on the phage-pathogen interaction. Using *Streptococcus pyogenes* as a model, we demonstrate, both in vitro and in vivo, that the eukaryote mediates the efficient induction of toxin-encoding temperate phage and the resultant conversion of Tox^- flora to Tox^+. Furthermore, we show that both phage induction and subsequent conversion need not happen in the same mammalian host, as host-to-host phage transmission can result in toxigenic conversion within the secondary host. Ultimately, our findings demonstrate that the eukaryotic host serves as an essential component in the phage-mediated evolution of virulence within the microbial population.

5. **The future of bacteriophage biology. Campbell, A. (2003). *Nature reviews Genetics* 4:471-477.** After an illustrious history as one of the primary tools that established the foundations of molecular biology, bacteriophage research is now undergoing a renaissance in which the primary focus is on the phages themselves rather than the molecular mechanisms that they explain. Studies of the evolution of phages and their role in natural ecosystems are flourishing. Practical questions, such as how to use phages to combat human diseases that are caused by bacteria, how to eradicate phage pests in the food industry and what role they have in the causation of human diseases, are receiving increased attention. Phages are also useful in the deeper exploration of basic molecular and biophysical questions.

6. **Microbiological aspects of an urban river used for unrestricted irrigation in the semi-arid region of north-east Brazil. Ceballos, B. S. O., Soares, N. E., Moraes, M. R., Catao, R. M. R., Konig, A. (2003). *Water Science and Technology* 47:51-57.** This study compared the behaviour of pathogenic bacteria (*Salmonella* and *Listeria*),

faecal indicators (faecal coliforms FC and faecal streptococci FS), somatic coliphages and F-specific bacteriophages in an urban river contaminated with domestic sewage and surface run-off from agricultural and cattle grazing lands. The influence of physical and chemical parameters was also investigated as well as *Salmonella* and *Listeria* serotype diversity and drug resistance patterns. Faecal contamination was high (FC = 5×10^6 - 4×10^3 CFU/100 mL; FS = 4×10^5 - 2×10^2 CFU/100 mL) but decreased along the river by up to 99.5% following 47% reduction of BOD5 and 91% increase of DO, both associated with the self purification process. Somatic coliphages (6.9×10^5 - 1×10^3 PFU/100 mL) and F-specific bacteriophages (5.8×10^4 - 65 PFU/100 mL) behaved similarly with reductions of 99.85%. *Salmonella* and *Listeria* were isolated at all sampling points with highest frequencies (91-100%) at those with sewage discharge and rural water run-off. The lowest value (35%) occurred at the end of the river where it was (a) wider and shallower, (b) it ran slower and was warmer (29-33ºC), (c) the pH was alkaline (8.2-9.9), (d) electrical conductivity (2,200-5,800 microS/cm) and DO (6-13 mg/L) were highest. Pathogen decline did not follow exactly FC and FS reduction patterns, while physical and chemical parameters apparently did not interfere with *Salmonella* and *Listeria* survival to the same extent as they did with FC and FS. Somatic coliphages and F-specific bacteriophages did not show more resistance than bacterial indicators. Catchment area contribution seemed to be more significant for pathogens than for indicators and rainy periods increased pathogenic isolation frequency. Five *Salmonella* serotypes and five serogroups were identified. *S. hadar* and serogroup E were predominant (50%); both are increasing in Brazil apparently from animal sources. Nearly 25% of *Salmonella* strains were resistant to at least one of twelve antimicrobials tested. Resistance to tetracycline was common (17%) followed by cefalotine (3%). Five Listeria serogroups were isolated and L. grayi (43%) and L. monocytogenes (9%) were present at all points. Listeria drug resistance rates were 100% for oxaciline followed by clindamicine (97%), tetracycline (34%) and vancomycin (32%). Both pathogenic bacterial strains presented resistance to the same drugs observed in humans and warm blood animals but the high number of sensitive strains and the low numbers of strains resistant to more than one drug was not expected because of the heavy anthropogenic impact in this basin.

7. **Comparative electrochemical inactivation of bacteria and bacteriophage. Drees, K. P., Abbaszadegan, M., Maier, R. M. (2003). *Water Research* 37:2291-2300.** Electric fields and currents have been shown to be capable of disinfecting drinking water and reducing the numbers of bacteria and yeast in food. However, little research has been conducted regarding the effectiveness of electric fields and currents in the inactivation of viruses. The objective of this study was to compare the ability of bacteria and bacteriophage to survive exposure to direct electric current in an electrochemical cell, where they would be subject to irreversible membrane permeabilization processes, direct oxidation of cellular/viral constituents by electric current, and disinfection by electrochemically generated oxidants. Suspensions of the bacteria Escherichia coli and Pseudomonas aeruginosa and bacteriophage MS2 and PRD1 at both high (approximately 1×10^6CFU or PFU/mL) and low (approximately 1×10^3CFU or PFU/mL) population densities were exposed to currents ranging from 25 to 350mA in 5 s pulses. Post-exposure plaque counts of the bacteriophage were proportionally higher than bacterial culturable counts at corresponding current exposures. E. coli and MS2 were then exposed to 5mA for 20 min at both high and low population densities. The inactivation rate of E. coli was 2.1-4.3 times greater than that of MS2. Both bacteria and bacteriophage were more resistant to exposure to direct current at higher population densities. Also, amelioration of inactivation within the electrochemical cell by the reducing agent glutathione indicates the major mechanism of inactivation in the electrochemical cell is disinfection by electrochemically generated oxidants. The implications of these results are that technologies relying upon direct current to reduce the numbers of microbes in food and water may not be sufficient to reduce the numbers of potentially pathogenic viruses and ensure the safety of the treated food or water.

8. **Application of a novel immunomagnetic separation-bacteriophage assay for the detection of *Salmonella enteritidis* and *Escherichia coli* O157:H7 in food. Favrin, S. J., Jassim, S. A., Griffiths, M. W. (2003). *International Journal of Food Microbiology* 85:63-71.** *Salmonella* infection is the second most prevalent cause of foodborne illness in most developing countries. Meat, poultry, and dairy products are frequently implicated in outbreaks. The objective of this study was to apply a novel immunomagnetic separation (IMS)-bacteriophage assay to the detection of *Salmonella enteritidis* in artificially inoculated skimmed milk powder, chicken rinses, and ground beef. In all food types tested, the IMS-bacteriophage assay was able to detect an average of 3 CFU of *S. enteritidis* in 25 g or ml of food sample. Total assay time including pre-enrichment is about 20 h. The results indicate that the IMS-bacteriophage assay is a rapid and sensitive means of detecting *S. enteritidis* in these foods. The assay was successfully adapted to the detection of *Escherichia coli* O157:H7 and was able to detect *E. coli* in ground beef at the lowest inoculation level tested, 2 CFU/g. The assay was also adapted to the simultaneous detection of *S. enteritidis* and *E. coli*. The results indicate that the IMS-bacteriophage assay shows promise for the simultaneous detection of these pathogens, but further development work would be necessary to improve sensitivity and produce reliable results at low inoculation levels.

9. **Evaluation of potential indicators of viral contamination in shellfish and their applicability to diverse geographical areas. Formiga-Cruz, M., Allard, A. K., Conden-Hansson, A. C., Henshilwood, K., Hernroth, B. E., Jofre, J., Lees, D. N., Lucena, F., Papapetropoulou, M., Rangdale, R. E., Tsibouxi, A., Vantarakis, A., Girones, R. (2003). *Applied and Environmental Microbiology* 69:1556-1563.** The distribution of the concentration of potential indicators of fecal viral pollution in shellfish was analyzed under diverse conditions over 18 months in diverse geographical areas. These microorganisms have been evaluated in relation to contamination by human viral pathogens detected in parallel in the analyzed shellfish samples. Thus, significant shellfish-growing areas from diverse countries in the north and south of Europe (Greece, Spain, Sweden, and the United Kingdom) were defined and studied by analyzing different physicochemical parameters in the water and the levels of *Escherichia coli*, F-specific RNA bacteriophages, and phages infecting *Bacteroides fragilis* strain RYC2056 in the shellfish produced, before and after depuration treatments. A total of 475 shellfish samples were studied, and the results were statistically analyzed. According to statistical analysis, the presence of human viruses seems to be related to the presence of all potential indicators in the heavily contaminated areas, where *E. coli* would probably be suitable as a fecal indicator. The F-RNA phages, which are present in higher numbers in Northern Europe, seem to be significantly related to the presence of viral contamination in shellfish, with a very weak predictive value for hepatitis A virus, human adenovirus, and enterovirus and a stronger one for Norwalk-like virus. However, it is important to note that shellfish produced in A or clean B areas can sporadically contain human viruses even in the absence of *E. coli* or F-RNA phages. The data presented here will be useful in defining microbiological parameters for improving the sanitary control of shellfish consumed raw or barely cooked.

10. **Improvement of phage defence in *Lactococcus lactis* by introduction of the plasmid encoded restriction and modification system LlaAI. Gabs, S., Josephsen, J. (2003). *Letters in Applied Microbiology* 36:332-336.** AIMS: To study the ability of the plasmid-encoded restriction and modification (R/M) system LlaAI to function as a bacteriophage resistance mechanism in *Lactococcus lactis* during milk fermentations. METHODS AND RESULTS: Plasmid pAIcat4, carrying the R/M system LlaAI and a chloramphenicol resistance cassette, was introduced into the plasmid-free strain *L. lactis* MG1614 and the industrial strain *L. lactis* 964. By measuring changes in conductivity the influence of different phage on the growth was determined. CONCLUSIONS: The plasmid-encoded R/M system LlaAI significantly improves the bacteriophage resistance of *L. lactis* during milk fermentations. SIGNIFICANCE AND IMPACT OF THE STUDY: It is essential to determine the potential of a phage defence mechanism in *L. lactis* starter culture strains during growth in milk before steps are taken to improve starter cultures. This study shows that LlaAI is useful for improvement of starter cultures.

11. **Bacteriophages as viral indicators for radiation processing of water: a chemical approach. Gehringer, P., Eschweiler, H., Leth, H., Pribil, W., Pfleger, S., Cabaj, A., Haider, T., Sommer, R. (2003). *Applied Radiation and Isotopes* 58:651-656.** Inactivation of the bacteriophages PHI X 174 (somatic coliphage), MS2 (F-specific coliphage) and B40-8 (phage infecting *Bacteroides fragilis*) suspended in tap water was studied applying gamma and electron beam irradiation as well. PHI X 174 phage was found to be a suitable viral indicator for water disinfection by means of ionizing radiation. The nutrient broths introduced simultaneously with the bacteriophages into the water when it is spiked with the phages for the experiments did not significantly change the scavenging capacity of the water matrix. No dose rate effect was observed with MS2 and B40-8 phages but PHI X 174 phage showed a clear dose rate effect. It was found that in water MS2 phage is significantly more sensitive to ionizing radiation than *Escherichia coli*.

12. **Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. Goode, D., Allen, V. M., Barrow, P. A. (2003). *Applied and Environmental Microbiology* 69:5032-5036.** Lytic bacteriophages, applied to chicken skin that had been experimentally contaminated with *Salmonella enterica* serovar Enteritidis or *Campylobacter jejuni* at a multiplicity of infection (MOI) of 1, increased in titer and reduced the pathogen numbers by less than 1 log10 unit. Phages applied at a MOI of 100 to 1,000 rapidly reduced the recoverable bacterial numbers by up to 2 $\log_{10}$ units over 48 h. When the level of *Salmonella* contamination was low (< $\log_{10}$ 2 per unit area of skin) and the MOI was 10^5, no organisms were recovered. By increasing the number of phage particles applied (i.e., MOI of 10^7), it was also possible to eliminate other *Salmonella* strains that showed high levels of resistance because of restriction but to which the phages were able to attach.

13. **Morphological, host range, and genetic characterization of two coliphages. Goodridge, L., Gallaccio, A., Griffiths, M. W. (2003). *Applied and Environmental Microbiology* 69:5364-5371.** Two coliphages, AR1 and LG1, were characterized based on their morphological, host range, and genetic properties. Transmission electron microscopy showed that both phages belonged to the *Myoviridae*; phage particles of LG1 were smaller than those of AR1 and had an isometric head 68 nm in diameter and a complex contractile tail 111 nm in length. Transmission electron micrographs of AR1 showed phage particles consisting of an elongated isometric head of 103 by 74 nm and a complex contractile tail 116 nm in length. Both phages were extensively tested on many strains of *Escherichia coli* and other enterobacteria. The results showed that both phages could infect many serotypes of *E. coli*. Among the enterobacteria, *Proteus mirabilis*, *Shigella dysenteriae*, and two *Salmonella* strains were lysed by the phages. The genetic material of AR1 and LG1 was characterized. Phage LG1 had a genome size of 49.5 kb compared to 150 kb for AR1. Restriction endonuclease analysis showed that several restriction enzymes could degrade DNA from both phages. The morphological, genome size, and restriction endonuclease similarities between AR1 and phage T4 were striking. Southern hybridizations showed that AR1 and T4 are genetically related. The wide host ranges of phages AR1 and LG1 suggest that they may be useful as biocontrol, therapeutic, or diagnostic agents to control and detect the prevalence of *E. coli* in animals and food.

14. **The complete sequence of marine bacteriophage VpV262 infecting *Vibrio parahaemolyticus* indicates that an ancestral component of a T7 viral supergroup is widespread in the marine environment. Hardies, S. C., Comeau, A. M., Serwer, P., Suttle, C. A. (2003). *Virology* 310:359-371.** The 46,012-bp sequence of the marine bacteriophage VpV262 infecting the bacterium *Vibrio parahaemolyticus* is reported. The VpV262 sequence reveals that it is a distant relative of marine Roseophage SIO1, and an even more distant relative of coliphage T7. VpV262 and SIO1 appear to represent a widespread marine phage group that lacks an RNA polymerase gene and is ancestral to the T7-like phages. We propose that this group together with the T7-like phages be designated as the T 7 supergroup. The ancestral head structure gene module for the T7 supergroup was reconstructed by using sensitive biased Psi-blast searches supplemented by statistical support derived from gene order. In the early and replicative segments, these phages have participated in extensive interchange with the viral gene pool. VpV262 carries a different replicative module than SIO1 and the T7-like phages.

15. **Can an arbitrary sequence evolve towards acquiring a biological function? Hayashi, Y., Sakata, H., Makino, Y., Urabe, I., Yomo, T. (2003). *Journal of Molecular Evolution* 56:162-168.** To explore the possibility that an arbitrary sequence can evolve towards acquiring functional role when fused with other pre-existing protein modules, we replaced the D2 domain of the fd-tet phage genome with the soluble random polypeptide RP3-42. The replacement yielded an fd-RP defective phage that is six-order magnitude lower infectivity than the wild-type fd-tet phage. The evolvability of RP3-42 was investigated through iterative mutation and selection. Each generation consists of a maximum of ten arbitrarily chosen clones, whereby the clone with highest infectivity was selected to be the parent clone of the generation that followed. The experimental evolution attested that, from an initial single random sequence, there will be selectable variation in a property of interest and that the property in question was able to improve over several generations. fd-7, the clone with highest infectivity at the end of the experimental evolution, showed a 240-fold increase in infectivity as compared to its origin, fd-RP. Analysis by phage ELISA using anti-M13 antibody and anti-T7 antibody revealed that about 37-fold increase in the infectivity of fd-7 was attributed to the changes in the molecular property of the single polypeptide that replaced the D2 domain of the g3p protein. This study therefore exemplifies the process of a random polypeptide generating a functional role in rejuvenating the infectivity of a defective bacteriophage when fused to some preexisting protein modules, indicating that an arbitrary

sequence can evolve toward acquiring a functional role. Overall, this study could herald the conception of new perspective regarding primordial polypeptides in the field of molecular evolution.

16. **Evaluation of biotracers to monitor effluent retention time in constructed wetlands. Hodgson, C. J., Perkins, J., Labadz, J. C. (2003). *Letters in Applied Microbiology* 36:362-371.** AIMS: With concern surrounding the environmental impact of chemical tracers on the aquatic environment, this paper presents the initial evaluation of biotracers used to determine the effluent retention time, an important performance indicator, in a Free Water Surface Constructed Wetland. METHODS AND RESULTS: Production of the biotracers, coliphage MS2, and the bacteriophage of *Enterobacter cloacae* and antibiotic resistant endospores of *Bacillus globigii* is described in detail. Their subsequent use in three separate tracer experiments - January, March and June (2000) - revealed the variability of retention time with respect to effluent flow. The biotracer MS2 showed the constructed wetland had a retention time of 8-9 h at a mean discharge of 0.9 $l\ s^{-1}$, increasing to 10-12 h at a mean discharge 0.3 $l\ s^{-1}$. A similar retention of 9-10 h at a mean discharge of 0.3 $l\ s^{-1}$ was calculated for the Ent. cloacae phage. In contrast, use of endospores revealed considerably longer retention times at these mean discharge rates; 12-24 h and 36-48 h, respectively. CONCLUSION: Biotracers could provide a useful and environmentally friendly technique to monitor effluent retention in constructed wetlands. At this stage the phage tracers appear particularly promising due to ease of isolation and recovery. SIGNIFICANCE AND IMPACT OF THE STUDY: Initial results are encouraging and have highlighted the potential of biotracers as alternatives to chemical tracers, even in microbially-rich waters.

17. **Evaluation of aerosol spray and intramuscular injection of bacteriophage to treat an *Escherichia coli* respiratory infection. Huff, W. E., Huff, G. R., Rath, N. C., Balog, J. M., Donoghue, A. M. (2003). *Poultry science* 82:1108-1112.** Two studies were conducted to determine the efficacy of either aerosol or i.m. injection of bacteriophage to treat an *Escherichia coli* respiratory infection in broiler chickens. An additional two studies were conducted to enumerate the bacteriophage in the blood of birds at 1, 2, 3, 4, 5, 6, 24, and 48 h after being sprayed or injected i.m. with bacteriophage. Five birds were bled at each period. In study 1, there were 10 treatments with three replicate pens of 10 birds. The treatments consisted of an untreated control, heat-killed bacteriophage spray, active bacteriophage spray, *E. coli* challenge at 7 d of age, and *E. coli* challenge followed by spraying the birds with heat-killed bacteriophage or active bacteriophage at 2, 24, or 48 h after challenge. In study 2 there were 11 treatments with three replicate pens of 10 birds per pen. The treatments were untreated controls, birds injected i.m. in the thigh with heat-killed or active bacteriophage, *E. coli* challenge at 7 d of age, PBS challenge, *E. coli* challenge followed by injection of heat-killed or active bacteriophage immediately after challenge or at 24 or 48 h after challenge. In both studies the *E. coli* challenge consisted of injecting 10(4) cfu into the thoracic air sac. Treatment of this severe *E. coli* infection with the bacteriophage aerosol spray significantly reduced mortality from 50 to 20% when given immediately after the challenge but had little treatment efficacy when administered 24 or 48 h after challenge. The i.m. injection of bacteriophage significantly reduced mortality from 53 to 17%, 46 to 10%, and 44 to 20% when given immediately, 24, or 48 h after challenge, respectively. Only a few birds sprayed with bacteriophage had detectable bacteriophage in their blood with an average of 96 pfu/mL 1 h after bacteriophage administration, and no bacteriophage was detected 24 and 48 h after bacteriophage administration. All birds injected i.m. with bacteriophage had detectable levels of bacteriophage in their blood at levels of 10(4) pfu/mL of blood up to 6 h after bacteriophage administration, and four of the five birds had detectable bacteriophage in their blood at an average level of 70 pfu/mL of blood 24 h after bacteriophage administration. The relative inefficiency of the spray treatment to the i.m. injection treatment may be due to the inability to get bacteriophage into the blood at high concentrations when the birds are sprayed versus the consistent high titers achieved with the i.m. injection of bacteriophage. These data provide support to the concept that bacteriophage may be an effective alternative to antibiotics in animal production when they are administered in a way that delivers high titers of the bacteriophage to the critical site of the bacterial infection.

18. **[Bacteriophage therapy]. Huovinen, P. (2003). *Duodecim; laaketieteellinen aikakauskirja* 119:581-583.**

19. **Efficient release of overproduced gene products from *Escherichia coli* BL21(DE3) by lytic infection with newly isolated bacteriophages. Iida, Yuichiro, Matsuda, Yoshinori, Saito, Ryuichiro, Nakasato, Masanori, Nonomura, Teruo, Kakutani, Koji, Tosa, Yukio, Mayama, Shigeyuki, Toyoda, H. (2003). *Bioscience Biotechnology and Biochemistry* 67:198-202.** Overproduced proteins from *Escherichia coli* BL21(DE3) were efficiently released with virulent bacteriophages. Leviviridae-like bacteriophages were isolated from soil and used to lyse BL21(DE3) cells transformed with beta-glucosidase, chitinase, or chitosanase genes. This method caused lysis of bacterial cells similar to that by conventional sonication and enabled us to effectively recover and purify the enzymes.

20. **The vertical distribution and diversity of marine bacteriophage at a station off Southern California. Jiang, S., Fu, W., Chu, W., Fuhrman, J. A. (2003). *Microbial Ecology* 45:399-410.** Sixty-two bacteriophages were isolated on eight indigenous bacteria from a Pacific Ocean station spanning 887-m vertical depth, on two occasions between 1999 and 2000. On the basis of 16S rRNA sequences, six hosts were tentatively identified to be in the genus *Vibrio* and the other two were closely related to *Altermonas macleodii* (W9a) and *Pseudoalteromonas* spp. (W13a). Restriction fragment length polymorphism (RFLP) analysis of phage genomes using AccI and HapI showed that 16 phages infecting host C4a (*Vibrio*) displayed 14 unique RFLP patterns. However, identical phages infecting host C4b, C6a, and C6b (all *Vibrio*) were obtained from both the surface layer and the hypoxic zone at 850 m. Most phage isolates from the second year had a different RFLP pattern but shared genetic similarity to the phages infecting the same host from the previous year based on a hybridization study using phage genome probes. Cluster analysis of RFLP patterns and hybridization results also indicated that phages infecting the same or genetically related hosts, in general, shared higher degrees of homology in spite of the diverse RFLP patterns. Pulsed field gel electrophoresis (PFGE) analysis of native viral genomes indicated a range in genome size from less than 40 to 200 kb, and the dominant band shifted up by about 5-10 kb in the deep samples compared to the shallow ones. Hybridization of phage genome probes with total viral community DNA from various depths suggests these isolates, or at least some of their genes, represent a detectable portion of the natural viral community and were distributed throughout the water column. Thus, the results of this study demonstrated that the genetic diversity of bacteriophage in the ocean is far greater than that of their bacterial hosts. However, host range may have contributed to the evolution of the diverse phage population in the marine environment.

21. **Lack of correlation between O-serotype, bacteriophage susceptibility and genomovar status in the *Burkholderia cepacia* complex. Kenna, D. T., Barcus, V. A., Langley, R. J., Vandamme, P., Govan, J. R. W. (2003). *FEMS Immunology and Medical Microbiology* 35:87-92.** The Burkholderia cepacia complex comprises at least nine phylogenetically related genomic species (genomovars) which cause lifethreatening infection in immunocompromised humans, particularly individuals with cystic fibrosis or chronic granulomatous disease. Prior to recognition that '*B. cepacia*' comprise multiple species, in vitro studies revealed that the lipopolysaccharide (LPS) of these Gramnegative bacteria is strongly endotoxic. In this study, we used 117 *B. cepacia* complex isolates to determine if there is a correlation between O-antigen serotype and genomovar status. Isolates were also tested for their ability to act as bacterial hosts for the LPS-binding bacteriophages NS1 and NS2. The absence of genomovar II (*Burkholderia multivorans*) in 'historical *B. cepacia*' isolates was notable. Neither O-serotype nor phage susceptibility correlated with genomovar status. We conclude that variability in LPS may contribute to the success of these highly adaptable bacteria as human pathogens.

22. **The role of horizontal gene transfer by bacteriophages in the origin of pathogenic bacteria. Krylov, V. (2003). *Russian Journal of Genetics* 39:483-504.** The review considers the involvement of bacteriophages in transferring genes, which determine bacterial pathogenicity, and the increasing role of comparative genomics and genetics of bacteria and bacteriophages in detecting new cases of horizontal gene transfer. Examples of phage participation in this process proved to a different extent are described. Emphasis is placed on the original work carried out in Russia and focused on bacteriophages (temperate transposable phages and giant virulent fKZ-like phages) of conditional pathogen *Pseudomonas aeruginosa.* Consideration is given to the possible lines of further research of the role of bacteriophages in the infection process and, in particular, the role of virulent phages, whose products are sim-ilar to those of pathogenic bacteria, in modification of clinical signs of infectious diseases and in evolution. An attempt is made to predict the possible direction of pathogen evolution associated with development of new treatment strategies and generation of new specific niches.

23. **[Role of horizontal gene transfer by bacteriophages in the origin of pathogenic bacteria]. Krylov, V. N. (2003). *Genetika* 39:595-620.** The review considers the involvement of bacteriophages in transferring genes, which determine bacterial pathogenicity, and the increasing role of comparative genomics and genetics of bacteria and bacteriophages in detecting new cases of horizontal gene transfer. Examples of phage participation in this process proved to a different extent are described. Emphasis is placed on the original work carried out in Russia and focused on bacteriophages (temperate transposable phages and giant virulent phi KZ-like phages) of conditional pathogen Pseudomonas aeruginosa. Consideration is given to the possible lines of further research of the role of bacteriophages in the infection process and, in particular, the role of virulent phages, whose products are similar to those of pathogenic bacteria, in modification of clinical signs of infectious diseases and in evolution. An attempt is made to predict the possible direction of pathogen evolution associated with development of new treatment strategies and generation of new specific niches.

24. **Lysogeny and bacteriophage host range within the *Burkholderia cepacia* complex. Langley, R., Kenna, D. T., Vandamme, P., Ure, R., Govan, J. R. W. (2003). *Journal of Medical Microbiology* 52:483-490.** The *Burkholderia cepacia* complex comprises a group of nine closely related species that have emerged as life-threatening pulmonary pathogens in immunocompromised patients, particularly individuals with cystic fibrosis or chronic granulomatous disease. Attempts to explain the genomic plasticity, adaptability and virulence of the complex have paid little attention to bacteriophages, particularly the potential contribution of lysogenic conversion and transduction. In this study, lysogeny was observed in 10 of 20 representative strains of the *B. cepacia* complex. Three temperate phages and five lytic phages isolated from soils, river sediments or the plant rhizosphere were chosen for further study. Six phages exhibited T-even morphology and two were lambda-like. The host range of individual phages, when tested against 66 strains of the *B. cepacia* complex and a representative panel of other pseudomonads, was not species-specific within the *B. cepacia* complex and, in some phages, included *Burkholderia gladioli* and *Pseudomonas aeruginosa*. These new data indicate a potential role for phages of the *B. cepacia* complex in the evolution of these soil bacteria as pathogens of plants, humans and animals, and as novel therapeutic agents.

25. **[Bacteriophages for treatment and prophylaxis of infectious diseases]. Lazareva, E. B. (2003). *Antibiotiki i Khimioterapiya* 48:36-40.**

26. **Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. Leverentz, B., Conway, W. S., Camp, M. J., Janisiewicz, W. J., Abuladze, T., Yang, M., Saftner, R., Sulakvelidze, A. (2003). *Applied and Environmental Microbiology* 69:4519-4526.** The fresh-cut produce industry has been the fastest-growing portion of the food retail market during the past 10 years, providing consumers with convenient and nutritious food. However, fresh-cut fruits and vegetables raise food safety concerns, because exposed tissue may be colonized more easily by pathogenic bacteria than intact produce. This is due to the higher availability of nutrients on cut surfaces and the greater potential for contamination because of the increased amount of handling. We found that applied *Listeria monocytogenes* populations survived and increased only slightly on fresh-cut Red Delicious apples stored at 10°C but increased significantly on fresh-cut honeydew melons stored at 10°C over 7 days. In addition, we examined the effect of lytic, *L. monocytogenes*-specific phages via two phage application methods, spraying and pipetting, on *L. monocytogenes* populations in artificially contaminated fresh-cut melons and apples. The phage mixture reduced *L. monocytogenes* populations by 2.0 to 4.6 log units over the control on honeydew melons. On apples, the reduction was below 0.4 log units. In combination with nisin (a bacteriocin), the phage mixture reduced *L. monocytogenes* populations by up to 5.7 log units on honeydew melon slices and by up to 2.3 log units on apple slices compared to the control. Nisin alone reduced *L. monocytogenes* populations by up to 3.2 log units on honeydew melon slices and by up to 2.0 log units on apple slices compared to the control. The phage titer was stable on melon slices, but declined rapidly on apple slices. The spray application of the phage and phage plus nisin reduced the bacterial numbers at least as much as the pipette application. The effectiveness of the phage treatment also depended on the initial concentration of *L. monocytogenes*.

27. **A role for bacteriophage T4 *rI* gene function in the control of phage development during pseudolysogeny and in slowly growing host cells. Los, M., Wegrzyn, G., Neubauer, P. (2003). *Research in Microbiology* 154:547-552.** Although most studies on bacteriophages have been performed under laboratory conditions that are

optimal for host cell growth, in nature, bacteria and bacteriophages coexist in different habitats. Here, by using different growth rates in carbon-limited chemostats, we investigated the development of phage T4 in its host *Escherichia coli*. Our results strongly suggest that T4 can form pseudolysogens not only when bacterial growth is completely inhibited, but also in growing host cells. The *rI* gene, previously known to be indispensable for lysis inhibition, seems to play an important role in optimization of phage development in slowly growing cells as well as during establishment and maintenance of pseudolysogeny.

28. **Isolation and characterization of a *Lactobacillus plantarum* bacteriophage, phiJL-1, from a cucumber fermentation. Lu, Z., Breidt, F. Jr, Fleming, H. P., Altermann, E., Klaenhammer, T. R. (2003). *International Journal of Food Microbiology* 84:225-235.** A virulent *Lactobacillus plantarum* bacteriophage, PhiJL-1, was isolated from a commercial cucumber fermentation. The phage was specific for two related strains of *L. plantarum*, BI7 and its mutant (deficient in malolactate fermenting ability) MU45, which have been evaluated as starter cultures for controlled cucumber fermentation and as biocontrol microorganisms for minimally processed vegetable products. The phage genome of PhiJL-1 was sequenced to reveal a linear, double-stranded DNA (36.7 kbp). Sodium dodecyl sulfate-polyacryamide gel electrophoresis (SDS-PAGE) profiles indicated that PhiJL-1 contains six structural proteins (28, 34, 45, 50, 61, and 76 kDa). Electron microscopy revealed that the phage has an isometric head (59 nm in diameter), a long non-contractile tail (182 nm in length and 11 nm in width), and a complex base plate. The phage belongs to the Bradley group B1 or Siphoviridae family. One-step growth kinetics of the phage showed that the latent period was 35 min, the rise period was 40 min, and the average burst size was 22 phage particles/infected cell. Phage particles (90%) adsorbed to the host cells 20 min after infection. Calcium supplementation (up to 30 mM CaCl(2)) in MRS media did not affect the first cycle of phage adsorption, but promoted rapid phage propagation and cell lysis in the infection cycle subsequent to adsorption. The D values of PhiJL-1 at pH 6.5 were estimated to be 2.7 min at 70 °C and 0.2 min at 80 °C by a thermal inactivation experiment. Knowledge of the properties of L. plantarum bacteriophage PhiJL-1 may be important for the development of controlled vegetable fermentations.

29. **Bacteriophage ecology in commercial sauerkraut fermentations. Lu, Z., Breidt, F., Plengvidhya, V., Fleming, H. P. (2003). *Applied and Environmental Microbiology* 69:3192-3202.** Knowledge of bacteriophage ecology in vegetable fermentations is essential for developing phage control strategies for consistent and high quality of fermented vegetable products. The ecology of phages infecting lactic acid bacteria (LAB) in commercial sauerkraut fermentations was investigated. Brine samples were taken from four commercial sauerkraut fermentation tanks over a 60- or 100-day period in 2000 and 2001. A total of 171 phage isolates, including at least 26 distinct phages, were obtained. In addition, 28 distinct host strains were isolated and identified as LAB by restriction analysis of the intergenic transcribed spacer region and 16S rRNA sequence analysis. These host strains included *Leuconostoc*, *Weissella*, and *Lactobacillus* species. It was found that there were two phage-host systems in the fermentations corresponding to the population shift from heterofermentative to homofermentative LAB between 3 and 7 days after the start of the fermentations. The data suggested that phages may play an important role in the microbial ecology and succession of LAB species in vegetable fermentations. Eight phage isolates, which were independently obtained two or more times, were further characterized. They belonged to the family Myoviridae or Siphoviridae and showed distinct host ranges and DNA fingerprints. Two of the phage isolates were found to be capable of infecting two *Lactobacillus* species. The results from this study demonstrated for the first time the complex phage ecology present in commercial sauerkraut fermentations, providing new insights into the bioprocess of vegetable fermentations.

30. **Phages of the marine cyanobacterial picophytoplankton. Mann, N. H. (2003). *FEMS Microbiology Reviews* 27:17-34.** Cyanobacteria of the genera *Synechococcus* and *Prochlorococcus* dominate the prokaryotic component of the picophytoplankton in the oceans. It is still less than 10 years since the discovery of phages that infect marine *Synechococcus* and the beginning of the characterisation of these phages and assessment of their ecological impact. Estimations of the contribution of phages to *Synechococcus* mortality are highly variable, but there is clear evidence that phages exert a significant selection pressure on *Synechococcus* community structure. In turn, there are strong selection pressures on the phage community, in terms of both abundance and composition. This review focuses on the factors affecting the diversity of cyanophages in the marine environment, cyanophage interactions with their hosts, and the selective pressures in the marine environment that affect cyanophage evolutionary biology.

31. **Bacterial photosynthesis genes in a virus. Mann, N. H., Cook, A., Millard, A., Bailey, S., Clokie, M. (2003). *Nature* 424:741.** A bacteriophage may protect itself and its host against a deadly effect of bright sunlight.

32. **[Coliphages as indicators of fecal contamination in sea water]. Meloni, P., Isola, D., Loi, N., Schintu, M., Contu, A. (2003). *Annali di igiene : medicina preventiva e di comunita* 15:111-116.** Assessment of water quality has traditionally relied on faecal indicator organisms, which however do not necessarily correlate well with the presence of pathogenic organisms. Coliphages are regarded as possible alternative indicators. Although they can be detected in water by rapid, simple and reliable procedures, any agreement about a standard method has not yet been reached. Moreover guidelines for the levels of bacteriophages have not yet been set as for coliform bacteria, making difficult to evaluate results. In this work both bacteriophages anti *E. coli* and traditional indicators of fecal contamination were detected on 274 seawater samples taken from 23 sampling stations located along the coast of southern Sardinia (Italy). The results confirm the usefulness of coliphages as indicators of fecal contamination and suggest a level which could be considered a guideline value for their presence in seawater.

33. **[Development of cyanobacterial phages at the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine (History and perspectives)]. Mendzhul, M. I., Lysenko, T. G., Syrchin, S. A. (2003). *Mikrobiologichnyi Zhurnal* 65:133-140.** The paper deals with the basic trends of fundamental investigations of the Department of Algae Viruses in the field of cyanophagia-ecology, biological and physico-chemical properties of cyanophages as well as interrelation with the host cells. Such problems as a possibility to use the system cyanophage-cyanobacteria as the experimental model for development of the unified functional model of productive infection, efficient methods of prophylaxis and therapy of virus infections as well as the solution of various biotechnological problems are discussed.

34. **Bacterial host strains that support replication of somatic coliphages. Muniesa, M., Moce-Llivina, L., Katayama, H., Jofre, J. (2003). *Antonie van Leeuwenhoek* 83:305-315.** Somatic coliphages detected by

Escherichia coli strain WG5 have been proposed as potential indicators of water quality. Their potential replication in the water environment is considered a drawback for their use as indicators. However, the contribution of replication outside the gut to the total numbers has never been quantified. It has not been determined either the fraction of bacterial strains that might support replication of phages detected by strain WG5 in the water environment. We examined the sensitivity of 291 host strains to 25 phages by streaking slants of the presumptive host strain onto an agar layer that contains bacteriophages, which gives a total of 7275 combinations (sensitivity tests). Only a 3.02% of the tests showed sensitivity. Additionally, six environmental strains were used as hosts to count phages in sewage and seawater. Phages isolated on these strains were used to infect strain WG5. The environmental strains detected 1 $\log_{10}$ fewer phages than strain WG5 in sewage and seawater. The fraction of phages that were detected by the six strains and that also infected strain WG5 ranged from < 0.07% to < 2.0% of the total amount of bacteriophages detected by strain WG5 in the same samples. Our results confirm that less than 3% of naturally occurring hosts support replication of phages infecting E. coli. We conclude that the contribution of replication to the number of somatic coliphages detected in the aquatic environment is negligible.

35. **Genomic sequence of C1, the first streptococcal phage. Nelson, D., Schuch, R., Zhu, S., Tscherne, D. M., Fischetti, V. A. (2003).** ***Journal of Bacteriology*** **185:3325-3332.** C(1), a lytic bacteriophage infecting group C streptococci, is one of the earliest-isolated phages, and the method of bacterial classification known as phage typing was defined by using this bacteriophage. We present for the first time a detailed analysis of this phage by use of electron microscopy, protein profiling, and complete nucleotide sequencing. This virus belongs to the Podoviridae family of phages, all of which are characterized by short, noncontractile tails. The C(1) genome consists of a linear double-stranded DNA molecule of 16,687 nucleotides with 143-bp inverted terminal repeats. We have assigned functions to 9 of 20 putative open reading frames based on experimental substantiation or bioinformatic analysis. Their products include DNA polymerase, holin, lysin, major capsid, head-tail connector, neck appendage, and major tail proteins. Additionally, we found one intron belonging to the HNH endonuclease family interrupting the apparent lysin gene, suggesting a potential splicing event yielding a functional lytic enzyme. Examination of the C(1) DNA polymerase suggests that this phage utilizes a protein-primed mechanism of replication, which is prominent in the phi29-like members of Podoviridae. Consistent with this evidence, we experimentally determined that terminal proteins are covalently attached to both 5' termini, despite the fact that no homology to known terminal proteins could be elucidated in any of our open reading frames. Likewise, comparative genomics revealed no close evolutionary matches, suggesting that the C(1) bacteriophage is a unique member of the Podoviridae

36. **[How do bacteria acquire the resistance to antibiotics]. Ohno, A. (2003).** ***Nippon Rinsho - Japanese Journal of Clinical Medicine*** **61 Suppl 3:158-163.**

37. **Yersiniophages. Special reference to phi YeO3-12. Pajunen, M. I., Molineux, I. J., Skurnik, M. (2003).** ***Advances in experimental medicine and biology*** **529:233-240.**

38. **Origins of highly mosaic mycobacteriophage genomes. Pedulla, M. L., Ford, M. E., Houtz, J. M., Karthikeyan, T., Wadsworth, C., Lewis, J. A., Jacobs-Sera, D., Falbo, J., Gross, J., Pannunzio, N. R., Brucker, W., Kumar, V., Kandasamy, J., Keenan, L., Bardarov, S., Kriakov, J., Lawrence, J. G., Jacobs, W. R., Jr., Hendrix, R. W., Hatfull, G. F. (2003).** ***Cell*** **113:171-182.** Bacteriophages are the most abundant organisms in the biosphere and play major roles in the ecological balance of microbial life. The genomic sequences of ten newly isolated mycobacteriophages suggest that the bacteriophage population as a whole is amazingly diverse and may represent the largest unexplored reservoir of sequence information in the biosphere. Genomic comparison of these mycobacteriophages contributes to our understanding of the mechanisms of viral evolution and provides compelling evidence for the role of illegitimate recombination in horizontal genetic exchange. The promiscuity of these recombination events results in the inclusion of many unexpected genes including those implicated in mycobacterial latency, the cellular and immune responses to mycobacterial infections, and autoimmune diseases such as human lupus. While the role of phages as vehicles of toxin genes is well established, these observations suggest a much broader involvement of phages in bacterial virulence and the host response to bacterial infections.

39. **Virus removal during simulated soil-aquifer treatment. Quanrud, D. M., Carroll, S. M., Gerba, C. P., Arnold, R. G. (2003).** ***Water Research*** **37:753-762.** Removals of indigenous coliphage and seeded poliovirus type 1 during simulated soil-aquifer treatment were evaluated during transport of secondary effluent under unsaturated flow conditions in 1-m soil columns. Independent variables included soil type (river sand or sandy loam) and infiltration rate. Removal of coliphage was in all cases less than removal of poliovirus type 1 (strain LSc-2ab), supporting contentions that indigenous coliphage can act as a conservative indicator of groundwater contamination by viral pathogens of human origin. Coliphage retention was significantly more efficient ($p<0.001$) in the finer-grained sandy loam (93%) than in sand (76%). Increasing reactor detention time from 5 to 20 h increased coliphage attenuation from 70% to 99% in a 1-m sand column. There was a significant linear correlation (p=0.012) between log-transformed (fractional) coliphage concentration [log(C/C(0))] and reactor detention time. Re-mobilization of attached coliphage occurred during simulated rainfall using low-ionic-strength water. Inhibition of aerobic respiration resulted in significantly less efficient coliphage attenuation ($p=0.033$), suggesting the involvement of aerobic microorganisms in the survival/retention of this virus.

40. **Inactivation of** ***Lactobacillus delbrueckii*** **bacteriophages by heat and biocides. Quiberoni, A., Guglielmotti, D. M., Reinheimer, J. A. (2003).** ***International Journal of Food Microbiology*** **84:51-62.** The effect of several biocides and thermal treatments on the viability of four *Lactobacillus delbrueckii* phages was investigated. Time to achieve 99% inactivation of phages at 63 and 72 degrees C in three suspension media (Tris Magnesium Gelatin (TMG) buffer, Man Rogosa Sharpe (MRS) broth and reconstituted nonfat dry skim milk (RSM)) was calculated. Thermal resistance depended on the phage considered, but a marked heat-resistance was exhibited by one phage (Ib(3)) since its high titre suspensions were completely inactivated only after 45 min at 72 degrees C or 15 min at 90 degrees C. A clear protective effect of the milk was revealed when the three suspension media were compared. As regards to the effects of biocides on phages, only peracetic acid was found to be effective for inactivating high titre suspensions. Ethanol, even at a concentration of 100%, was not suitable to assure no surviving phage particles and isopropanol turned out to be less effective than ethanol. Sodium hypochlorite at 200-400 ppm inactivated the phages completely, except phage Ib(3), which was only destroyed after treatments with 1200 ppm. The diversity observed in the heat and biocide resistance of L. delbrueckii phages is useful to establish a basis for adopting the most effective

thermal and chemical treatments for inactivating them in dairy plants and laboratory environments.

41. **Bacteriophages and *Clostridium* spores as indicator organisms for removal of pathogens by passage through saturated dune sand. Schijven, J. F., de Bruin, H. A. M., Hassanizadeh, S. M., Roda Husman, A. M. (2003). *Water Research* 37:2186-2194.** In a field study on the efficiency of dune recharge for drinking water production, bacteriophage MS2 was shown to be removed 8 log(10) by passage through the dune sand. The question of whether pathogenic viruses would be removed as much as MS2 was studied by comparing complete breakthrough curves of MS2 with those of the human viruses Coxsackievirus B4 (CB4) and Poliovirus 1 (PV1) in laboratory columns. The columns were designed to closely simulate the field conditions: same sand, water, porewater velocity and temperature. Employing a two-site kinetic model to simulate breakthrough curves, attachment/detachment to two types of kinetic sites as well as inactivation of free and attached viruses were evaluated. It was found that attachment to only one of the sites is of significance for determining overall removal. At field scale, removal of the less negatively charged PV1 was extrapolated to be about 30 times greater than that of MS2, but removal of CB4 would be only as much as that of MS2. Also, removal of spores of Clostridium perfringens D10, a potential surrogate for Cryptosporidium oocysts, was studied. The attachment rate coefficient of the spores was 7.5 times greater than that of MS2. However, this does not imply that the removal of the spores is 7.5 times greater than that of MS2. Due to negligible inactivation in combination with detachment of previously attached spores, the actual removal rate of the spores depends on the duration of contamination and eventually all spores will break through. Provided no irreversible attachment or physical straining occurs, this may also be the case for other persistent microorganisms, like oocysts of *Cryptosporidium*.

42. ***Escherichia coli* O157:H7 Shiga toxin-encoding bacteriophages: integrations, excisions, truncations, and evolutionary implications. Shaikh, N., Tarr, P. I. (2003). *Journal of Bacteriology* 185:3596-3605.** As it descended from *Escherichia coli* O55:H7, Shiga toxin (Stx)-producing *E. coli* (STEC) O157:H7 is believed to have acquired, in sequence, a bacteriophage encoding Stx2 and another encoding Stx1. Between these events, sorbitol-fermenting *E. coli* O157:H$^-$ presumably diverged from this clade. We employed PCR and sequence analyses to investigate sites of bacteriophage integration into the chromosome, using evolutionarily informative STEC to trace the sequence of acquisition of elements encoding Stx. Contrary to expectations from the two currently sequenced strains, truncated bacteriophages occupy yehV in almost all *E. coli* O157:H7 strains that lack stx_1 (stx_1-negative strains). Two truncated variants were determined to contain either GTT or TGACTGTT sequence, in lieu of 20,214 or 18,895 bp, respectively, of the bacteriophage central region. A single-nucleotide polymorphism in the latter variant suggests that recombination in that element extended beyond the inserted octamer. An stx_2 bacteriophage usually occupies wrbA in stx_1^+/stx_2^+ E. coli O157:H7, but wrbA is unexpectedly unoccupied in most stx_1-negative/stx_2^+) *E. coli* O157:H7 strains, the presumed progenitors of stx_1^+)/stx_2^+ E. coli O157:H7. Trimethoprim-sulfamethoxazole promotes the excision of all, and ciprofloxacin and fosfomycin significantly promote the excision of a subset of complete and truncated stx bacteriophages from the *E. coli* O157:H7 strains tested; bile salts usually attenuate excision. These data demonstrate the unexpected diversity of the chromosomal architecture of *E. coli* O157:H7 (with novel truncated bacteriophages and multiple stx_2 bacteriophage insertion sites), suggest that stx_1 acquisition might be a multistep process, and compel the consideration of multiple exogenous factors, including antibiotics and bile, when chromosome stability is examined.

43. **Temporal dynamics of natural communities of marine algal viruses and eukaryotes. Short, S. M., Suttle, C. A. (2003). *Aquatic Microbial Ecology* 32:107-119.** The composition of algal virus communities in relation to temperature, salinity, chlorophyll *a* (chl *a*) concentration and eukaryotic community composition was monitored at a single location for 14 mo. Changes in algal virus and eukaryote communities were determined using polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) to generate genetic fingerprints. Sequence analysis of bands extracted from denaturing gradient gels revealed the presence of at least 5 distinct viruses as well as temporally dynamic and diverse communities of eukaryotes that included taxa from the viridiplantae, fungi and metazoa. Comparison of algal virus fingerprints with environmental conditions revealed that, at certain times, changes in algal virus community composition were coincident with changes in tide height, salinity or chl *a* concentration. However, algal virus community changes were not often coupled to eukaryote community changes. The lack of coincidence between changes in virus and eukaryote communities can be explained by the presence of organisms that were not hosts of the detected viruses. It is likely that the uncoupling of 18S and AVS fingerprints was due to succession among non-host eukaryotes. Although algal virus fingerprint patterns were stable throughout most of the study, stable eukaryote fingerprint patterns were observed only during the winter months. Furthermore, specific taxa of algal viruses persisted in fluctuating physical and biological environments. We concluded that the constant production of, and mortality from, some taxa of algal viruses provide further evidence that algal viruses affect phytoplankton community structure and dynamics.

44. **Cyanophages infecting the oceanic cyanobacterium *Prochlorococcus*. Sullivan, M. B., Waterbury, J. B., Chisholm, S. W. (2003). *Nature* 424:1047-1051.** *Prochlorococcus* is the numerically dominant phototroph in the tropical and subtropical oceans, accounting for half of the photosynthetic biomass in some areas. Here we report the isolation of cyanophages that infect Prochlorococcus, and show that although some are host-strain-specific, others cross-infect with closely related marine *Synechococcus* as well as between high-light- and low-light-adapted *Prochlorococcus* isolates, suggesting a mechanism for horizontal gene transfer. High-light-adapted *Prochlorococcus* hosts yielded Podoviridae exclusively, which were extremely host-specific, whereas low-light-adapted Prochlorococcus and all strains of *Synechococcus* yielded primarily Myoviridae, which has a broad host range. Finally, both *Prochlorococcus* and *Synechococcus* strain-specific cyanophage titres were low (< 10(3) ml(-1)) in stratified oligotrophic waters even where total cyanobacterial abundances were high (> 10^5) cells x ml^{-1}). These low titres in areas of high total host cell abundance seem to be a feature of open ocean ecosystems. We hypothesize that gradients in cyanobacterial population diversity, growth rates, and/or the incidence of lysogeny underlie these trends.

45. **A virus booster for game theory. Turner, P. E. (2003). *ASM News* 69:289-295.** Experiments with a selfish genotype of RNA bacteriophage f6-a parasite of a parasite-provide evidence for prisoner's dilemma.

46. **Transport and survival of bacterial and viral tracers through submerged-flow constructed wetland and sand-filter system. Vega, E., Lesikar, B., Pillai, S. D. (2003). *Bioresource Technology* 89:49-56.** Untreated or improperly treated wastewater has often been cited as the primary contamination source of groundwater. The use of decentralized wastewater treatment systems has applicability around the world since it obviates the need for extensive infrastructure development and expenditures. The use of a submerged flow constructed wetland (CW) and a sand filter to remove bacterial and viral pathogens from wastewater streams was evaluated in this study *Salmonella* sp. and a bacteriophages tracer were used in conjunction with the conservative bromide tracer to understand the fate and transport of these organisms in these treatment systems. Viral breakthrough numbers in the sand filter and CW were similar with a Spearman Rank correlation of 0.8 ($P<0.05$). In the CW, the virus exhibited almost a 3-log reduction, while in the sand filter, the viruses exhibited a 2-log reduction. The bacterial tracers, however, did not exhibit similar reductions. Low numbers of bacteria and viruses were still detectable in the

effluent streams suggesting that disinfection of the effluent is critical. The survival of the tracer bacteria and viruses was as expected dependent on the biotic and abiotic conditions existing within the wastewater. The results suggest that the microbial removal characteristics of decentralized wastewater treatment systems can vary and depend on factors such as adsorption, desorption and inactivation which in turn depend on the design specifics such as filter media characteristics and local climatic conditions.

47. **Integration and distribution of *Lactobacillus johnsonii* prophages. Ventura, M., Canchaya, C., Pridmore, D., Berger, B., Brüssow, H. (2003). *Journal of Bacteriology* 185:4603-4608.** In *Lactobacillus johnsonii* strain NCC533, two prophages were integrated into tRNA genes and one was disrupted by integration. In a survey, the prophages were restricted to strains sharing an essentially identical restriction pattern. Microarray analysis showed that the prophage DNA represents about 50% of the NCC533 strain-specific DNA.

48. **Three *Bacillus anthracis* bacteriophages from topsoil. Walter, M. H., Baker, D. D. (2003). *Current Microbiology* 47:55-58.** Three *Bacillus anthracis* bacteriophages from Iowa topsoil are characterized as to latent period, morphology, structural proteins, DNA size, and restriction endonuclease digestion. Electron micrographs indicate that the three isolates include two members of the Myoviridae and one smaller phage belonging to the Podoviridae. Phages Nk and DB resemble Myoviridae phage SP50 in morphology, but host range studies, protein, and DNA analysis indicate that both differ from SP50. Phage MH is very similar to phage phi 29, but differs in terms of host range, structural protein, and DNA characteristics.

49. **Cell death in *Pseudomonas aeruginosa* biofilm development. Webb, J. S., Thompson, L. S., James, S., Charlton, T., Tolker-Nielsen, T., Koch, B., Givskov, M., Kjelleberg, S. (2003). *Journal of Bacteriology* 185:4585-4592.** Bacteria growing in biofilms often develop multicellular, three-dimensional structures known as microcolonies. Complex differentiation within biofilms of *Pseudomonas aeruginosa* occurs, leading to the creation of voids inside microcolonies and to the dispersal of cells from within these voids. However, key developmental processes regulating these events are poorly understood. A normal component of multicellular development is cell death. Here we report that a repeatable pattern of cell death and lysis occurs in biofilms of *P. aeruginosa* during the normal course of development. Cell death occurred with temporal and spatial organization within biofilms, inside microcolonies, when the biofilms were allowed to develop in continuous-culture flow cells. A subpopulation of viable cells was always observed in these regions. During the onset of biofilm killing and during biofilm development thereafter, a bacteriophage capable of superinfecting and lysing the *P. aeruginosa* parent strain was detected in the fluid effluent from the biofilm. The bacteriophage implicated in biofilm killing was closely related to the filamentous phage Pf1 and existed as a prophage within the genome of *P. aeruginosa*. We propose that prophage-mediated cell death is an important mechanism of differentiation inside microcolonies that facilitates dispersal of a subpopulation of surviving cells.

50. **Prokaryotic and viral diversity patterns in marine plankton. Fuhrman, J. A., Griffith, J., Schwalbach, M. (2002). *Ecological Research* 17:183-194.** Prokaryotes and viruses play critical roles in marine ecosystems, where they are both highly abundant and active. Although early work on both prokaryotes and viruses revealed little of their diversity, molecular biological approaches now allow us to break apart these 'black boxes.' The most revealing methods have been cloning and sequencing of 16S rRNA genes, community fingerprinting (such as terminal restriction fragment length polymorphism; TRFLP), and fluorescent *in situ* hybridization. Viral diversity can now be analyzed by pulsed field gel electrophoresis (PFGE) of viral genomes. The present paper summarizes recent advances in bacterial and virus diversity studies, and presents examples of measurements from polar, tropical, and temperate marine waters. Terminal restriction fragment length polymorphism shows that many of the same operationally defined prokaryotic taxa are present in polar and tropical waters, but there are also some unique to each environment. By one measure, a sample from over a Philippine coral reef had about 100 operationally defined taxa, whereas one from the open tropical Atlantic had about 50 and from the icy Weddell Sea, about 60. Pulsed field gel electrophoresis of two depth profiles, to 500 m, from Southern California, measured 2 months apart, shows striking similarities in viral genome length diversity over time, and some distinct differences with depth. The euphotic zone samples had extremely similar apparent diversity, but samples from 150 m and 500 m were different. An obvious next step is to compare the bacterial and viral diversity patterns, because theory tells us they should be related.

51. **Sunlight inactivation of human enteric viruses and fecal bacteria. Fujioka, R. S., Yoneyama, B. S. (2002). *Water Science and Technology* 46:291-295.** Three human enteric viruses (poliovirus, echovirus, coxsackievirus) suspended in seawater or buffer were stable for 6 hr in the absence of sunlight but were inactivated at the same rate in the presence of sunlight. Under summer sunlight conditions, at least 3 logs of these viruses were inactivated by one-hit kinetics while under winter sunlight conditions only 1 log of these viruses was inactivated by two-hit kinetics. Under these same conditions, 6 logs of *E. coli* were inactivated within 1 hr by one-hit kinetics under summer and winter conditions. In comparison, *E. faecalis* was inactivated by two-hit kinetics and only 2.5 logs of inactivation were observed after 4 hr of exposure to winter sunlight. Since human enteric viruses are considerably more resistant to sunlight inactivation than *E. coli* and moderately more resistant than *E. faecalis*, marine recreational water quality standards should be based on concentrations of enterococci and not on coliform bacteria. Since the mechanism and rate of inactivation of coliphage and human enteric viruses are similar, coliphages appear to be the best indicator for the presence of human enteric viruses in recreational waters, especially coastal waters where abundant sunshine is available.

52. **Bacteriophage replication and reactivation in stationary phase hosts. Gallimore, W. H., Burgess, J. M., Kokjohn, T. A. (2002). *Research Signpost* 6:467-476.** Bacteriophage dynamics in stationary phase or stressed bacterial hosts are poorly understood. Using one-step growth experiments we have demonstrated that stationary phase does not constitute an absolute block to phage multiplication, although latent periods are extended and burst sizes decreased substantially compared to exponential phase infections. Using infectious center assays to quantify lysogen responses to DNA damage revealed that while there was a range of sensitivity to ultraviolet (UV) radiation, no prophages were induced by sunlight exposure. Comparing the capacity of exponential phase and stationary phase cells to resist UV irradiation and reactivate UV-damaged phage revealed that cells maintained in the stationary phase at the time of infection expressed significantly higher levels of DNA repair. Photoreactivation experiments in stationary phase hosts revealed that light-mediated reversal of phage DNA damage definitely occurred in stationary phase bacterial hosts. Our experiments demonstrate that many bacteriophages multiply actively and are competent to reverse DNA damage in post exponential phase host cells. In order to establish the scope and significance of bacteriophages to aquatic ecosystem ecology a more complete understanding of virus dynamics in both growing and stationary phase hosts is essential.

53. **[The bacteriophages and their gene products as antimicrobial agents]. Garcia, E., Lopez, R. (2002). *Revista Espanola de Quimioterapia* 15:306-312.** The viruses that infect bacteria (bacteriophages or phages) were first isolated about 90 years ago. Phages have been fundamental tools in the development of molecular biology. Phages were early hypothesized as therapeutic agents for combatting pathogenic bacteria. However, the discovery and successful use of antibiotics to treat infectious diseases hindered this aim. the development of bacterial resistance to most available drugs has recently led researchers to test the possibilities of using phages as therapeutic agents. We review here recent achievements in this field, taking into consideration former bias in handling phages as well as previous achievements carried out in Eastern Europe where bacteriophages have been employed for decades as an alternative to antibiotics.

54. **[Effect of bacteriophage on the lipid peroxidation process and antioxidant protective enzymes in experimental uveitis]. Karimova, M. Kh, Bakhritdinova, F. A. (2002). *Vestnik oftalmologii* 118:38-40.** Experimental uveitis features distinct hyperlipoperoxidation in damaged eye tissues, blood serum and the liver. The activity of antioxidant defense (AOD) enzymes decreases in tissues and blood of experimental animals whereas catalase compensatorily activates in hepatic tissue. Experimental therapy of uveitis with gentamycin and bacteriophage results in reducing hyperlipoperoxidation, increased activity of AOD enzymes but no complete normalization is observed. This manifested in preservation of inflammations to a certain degree.

55. **Viral and bacterial production in the North Water polynya: In situ measurements, batch culture experiments, and characterization and distribution of a virus-host system. Middelboe, M., Nielson, T. G., Bjørnsen, P. K. (2002). *Deep-Sea***

***Research II* 49:5063-5079.** Growth and viral lysis of bacterioplankton at subzero temperatures were measured in the North Water polynya in July 1998. In situ measurements of bacterial carbon consumption in surface waters ranged from 15 to 63mgCl^{-1}d^{-1} in the eastern and 6 to 7mgCl^{-1}d^{-1} in the northern part of the polynya. Both bacterial abundance and activity appeared to increase in response to the decay of the phytoplankton bloom that developed in the North Water. Organic carbon was the limiting substrate for bacteria in the polynya since addition of glucose, but not inorganic nutrients, to batch cultures increased both the carrying capacity of the substrate and the growth rate of the bacteria. Bacterial growth rates ranged from 0.11 to 0.40d^{-1}, corresponding to bacterial generation times of 1.7-6.3d. The in situ viral production rate was estimated both from the frequency of visibly infected cells and from the rate of viral production in batch cultures; it ranged from 0.04 to 0.52d-1 and from 0.25 to 0.47d^{-1}, respectively. From 6% to 28% of bacterial production was found to be lost due to viral lysis. The average virus-bacteria ratio was 5.1±3.1, with the abundance of viruses being correlated positively with bacterial production. A *Pseudoalteromonas* sp. bacterial host and an infective virus were isolated from the polynya; characteristics and distribution of the virus-host system were examined. The *Pseudoalteromonas* sp. showed psychrotolerant growth and sustained significant production of viruses at 0°C. The virus-host system was found throughout the polynya. Overall the results suggested that a large amount of organic carbon released during the development and breakdown of the spring phytoplankton bloom was consumed by planktonic bacteria and that the microbial food web was an important and dynamic component of the planktonic food web in the North Water.

56. **Mobilization of the *Vibrio* pathogenicity island between *Vibrio cholerae* isolates mediated by CP-T1 generalized transduction. O'Shea, Yvonne A., Boyd, E Fidelma (2002). *FEMS Microbiology Letters* 214:153-157.** Pathogenicity islands are large chromosomal regions encoding virulence genes that were acquired by horizontal gene transfer and are found in a wide range of pathogenic bacteria. In toxigenic *Vibrio cholerae* isolates the receptor for the cholera toxin encoding filamentous phage CTX_{ϕ}, the toxin-coregulated pilus, is part of the Vibrio pathogenicity island (VPI). In this paper, we show that the VPI can be transferred between O1 serogroup strains, the predominant cause of epidemic cholera, via a generalized transducing phage CP-T1

57. **When phage, plasmids, and transposons collide: genomic islands, and conjugative- and mobilizable-transposons as a mosaic continuum. Osborn, A. M., Boltner, D. (2002). *Plasmid* 48:202-212.** Plasmids and bacteriophage represent the classical vectors for gene transfer within the horizontal gene pool. However, the more recent discovery of an increasing array of other mobile genetic elements (MGE) including genomic islands (GIs), conjugative transposons (CTns), and mobilizable transposons (MTns) which each integrate within the chromosome, offer an increasingly diverse assemblage contributing to bacterial adaptation and evolution. Molecular characterisation of these elements has revealed that they are comprised of functional modules derived from phage, plasmids, and transposons, and further that these modules are combined to generate a continuum of mosaic MGE. In particular, they are comprised of any one of three distinct types of recombinase, together with plasmid-derived transfer and mobilisation gene functions. This review highlights both the similarities and distinctions between these integrating transferable elements resulting from combination of the MGE toolbox.

58. **Evidence for a phage proliferation threshold? Payne, R. J. H., Jansen, V. A. A. (2002). *Journal of Virology* 76:13123.** [first paragraph] Both experiments (5) and theory (3, 4) have suggested that for a population of phage to increase in numbers requires the host cell population to surpass a critical density termed the "replication threshold" or the "proliferation threshold." However, recently in the *Journal of Virology*, Kasman et al. (1) argued that no such threshold exists. Why this discrepancy? For a population of phage to increase in numbers, not only must phage from the initial dose replicate but also progeny phage must survive long enough to sustain further replication. This in turn depends on the density of remaining uninfected cells and on the rate of loss of free phage. The proliferation threshold is that cell density above which the probability of a progeny phage replicating is greater than the probability of that phage being lost (4). From this we identify three ways to reconcile the apparent inconsistencies between Kasman et al. (1) and Wiggins and Alexander (5).

59. **Viral lysis of marine bacterioplankton: Potential implications for organic matter cycling and bacterial clonal composition. Riemann, L., Middelboe, M. (2002). *Ophelia* 56:57-68.**

60. **Spatial stability of bacterial and viral community compositions in Danish coastal waters as depicted by DNA fingerprinting techniques. Riemann, L., Middelboe, M. (2002). *Aquatic Microbial Ecology* 27:219-232.**

61. **Column experiments to study nonlinear removal of bacteriophages by passage through saturated dune sand. Schijven, J. F., Hassanizadeh, S. M., de Bruin, H. A. M. (2002). *Journal of contaminant hydrology* 58:243-259.** In a recent field study on dune recharge, bacteriophages MS2 and PRD1 were found to be removed 3 log10 over the first 2.4 m and only 5 log10 over the next 27 m. To understand the causes of this nonlinear removal, column experiments were carried out under conditions similar to the field: same recharge water, temperature (5 +/- 3 degrees C) and pore water velocity (1.5 m day^{-1}). Soil samples were taken along a streamline between the recharge canal and the first monitoring well. Bacteriophage phiX174 was included for comparison. The high initial removal in the field was found not to be due to heterogeneity of phage suspensions but to soil heterogeneity. Phage removal rates correlated strongly positively with soil organic carbon content, and relatively strongly positively with silt content and the presence of ferric oxyhydroxides. Soil organic carbon content, silt content and the presence of ferric oxyhydroxides were found to decrease exponentially with travel distance. Removal rates of phiX174 were found to be 3-10 times higher than those of MS2 and PRD1 due to the lower electrostatic repulsion that the less negatively charged phiX174 experiences. It is suggested that the high initial removal in the field is due to the presence of favorable sites for attachment formed by ferric oxyhydroxides that decrease exponentially with travel distance. Similar removal rates may be found at both laboratory and field scale. However, due to local variations at field scale detailed knowledge on soil heterogeneity may be needed to enable a reliable prediction of removal.

62. **Microbial source tracking: current methodology and future directions. Scott, T. M., Rose, J. B., Jenkins, T. M., Farrah, S. R., Lukasik, J. (2002). *Applied and Environmental Microbiology* 68:5796-5803.** [first paragraph] Maintenance of the microbiological quality and safety of water systems used for drinking, for recreating, and in the harvesting of seafood is imperative, as contamination of these systems can exact high risks to human health as well as result in significant economic losses due to closures of beaches and shellfish harvesting areas. Waters contaminated with human feces are generally regarded as a greater risk to human health, as they are more likely to contain human-specific enteric pathogens, including *Salmonella enterica* serovar Typhi, *Shigella* spp., hepatitis A virus, and Norwalk-group viruses. Animals can also serve as reservoirs for a variety of enteric pathogens (e.g., various serotypes of *Salmonella*, *Escherichia coli*, and *Cryptosporidium* spp.). Understanding the origin of fecal pollution is paramount in assessing associated health risks as well as the actions necessary to remedy the problem while it still exists. Traditional and alternative indicator microorganisms have been used for many years to predict the presence of fecal pollution in water; however, it is well established that the majority of these organisms are not limited to humans but also exist in the intestines of many other warm-blooded animals (55). Due to the ubiquitous nature of these organisms, the effectiveness of using traditional indicators to predict the presence of human or animal waste impact and subsequent health risks is limited. The usefulness of the microbial indicators as tools for risk assessment can be significantly enhanced by the development of testing methods and analysis techniques that can define specific sources of these organisms.

63. **Rapid detection of phylloplane bacterium *Enterobacter cloacae* based on chitinase gene transformation and lytic infection by specific bacteriophages. Takikawa, Y., Mori, H., Otsu, Y., Matsuda, Y., Nonomura, T., Kakutani, K., Tosa, Y., Mayama, S., Toyoda, H. (2002). *Journal of Applied Microbiology* 93:1042-1050.** AIMS: To establish a rapid and efficient method for detecting *Enterobacter cloacae* based on chitinase gene transformation and lytic infection by virulent bacteriophages. METHODS AND RESULTS: A phylloplane strain of *E. cloacae* was isolated from tomato leaves and transformed with a chitinase gene. Transformed bacteria were collected from single colonies and infected with newly isolated, virulent bacteriophages in the presence of the chitinase substrate 4-methylumbelliferon (4MU)-(GlcNac)3. To assay chitinase activity in the lysates, the product 4MU was measured spectrofluorophotometrically or visibly detected under u.v. irradiation. Chitinase gene-transformed bacteria obtained from single colonies could be specifically identified in 30 min by the emission of 4MU fluorescence following lysis caused by phage infection. CONCLUSIONS:

The chitinase gene was used as a reporter gene to construct a new system for easy and rapid monitoring of transgenic strains of *E. cloacae* released in the environment, in combination with specific recognition by virulent bacteriophages. SIGNIFICANCE AND IMPACT OF THE STUDY: The assay is simple, rapid, inexpensive, easy to perform and applicable to other strains. The system can be used for the routine monitoring of bacteria, which is important because of the increased use of transgenic strains of *E. cloacae* as an antagonistic biological control agent for plant diseases.

64. **Metal ion-induced lateral aggregation of filamentous viruses fd and M13. Tang, J. X., Janmey, P. A., Lyubartsev, A., Nordenskiold, L. (2002). *Biophysical Journal* 83:566-581.** We report a detailed comparison between calculations of inter-filament interactions based on Monte-Carlo simulations and experimental features of lateral aggregation of bacteriophages fd and M13 induced by a number of divalent metal ions. The general findings are consistent with the polyelectrolyte nature of the virus filaments and confirm that the solution electrostatics account for most of the experimental features observed. One particularly interesting discovery is resolubilization for bundles of either fd or M13 viruses when the concentration of the bundle-inducing metal ion Mg2+ or Ca2+ is increased to large (>100 mM) values. In the range of Mg^{2+} or Ca^{2+} concentrations where large bundles of the virus filaments are formed, the optimal attractive interaction energy between the virus filaments is estimated to be on the order of 0.01kT per net charge on the virus surface when a recent analytical prediction to the experimentally defined conditions of resolubilization is applied. We also observed qualitatively distinct behavior between the alkali-earth metal ions and the divalent transition metal ions in their action on the charged viruses. The understanding of metal ions-induced reversible aggregation based on solution electrostatics may lead to potential applications in molecular biology and medicine.

65. **Virus-like particle distribution and abundance in sediments and overlying waters along eutrophication gradients in two subtropical estuaries. Hewson, I., O'Neil, J. M., Fuhrman, J. A., Dennison, W. C. (2001). *Limnology and Oceanography* 46:1734-1746.** Viruses are recognized as ubiquitous components of marine ecosystems; however, there has been limited study of viral abundance and its ecological role in sediments. Viral abundance was determined in both the water column and sediments of a eutrophic (Brisbane River/Moreton Bay; 27º25'S, 153º5'E) and oligotrophic (Noosa River; 26º15'S, 153º0'E) estuary in subtropical Queensland, Australia. Viruses, bacteria, and microalgae from both water column and extracted sediment samples were enumerated using SYBR Green I staining and epifluorescence microscopy. Sediment viral abundance ranged from 10^7 to 10^9 particles cm^{-3} of sediment, bacterial abundance ranged from 10^7 to 10^8 cells cm^{-3} of sediment, and microalgal abundance ranged from 10^4 to 10^5 cells cm^{-3} sediment. Pelagic abundances for all microorganisms were 10-1,000-fold lower than sediment abundances. Correlations between viral abundances and suspended solids suggest that viruses sorbed to suspended material in the water column may settle out and contribute to the benthic viral population. Virus production was measured by a time course increase of viral abundance in seawater using a dilution technique. Virus production was highest in eutrophic waters of the Brisbane River, and addition of inorganic nutrients (NO_3^- + NH_4^+ + PO_4^{-3} + SiO_3) stimulated viral production rates at all stations by 14-52% above ambient, suggesting that inorganic nutrient availability may play a key role in aquatic viral abundance.

66. **Phage facts. Konforti, B. (2001). *Nature Structural Biology* 8:19-20.** [first paragraph] Often the simplest experiments lead to the most remarkable insights. So it was with the famous fluctuation experiments of Luria and Delbrück and the Waring blender experiments of Hershey and Chase for which they were awarded the Nobel Prize in Physiology or Medicine in 1969. While the results of these experiments are permanently etched into every first year biology student's brain, it is worth recalling what was known at the time these experiments were conducted and the conclusions the authors drew.

67. **Transfer of the *Salmonella* type III effector *sopE* between unrelated phage families. Mirold, S., Rabsch, W., Tschaepe, H., Hardt, W.-D. (2001). *Journal of Molecular Biology* 312:7-16.** *Salmonella* spp. are pathogenic enterobacteria that employ type III secretion systems to translocate effector proteins and modulate responses of host cells. The repertoire of translocated effector proteins is thought to define host specificity and epidemic virulence, and varies even between closely related *Salmonella* strains. Therefore, horizontal transfer of effector protein genes between *Salmonella* strains plays a key role in shaping the *Salmonella*-host interaction. Several effector protein genes are located in temperate phages. The P2-like phage SopEF encodes SopE and the ?-like GIFSY phages encode several effector proteins of the YopM/IpaH-family. Lysogenic conversion with these phages is responsible for much of the diversity of the effector protein repertoires observed among *Salmonella* spp. However, free exchange of effector proteins by lysogenic conversion can be restricted by superinfection immunity. To identify genetic mechanisms that may further enhance horizontal transfer of effector genes, we have analyzed *sopE* loci from *Salmonella* spp. that do not harbor P2-like sequences of SopEF. In two novel *sopE* loci that were identified, the 723 nt *sopE* gene is located in a conserved 1.2 kb cassette present also in SopEF. Most strikingly, in *Salmonella enterica* subspecies I serovars Gallinarum, Enteritidis, Hadar and Dublin, the *sopE*-cassette is located in a cryptic I-like prophage with similarity to the GIFSY phages. This provides the first evidence for transfer of virulence genes between different phage families. We show that such a mechanism can circumvent restrictions to phage-mediated gene transfer and thereby enhances reassortment of the effector protein repertoires in *Salmonella* spp.

68. **Attempts to utilize bacteriophage to combat *Salmonella enterica* serovar Enteritidis infection in chickens. Sklar, I. B., Joerger, R. D. (2001). *Journal of Food Safety* 21:15-29.** Bacteriophage capable of lysing a nalidixic acid-resistant *Salmonella enterica* serovar Enteritidis strain *(SeE* Nalr *)* were tested for the ability to reduce cecal *Salmonella* counts in young chickens infected with the bacterium. Qualitative analysis of cloacal swabs suggested that phage treatment can possibly reduce shedding of *SeE* Nalr, but average *SeE* Nalr counts of between 10^5 and 10^7 cfu of *SeE* Nalr per *g* of cecum were obtained even fiom phage-treated 14-day old birds and even when more than 10^7 plaque/onning units of phage were present per gram of cecal content. The average cecal SeE Nalr counts were generally between 0.3 and 1.3 orders of magnitude lower in phage-treated chickens than in untreated controls bird. The diference in counts was statistically not significant in three animal trials, but significant in two trials using feed particles as delivery vehicles for the phage. Although some of the *SeE* Nalr in the cecae of phage-treated chickens had developed resistance to some of the phage used, factors other than phage resistance must have contributed to the failure of the phage to substantially reduce *SeE* Nalr counts.

69. **Infectious CTXF and the vibrio pathogenicity island prophage in *Vibrio mimicus*: evidence for recent horizontal transfer between *V. mimicus* and *V. cholerae*. Boyd, E. F., Moyer, K. E., Shi, L., Waldor, M. K. (2000). *Infection and Immunity* 68:1507-1513.** *Vibrio mimicus* differs from *Vibrio cholerae* in a number of genotypic and phenotypic traits but like *V. cholerae* can give rise to diarrheal disease. We examined clinical isolates of *V. mimicus* for the presence of CTXF, the lysogenic filamentous bacteriophage that carries the cholera toxin genes in epidemic *V. cholerae* strains. Four *V. mimicus* isolates were found to contain complete copies of CTXF. Southern blot analyses revealed that *V. mimicus* strain PT5 contains two CTX prophages integrated at different sites within the *V. mimicus* genome whereas *V. mimicus* strains PT48, 523-80, and 9583 each contain tandemly arranged copies of CTXF. We detected the replicative form of CTXF, pCTX, in all four of these *V. mimicus* isolates. The CTX prophage in strain PT5 was found to produce infectious CTXF particles. The nucleotide sequences of CTXF genes orfU and zot from *V. mimicus* strain PT5 and *V. cholerae* strain N16961 were identical, indicating contemporary horizontal transfer of CTXF between these two species. The receptor for CTXF, the toxin-coregulated pilus, which is encoded by another lysogenic filamentous bacteriophage, VPIF, was also present in the CTXF-positive *V. mimicus* isolates. The nucleotide sequences of VPIF genes aldA and toxT from *V. mimicus* strain PT5 and *V. cholerae* N16961 were identical, suggesting recent horizontal transfer of this phage between *V. mimicus* and *V. cholerae*. In *V. mimicus*, the vibrio pathogenicity island prophage was integrated in the same chromosomal attachment site as in *V. cholerae*. These results suggest that *V. mimicus* may be a significant reservoir for both CTXF and VPIF and may play an important role in the emergence of new toxigenic *V. cholerae* isolates.

70. **Viral density and virus-to-bacterium ratio in deep-sea sediments of the Eastern Mediterranean. Danovaro, R., Serresi, M. (2000). *Applied and Environmental Microbiology* 66:1857-1861.** Viruses are now recognized as a key component in pelagic systems, but their role in marine sediment has yet to be assessed. In this study bacterial and viral densities were determined at nine deep-sea stations selected from three main sites (i.e., the Sporades Basin, the Cretan Sea, and the Ierapetra Trench at depths of 1,232, 1,840, and 4,235 m,

respectively) of the Eastern Mediterranean. The three areas were characterized by different phytopigment and biopolymeric carbon concentrations and by changes in the protein and carbohydrate pools. A gradient of increasing trophic conditions was observed from the Sporades Basin (North Aegean) to the Ierapetra Trench (South Aegean). Viral densities (ranging from 1 x 10^{9} to 2 x 10^{9} viruses ml of sediment^{-1}) were significantly correlated to bacterial densities (n = 9, r^2 = 0.647) and reached values up to 3 orders of magnitude higher than those generally reported for the water column. However, the virus-to-bacterium density ratio in deep-sea sediments was about 1 order of magnitude lower (range of 2 to 5, with a modal value of 2.6) than in pelagic environments. Virus density decreased vertically with depth in sediment cores at all stations and was below detection limits at the 10^{-cm} depth of the abyssal sediments of the Ierapetra Trench. Virus density in the sediment apparently reflected a gradient of particle fluxes and trophic conditions, displaying the highest values in the Sporades Basin. The low virus-to-bacterium ratios and their inverse relationship with station depth suggest that the role played by viruses in controlling deep-sea benthic bacterial assemblages and biogeochemical cycles is less relevant than in pelagic systems.

71. **Sensitivity of *Burkholderia cepacia* complex and *Pseudomonas aeruginosa* to transducing bacteriophages. Nzula, S., Vandamme, P., Govan, J. R. W. (2000). *FEMS Immunology and Medical Microbiology* 28:307-312.** *Burkholderia cepacia* is now recognised as a life-threatening pathogen among several groups of immunocompromised patients. In this context, the proposed large-scale use of these bacteria in agriculture has increased the need for a better understanding of the genetics of the species forming the B. cepacia complex. Until now, little information has been available on the bacteriophages of the *B. cepacia* complex. Transducing phages, named NS1 and NS2, were derived from the lysogenic B. cepacia strains ATCC 29424 and ATCC 17616. The frequency of transduction per phage particle ranged from 1.0 x 10^{-8} to 7.0 x 10^{-6} depending on the phage and recipient strain used. The host range of NS1 and NS2 differed but in each case included environmental and clinical isolates, and strains belonging to several species and genomovars of the B. cepacia complex. The host range of both phages also included *Pseudomonas aeruginosa*. Some *B. cepacia* complex isolates were sensitive to the well-characterised *P. aeruginosa* transducing phages, B3, F116L and G101. The lytic activity of NS1 and NS2 was inhibited by *B. cepacia* lipopolysaccharide suggesting that this moiety is a binding site for both phages. The molecular size of the NS1 and NS2 genomes was approximately 48 kb.

72. **Genome size distributions indicate variability and similarities among marine viral assemblages from diverse environments. Steward, G., Montiel, J., Azam, F. (2000). *Limnology and Oceanography* 45:1697-1706.**

73. **Significance of algal viruses and ecology of *Phaeocystis* hostvirus interactions. Bratbak, G., Heldal, M. (1999). in Bell, C. R., Brylinsky, M., Johnson-Green, P. (eds.) *Microbial Biosystems: New Frontiers*. Atlantic Canada Society for Microbial Ecology, Halifax, Canada.** The ecological significance of algal viruses is suggested by the presence of algal cells containing virus like particles, the presence of viruses infecting specific algae and the succession of phytoplankton and virus in natural aquatic ecosystems. The interaction between *Phaeocystis pouchetii* and the lytic virus PpV01 was investigated in laboratory microcosms. The experiments show that *P. pouchetii* is susceptible to virus infection in all stages of growth, and that nitrate, phosphate and light limitation of algal growth do not prevent virus reproduction and cell lysis. Inferior growth conditions caused a decrease in burst size of > 50% compared to more copious growth conditions. The length of the lytic cycle and the infectivity of the viruses was apparently not affected by the host's growth conditions. Viral infection did not affect photosynthesis until near the onset of cell lysis. Release of dissolved organic carbon was low in non-infected cultures, while in infected cultures the entire algal biomass was converted to DOC. The DOC released during viral lysis was rapidly and efficiently utilized for bacterial growth.

74. **Flagellar determinants of bacterial sensitivity to c-phage. Samuel, A. D., Pitta, T. P., Ryu, W. S., Danese, P. N., Leung, E. C., Berg, H. C. (1999). *Proceedings of the National Academy of Sciences, USA* 96:9863-9866.** Bacteriophage c is known to infect motile strains of enteric bacteria by adsorbing randomly along the length of a flagellar filament and then injecting its DNA into the bacterial cell at the filament base. Here, we provide evidence for a "nut and bolt" model for translocation of phage along the filament: the tail fiber of c fits the grooves formed by helical rows of flagellin monomers, and active flagellar rotation forces the phage to follow the grooves as a nut follows the threads of a bolt.

75. **Origin and evolution of viruses. Holland, J., Domingo, E. (1998). *Virus Genes* 16:13-21.** [first paragraph] The origin(s) of viruses can not be known with certainty. PCR and other sensitive molecular techniques will reveal some viral genome sequences from the relatively recent past, but very ancient viral genomes will remain a matter for speculation. Numerous theories have been advanced regarding virus origins (reviewed in 1) and all necessarily involve speculation. However, comparative sequence analysis strongly suggests that both RNA (2) and DNA (3) viruses have deep, archaic evolutionary roots both for genome structural organization and as regards certain genomic and protein domains. It is also clear that both DNA and RNAviruses can emerge and evolve by a variety of mechanisms including mutation, recombination and reassortment. This can involve point mutation, insertions and deletions, acquisition or loss of genes (and gene domains, or sets of genes), rearrangement of genomes and utilization of alternate reading frames or inverted reading frames (1±6).

76. **Evolution of Viral DNA-Dependent DNA Polymerases. Knopf, C. W. (1998). *Virus Genes* 16:47-58.** DNA viruses as their host cells require a DNA-dependent DNA polymerase (Pol) to faithfully replicate their genomic information. Large eukaryotic DNA viruses as well as bacterial viruses encode a specific Pol equipped with a proofreading 3'-5'-exonuclease, and other replication proteins. All known viral Pol belong to family A and family B Pol. Common to all viral Pol is the conservation of the 3'-5'-exonuclease domain manifested by the three sequence motifs Exo I, Exo II, and Exo III. The polymerase domain of family A and B Pol is clearly distinguishable. Family A Pol share 9 distinct consensus sequences, only two of them are convincingly homologous to sequence motif B of family B Pol. The putative sequence motifs A, B, and C of the polymerase domain are located near the C-terminus in family A Pol and more central in family B Pol. Thus, family A Pol show a significant greater spacing between the Exo III motif and the Pol motif A that is especially extended in the case of the mitochondrial Pol g. From each host and virus family whenever possible the consensus sequences of two distantly related polymerase species were aligned for assessment of phylogenetic trees, using both maximum parsimony and distance methods, and evaluated by bootstrap analysis. Three alternative methods yielded trees with identical major groupings. A subdivision of viral family B Pol was achieved resulting in a branch with Pol carrying out a protein-primed mechanism of DNA replication, including adenoviruses, bacteriophages and linear plasmids of plant and fungal origin. Archaebacterial Pol and cellular Pol e were consistently found at the base of this branch. Another major branch comprised alpha- and delta-like viral Pol from mammalian herpesviruses, fish lymphocystis disease virus, insect ascovirus, and chlorella virus. Due to a lower branch integrity Pol of T-even bacteriophages, poxviruses, African swine fever virus, fish herpesvirus, and baculoviruses were not clearly resolved and placed in alternate groupings. A composite and rooted tree of family A and B Pol shows that viral Pol with a protein-priming requirement represent the oldest viral Pol species suggesting that the protein-primed mechanism is one of the earliest modes of viral DNA replication.

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