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Bacteriophage Ecology Group (BEG) News

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

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April 1, 2002 issue (volume 12)

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Editorial

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

Shipping Phages

by Hans-Wolfgang Ackermann

It used to be that common, minor pathogens, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, could be exchanged between scientists, by mail, without fuss, loss of time, permits, or shipment via expensive courier services. No longer. Today the exchange of these materials has become more and more difficult, and this indirectly affects phage research.

I became alerted to the problem because I am the curator of the Felix d'Herelle Reference Center for Bacterial Viruses (acronym HER), a small collection but, nevertheless, perhaps the largest collection of bacteriophages in the world. The collection houses some 450 bacteriophages and about as many bacterial hosts. The hosts include many Group 2 and a few Group 3 pathogens. Importation permits are not required for phages in the U.S. However, anybody who wants to acquire and propagate phages with hosts of risk groups 2 and 3 automatically needs a permit for the hosts from the CDC in Atlanta.

Transportation regulations have, in fact, sprung up everywhere on both national and international levels. These regulations,

however, have nothing to do with legitimate concerns with germ warfare and the recent anthrax alert as they came into being in the 1980s. I believe, instead, that a monster has been created that seriously hampers research. The problem is not packaging regulations and not even paperwork or costs. I readily go along with restrictions on the circulation of Group 3 or 4 pathogens. The problem is that almost all microorganisms are classified as Group 2 and thus fall automatically under IATA (International Air Transport Association) Dangerous Goods Regulations and associated restrictions on the national level. I contend that much harm has been done to microbial research, that more is to come, and that these regulations are going to make our lives miserable.

The trend is to tighten regulations. In Canada, the Bureau for Laboratory Safety of the Health Ministry in Ottawa has produced extremely comprehensive lists of microorganisms that include *Escherichia coli*, *Mycobacerium smegmatis*, all members of the genus *Lactococcus*, all members of the genus *Bifidobacterium* and, on the fungal side, all members the genus *Saccharomyces*. Regulations of this type suggest that the Bureau must consider that the microorganisms producing cheese, bread, beer, and wine are dangerous. I surmise that few or no medical people have been involved in the relevant decisions. For example, of the approximately 12 members of the Bureau, none, except perhaps one person working in a medical school, seems to be a medical doctor. To my knowledge, microbiological societies have had little influence on regulations and the present situation has developed with minimal consultation of microbiologists. I am not impressed by the argument that some members of the normal flora are potentially and occasionally pathogenic. This relates to the current germ craze and has no rational basis.

Regulations and restrictions such as these have spawned a host of companies producing forms and packing material. Shipping companies and customs offices, at least in Canada, are required to control every shipment with a checklist of 50 items. Employees are literally going after every colon (with no pun intended) and, indeed, it happened to me that a shipment was nearly refused for a missing colon. Below are excerpts from messages that I received from New Zealand and Belgium, respectively:

> As a Ph.D student, several years ago, I regularly sent and received all manner of (innocuous) plasmids and bacterial strains through the post in eppendorf tubes without any permission from anyone. Now I can only import specifically allowed material without sending a book chapter of irrelevant material and a wad of money to a committee of people who barely passed high school to decide whether or not importation is ok. Much of the problem in New Zealand is due to an over reaction by politicians and the green movement, a few years ago, to the "discovery" that scientists in New Zealand were doing recombinant DNA work.
>
> It took the Belgian officials months to come to the following conclusion: "Hereby the Service of Biosafety and Biotechnology (SBB) confirms on the basis of the obtained information that phages D3, D3112, phiKZ, 7, 21, 68 (host: *Pseudomonas aeruginosa*), T4, T7, lambda (host: *Escherichia coli*) are neither genetically modified nor pathogens and are therefore not submitted to the Belgian regional decrees, in this case the decree of the Flemish region of 1 june 1995, on the contained use of genetically modified organisms and/or pathogens. Also the above mentioned phages cannot be considered as belonging to class 6.2 (infectious substances) or 9 (Miscellaneous Dangerous Substances and Articles, including genetically modified micro-organisms which are not dangerous for animals or humans, but which could modify animals, plants, microbiological substances and ecosystems in a way that does not occur naturally, and genetically modified organisms which are known or suspected to be dangerous to the environment), according to the UN recommendations on the transport of dangerous goods. Hence importation of these phages is not submitted to the conditions imposed by these regulations."

Can something be done? Perhaps if ASM makes representations at the CDC to exempt Group 2 microorganisms from import permits and Dangerous Goods declarations (not of the use of safe packaging material). If this cannot be done, then ASM should press for exemption of microorganisms of the normal flora and the environment, and of useful microbes. In turn, CDC must influence IATA to relax regulations on Group 2 pathogens. I would like to learn of your own experiences with respect to shipping phages, along with your suggestions.

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New BEG Members

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: abedon.1@osu.edu. Include:

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

Note that it is preferable that you include the full reference, including the abstract, if the reference is not already present in the BEG bibliography. Responsibility of members includes keeping the information listed on the BEG members 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: abedon.1@osu.edu and minimally include your name and e-mail address.

Please welcome our newest members

name (home page links)	status	e-mail	address
Ido Golding	---	igolding @princeton.edu	Lewis Thomas Fellow, Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544
	interests:	I'm a postdoc research fellow, working on various aspects of lambda phage "life cycle". My PhD was in physics, so my previous excursions into biology have been mainly theoretical. A list of my publications can be found on my website. (contents | BEG members | top of page)	
Esmail Samiwala	PI	samiwala @wockhardtin.com	Merind Limited (A Wockhardt Enterprise), Mulund Goregaon Link Road, Bhandup (w), Mumbai 400 078, INDIA
	interests:	We manufacture vitamin B12 which happens to be a fermentation process and highly prone to bacteriophage attacks. If the manufacturing site starts showing phage attacks, steps that need to be taken to counter the problem have to be studied in detail and implemented. (contents | BEG members | top of page)	
Emma Stanley	---	sbxecs @nottingham.ac.uk	Division of Food Microbiology, Sutton Bonnington Campus, University of Nottingham, Loughborough, Leicestershire, LE12 5RD
	interests:	Deveolping a detection assay for *Mycobacterium paratuberculosis* using bacteriophage amplification. (contents | BEG members | top of page)	
James Taylor	---	Jtaylor @bio.warwick.ac.uk	Dept. Of Biology, University of Warwick, Coventry, Warwickshire. CV4 7AL
	interests:	Ecology of *Listeria monocytogenes* bacteriophages. Use of Listeriaphage in the treatment of silage and milk products. (contents | BEG members | top of page)	

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New Links

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

No Entry.

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New Features

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

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Meetings

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

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

I'm not aware of anything new since the listing in *BEG News* issue 11.

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Jobs

Looking for job? Looking to fill a position? Please send advertisement and information to abedon.1@osu.edu or to "Jobs", 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 information as text (e.g., as an e-mail) or as Microsoft Word documents, if possible (I'll let you know if I have trouble converting any other document formats), and in English. I will update this section as I receive material, regardless of what date this issue of BEG News goes live.

Click here for International Society for Microbial Ecology Employment Listings.

Click here for American Association for the Advancement of Science Employment Listings.

Click here for AAAS "Microbial Ecology" Search.

Click here for AAAS "Ecology and Microbiology" Search.

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Submissions

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

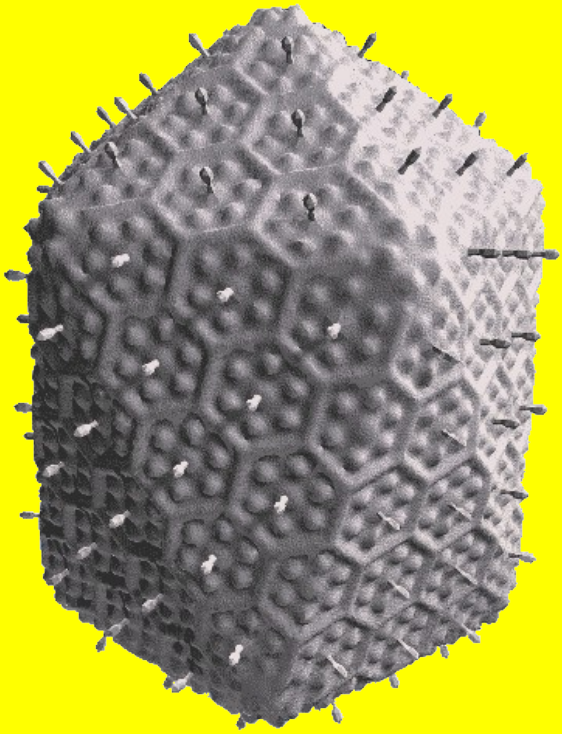
Rendering Phage Heads

by Steven McQuinn

The construction of T4 in 3D is an ongoing project. These first pictures, mostly of the phage T4 head, are high-polygon-count meshes modeled to approximate published, data-derived imagery. Researchers who are teasing out the fine details of T4 morphology, from the capsid head to the tail-fiber toes, will eventually publish more accurate meshes generated directly from their analyses. Nevertheless, there is some value in graphical interpretation, especially when showing functionality through animation. I am directing my efforts accordingly, eventually turning toward the creation of simplified low polygon meshes that can be used with interactive web3D formats such as Viewpoint.

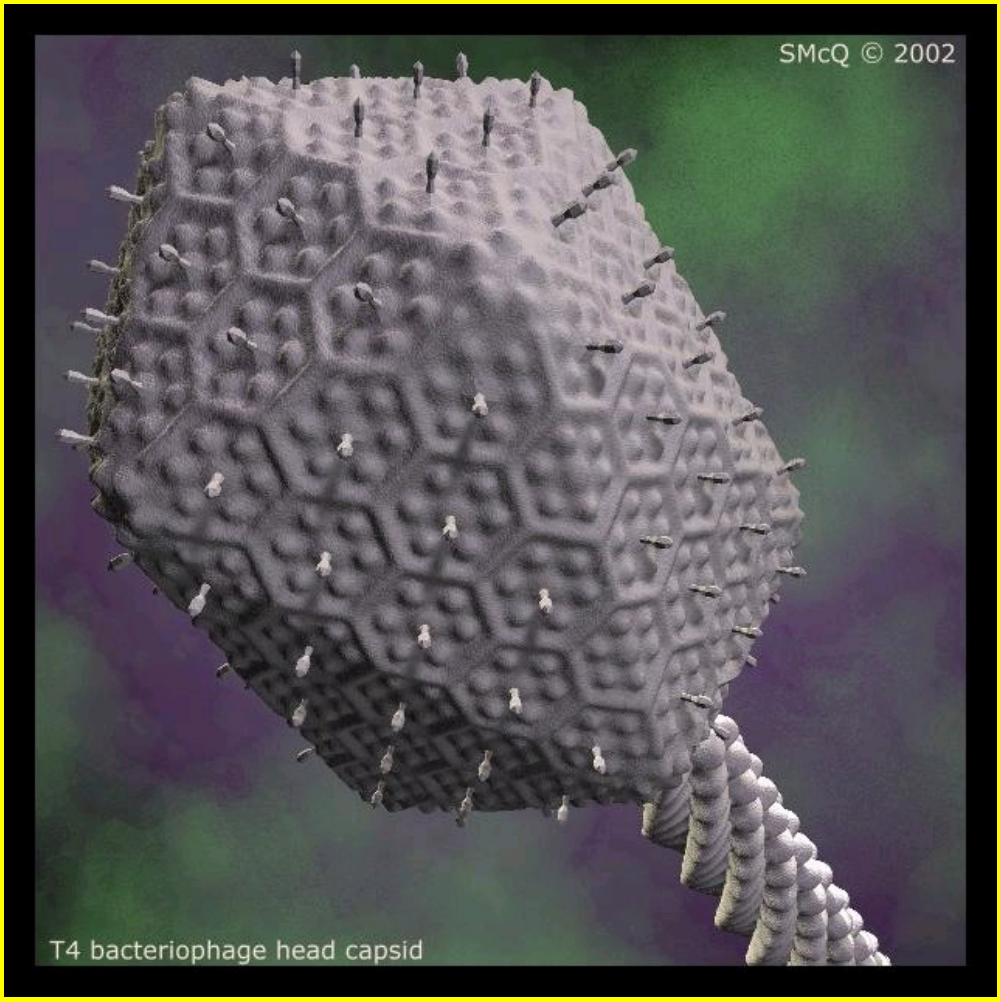
Click on the following 2D images to view in a separate window, which in newer browsers should be more-easily scaled to your monitor's resolution.

Below is a full-relief gif of T4's head on a transparent background.

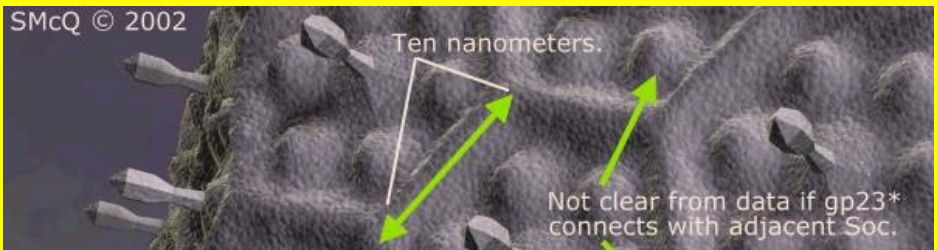


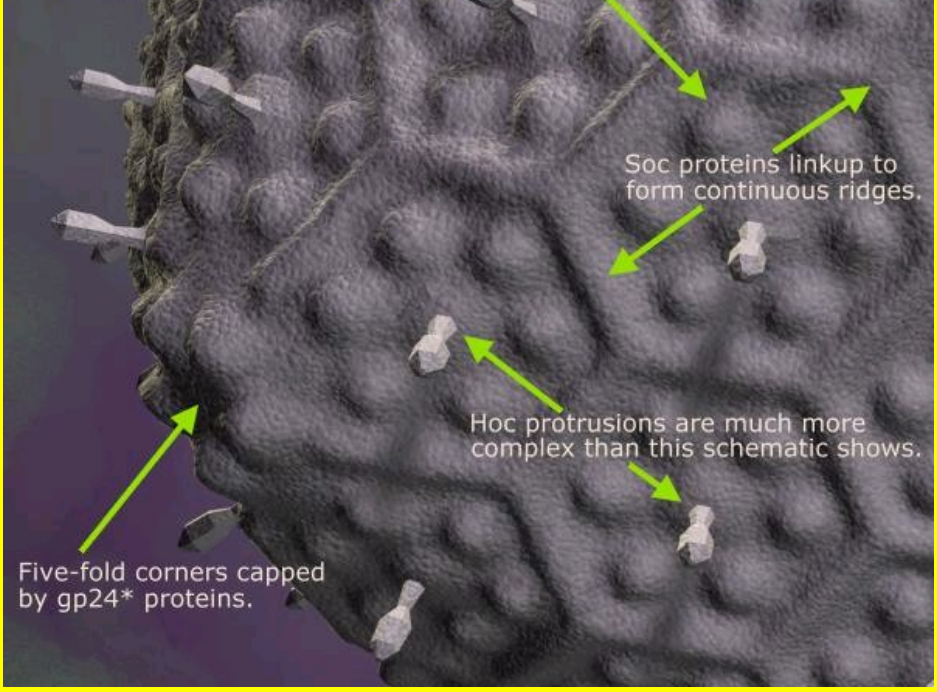
SMcQ © 2002

Below is the T4 head, with tail attached. For more on the T4 tail, see this month's phage image.



Below is a close up of the head structure with various proteins labeled.





For a paper-cutout model of the T4 icosahedral head, click here (warning, PDF file is large: 1.6 megabytes). Below is an animated rendering of the paper-cutout model.

References

1. Baschong, W.C. *et al.* (1988). Head structure of bacteriophages T2 and T4. *J. Ultrastruct. Res.* 99:189-202.
2. Mosig, G., Eiserling, F. (1988). In *The Bacteriophages* Plenum Press, New York 2:521.
3. Olson, N. *et al.* (2001). The structure of isometric capsids of bacteriophage T4. *Virology* 279:385-391.
4. Qu, C. The Icosahedral Server. mmtsb.scripps.edu/viper/chunxuqu/.

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Letters & Questions

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

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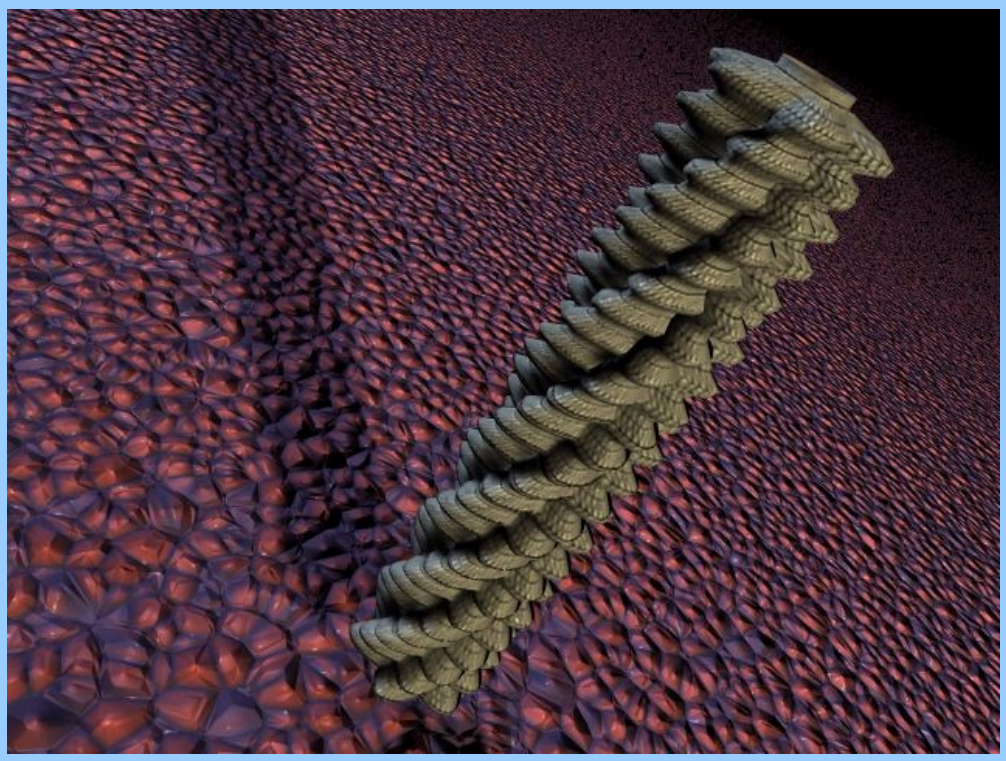
Phage Images

Please send any phage images that you would like to present in this section to "Phage Images," *The Bacteriophage Ecology Group*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Alternatively, you may scan the images yourself and send them as an attachment to abedon.1@osu.edu. Please save all scans in gif or jpg formats and preferably with an image size (in terms of width, height, and kbytes) that will readily fit on a standard web page. No copyrighted material without permission, please!



This is a modeled image of the T4 phage contractile tail sheath

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

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New Publications

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

1. Steps toward mapping the human vasculature by phage display. Arap, W., Kolonin, M. G., Trepel, M., Lahdenranta, J., Cardo-Vila, M., Giordano, R. J., Mintz, P. J., Ardelt, P. U., Yao, V. J., Vidal, C. I., Chen, L., Flamm, A., Valtanen, H., Weavind, L. M., Hicks, M. E., Pollock, R. E., Botz, G. H., Bucana, C. D., Koivunen, E., Cahill, D., Troncoso, P., Baggerly, K. A., Pentz, R. D., Do, K. A., Logothetis, C. J., Pasqualini, R. (2002). *Nature Medicine* 8:121-127. [PRESS FOR ABSTRACT]

2. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. Biswas, B., Adhya, S., Washart, P., Paul, B., Trostel, A. N., Powell, B., Carlton, R., Merril, C. R. (2002). *Infection and Immunity* 70:204-210. [PRESS FOR ABSTRACT]

3. Phage genomics: Small is beautiful. Brussow, H., Hendrix, W. (2002). *Cell* 108:13-16. [PRESS FOR ABSTRACT]

4. Evaluation of the international phage typing set and some experimental phages for typing of *Listeria monocytogenes* from poultry in Spain. Capita, R., Alonso-Calleja, C., Mereghetti, L., Moreno, B., del Camino, G. (2002). *Journal of Applied Microbiology* 92:90-96. [PRESS FOR ABSTRACT]

5. A conductance method for the identification of *Escherichia coli* O157:H7 using bacteriophage AR1. Chang, T. C., Ding, H. C., Chen, S. (2002). *Journal of Food Protection* 65:12-17. [PRESS FOR ABSTRACT]

6. Removal and inactivation of indicator bacteriophages in fresh waters. Duran, A. E., Muniesa, M., Mendez, X, Valero, F., Lucena, F., Jofre, J. (2002). *Journal of Applied Microbiology* 92:338-347. [PRESS FOR ABSTRACT]

7. Enteric bacteriophages as potential fecal indicators in ground beef and poultry meat. Hsu, F. C., Shieh, Y. S. C., Sobsey, M. D. (2002). *Journal of Food Protection* 65:93-99. [PRESS FOR ABSTRACT]

8. Identification and characterization of phage-resistance genes in temperate lactococcal bacteriophages. McGrath, S., Fitzgerald, G. F., van Sinderen, D. (2002). *Molecular Microbiology* 43:509-520. [PRESS FOR ABSTRACT]

9. Bacteriophage therapy of infectious disease in aquaculture. Nakai, T., Park, S. C. (2002). *Research in Microbiology* 153:13-18. [PRESS FOR ABSTRACT]

10. The nucleotide sequence of shiga toxin (Stx) 2e-encoding phage fP27 is not related to other Stx phage genomes, but the modular genetic structure is conserved. Recktenwald, J., Schmidt, H. (2002). *Infection and Immunity* 70:1896-1908. [PRESS FOR ABSTRACT]

11. Expression of antisense RNA targeted against *Streptococcus thermophilus* bacteriophages. Sturino, J. M., Klaenhammer, T. R. (2002). *Applied and Environmental Microbiology* 68:588-596. [PRESS FOR ABSTRACT]

12. Viruses stop antibiotic-resistant bacteria. Travis, J. (2002). *Science News* 161:???-??? [PRESS FOR ABSTRACT]

13. Isolation and characterization of bacteriophages from fermenting sauerkraut. Yoon, S. S., Barrangou-Poueys, R., Breidt, F., Jr., Klaenhammer, T. R., Fleming, H. P. (2002). *Applied and Environmental Microbiology* 68:973-976. [PRESS FOR ABSTRACT]

14. Validation of the use of gamma phage for identifying *Bacillus anthracis*. Abshire, T. G., Brown, J. E., Allan, C. M., Redus, S. L., Teska, J. D., Ezzell, J. W. (2001). *Abstracts of the General Meeting of the American Society for Microbiology* 101:176. [PRESS FOR ABSTRACT]

15. *Vibrio cholerae* VPIPHI/CTXPHI/TCP: Interactions of phage-phage-bacterium. Ai, Y.-C., Meng, F. (2001). *Weishengwu Xuebao* 41:510-512. [no abstract]

16. Isolation and characterisation of *Campylobacter*-specific bacteriophage from retail poultry. Atterbury, R., Connerton, P., Dodd, C., Rees, C., Connerton, I (2001). *International Journal of Medical Microbiology* 291:79-80. [no abstract]

17. Persistence of viral pathogens and bacteriophages during sewage treatment: Lack of correlation with indicator bacteria. Baggi, F., Demarta, A., Peduzzi, R. (2001). *Research in Microbiology* 152:743-751. [PRESS FOR ABSTRACT]

18. Luciferase reporter mycobacteriophages for detection, identification, and antibiotic susceptibility testing of *Mycobacterium tuberculosis* in Mexico. Banaiee, N., Bobadilla-del-Valle, M., Bardarov, S., Jr., Riska, P. F., Small, P. M., Ponce-de-Leon, A., Jacobs, W. R., Jr., Hatfull, G. F., Sifuentes-Osornio, J. (2001). *Journal of Clinical Microbiology* 39:3883-3888. [PRESS FOR ABSTRACT]

19. Proteins PblA and PblB of *Streptococcus mitis*, which promote binding to human platelets, are encoded within a lysogenic bacteriophage. Bensing, B. A., Siboo, I. R., Sullam, P. M. (2001). *Infection and Immunity* 69:6186-6192. [PRESS FOR ABSTRACT]

20. Bacteriophages transducing antibiotic resistance from a cluster of lysogenic strains of *Pseudomonas aeruginosa* isolated from

patients. Blahova, J., Kralikova, K., Krcmery, V., Sr., Schafer, V (2001). *Journal of Chemotherapy* 13:331-333. [no abstract]

21. Faecal bacteria and bacteriophage inactivation in a full-scale UV disinfection system used for wastewater reclamation. Bourrouet, A., Garcia, J., Mujeriego, R., Penuelas, G. (2001). *Water Science and Technology* 43:187-194. [PRESS FOR ABSTRACT]

22. Collective action in an RNA virus. Brown, S. P. (2001). *Journal of Evolutionary Biology* 14:821-828. [PRESS FOR ABSTRACT]

23. A general mechanism for viral resistance to suicide gene expression. Bull, J. J., Badgett, M. R., Molineux, I. J. (2001). *Journal of Molecular Evolution* 53:47-54. [PRESS FOR ABSTRACT]

24. Water quality improvement of treated wastewater by intermittent soil percolation. Castillo, G., Mena, M. P., Dibarrart, F., Honeyman, G. (2001). *Water Science and Technology* 43:187-190. [PRESS FOR ABSTRACT]

25. Rapid Identification of *Escherichia coli* O157:H7. Chang, T. C., Chen, S., Ding, H. C. (2001). *Official Gazette of the United States Patent and Trademark Office Patents* 1245:No. [PRESS FOR ABSTRACT]

26. Bacteriophage T4 multiplication in a glucose-limited *Escherichia coli* biofilm. Corbin, B. D., McLean, R. J. C., Aron, G. M. (2001). *Canadian Journal of Microbiology* 47:680-684. [PRESS FOR ABSTRACT]

27. Clinical and environmental isolates of *Vibrio cholerae* serogroup O141 carry the CTX phage and the genes encoding the toxin-coregulated pili. Dalsgaard, A., Serichantalergs, O., Forslund, A., Lin, W., Mekalanos, J., Mintz, E., Shimada, T., Wells, J. G. (2001). *Journal of Clinical Microbiology* 39:4086-4092. [PRESS FOR ABSTRACT]

28. Comparative genomics of lactococcal phages: insight from the complete genome sequence of *Lactococcus lactis* phage BK5-T. Desiere, F., Mahanivong, C., Hillier, A. J., Chandry, P. S., Davidson, B. E., Brussow, H. (2001). *Virology* 283:240-252. [PRESS FOR ABSTRACT]

29. Bacteriophages of *Borrelia burgdorferi* and other spirochetes. Eggers, Christian H., Casjens, Sherwood, Samuels, D. S., Saier, Milton H., Jr., Garcia-Lara, Jorge Eds (2001). *JMMB Symposium Series. The spirochetes: Molecular and cellular biology.* 35-44. [no abstract]

30. Adsorption and survival of faecal coliforms, somatic coliphages and F-specific RNA phages in soil irrigated with wastewater. Gantzer, C., Gillerman, L., Kuznetsov, M., Oron, G. (2001). *Water Science and Technology* 43:117-124. [PRESS FOR ABSTRACT]

31. Bacteriophages: Update on application as models for viruses in water. Grabow, W. O. K. (2001). *Water SA (Pretoria)* 27:251-268. [PRESS FOR ABSTRACT]

32. Cheese making with bacteriophage resistant bacteria. Hicks, C. L. (2001). *Official Gazette of the United States Patent and Trademark Office Patents* 1251:No. [PRESS FOR ABSTRACT]

33. Seasonal dynamics of viruses in an alpine lake: Importance of filamentous forms. Hofer, J. S., Sommaruga, R. (2001). *Aquatic Microbial Ecology* 26:1-11. [PRESS FOR ABSTRACT]

34. Profiles of adaptation in two similar viruses. Holder, K. K., Bull, J. J. (2001). *Genetics* 159:1393-1404. [PRESS FOR ABSTRACT]

35. Application of rapid detection for *Mycobacterium tuberculosis* with phage splitting assay. Hu, Z., Pang, M., Jin, A. (2001). *Zhonghua Jiehe He Huxi Zazhi* 24:611-613. [PRESS FOR ABSTRACT]

36. Lytic and lysogenic infection of diverse *Escherichia coli* and *Shigella* strains with a verocytotoxigenic bacteriophage. James, C. E., Stanley, K. N., Allison, H. E., Flint, H. J., Stewart, C. S., Sharp, R. J., Saunders, J. R., McCarthy, A. J. (2001). *Applied and Environmental Microbiology* 67:4335-4337. [PRESS FOR ABSTRACT]

37. Effects of disinfectants on Shiga-like toxin converting phage from enterohemorrhagic *Escherichia coli* O157 : H7. Kajiura, T, Tanaka, M., Wada, H., Ito, K., Koyama, Y., Kato, F. (2001). *Journal of Health Science* 47:203-207. [PRESS FOR ABSTRACT]

38. Phage conversion of staphylococcal bi-component toxin. Kaneko, J. (2001). *Nippon Nogeikagaku Kaishi* 75:939-947. [no abstract]

39. Behavior of restriction-modification systems as selfish mobile elements and their impact on genome evolution. Kobayashi, I (2001). *Nucleic Acids Research* 29:3742-3756. [PRESS FOR ABSTRACT]

40. The bacteriophage lambda attachment site in wild strains of *Escherichia coli*. Kuhn, J., Campbell, A. (2001). *Journal of Molecular Evolution* 53:607-614. [PRESS FOR ABSTRACT]

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106. Bacteriophage genotypically modified to delay inactivations by the host defense system. Merril, C. R., Carlton, R. M., Adhya, S. L. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1214:4125. [no abstract]

107. Antibacterial therapy with bacteriophage genotypically modified to delay inactivation by the host defense system together with an antibiotic. Merril, C. R., Carlton, R. M., Adhya, S. L. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1211:2866-2867. [no abstract]

108. Phage-resistant streptococcus. Mollet, B., Pridmore, D., Zwahien, M. C. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1211:2869. [no abstract]

109. Nucleic acid sequence and plasmids comprising at least one phage resistence mechanism, bacteria containing them and their use. Prevots, F., Tolou, S., Daloyau, M. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1206:2923. [no abstract]

110. Genetically engineered reporter bacteria for the detection of bacteriophage. Rees, C. E. D., Rostas-Mulligan, K., Park, S. F., Denyer, S. P., Anderson, G. S., Stewart, B., Jassim, S. A. A. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1208:410. [no abstract]

111. Detection of listeria by means of recombinant bacteriophages. Scherer, S., Loessner, M. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1215:2969. [no abstract]

112. A theoretical approach to structuring mechanisms in the pelagic food web. Thingstad, T. F. (1998). *Hydrobioligia* 363:59-72. [PRESS FOR ABSTRACT]

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New Publications with Abstracts

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

1. **Steps toward mapping the human vasculature by phage display. Arap, W., Kolonin, M. G., Trepel, M., Lahdenranta, J., Cardo-Vila, M., Giordano, R. J., Mintz, P. J., Ardelt, P. U., Yao, V. J., Vidal, C. I., Chen, L., Flamm, A., Valtanen, H., Weavind, L. M., Hicks, M. E., Pollock, R. E., Botz, G. H., Bucana, C. D., Koivunen, E., Cahill, D., Troncoso, P., Baggerly, K. A., Pentz, R. D., Do, K. A., Logothetis, C. J., Pasqualini, R. (2002). *Nature Medicine* 8:121-127.** The molecular diversity of receptors in human blood vessels remains largely unexplored. We developed a selection method in which peptides that home to specific vascular beds are identified after administration of a peptide library. Here we report the first in vivo screening of a peptide library in a patient. We surveyed 47,160 motifs that localized to different organs. This large-scale screening indicates that the tissue distribution of circulating peptides is nonrandom. High-throughput analysis of the motifs revealed similarities to ligands for differentially expressed cell-surface proteins, and a candidate ligand-receptor pair was validated. These data represent a step toward the construction of a molecular map of human vasculature and may have broad implications for the development of targeted therapies

2. **Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. Biswas, B., Adhya, S., Washart, P., Paul, B., Trostel, A. N., Powell, B., Carlton, R., Merril, C. R. (2002). *Infection and Immunity* 70:204-210.** Colonization of the gastrointestinal tract with vancomycin-resistant *Enterococcus faecium* (VRE) has become endemic in many hospitals and nursing homes in the United States. Such colonization predisposes the individual to VRE bacteremia and/or endocarditis, and immunocompromised patients are at particular risk for these conditions. The emergence of antibiotic-resistant bacterial strains requires the exploration of alternative antibacterial therapies, which led our group to study the ability of bacterial viruses (bacteriophages, or phages) to rescue mice with VRE bacteremia. The phage strain used in this study has lytic activity against a wide range of clinical isolates of VRE. One of these VRE strains was used to induce bacteremia in mice by intraperitoneal (i.p.) injection of 10^9 CFU. The resulting bacteremia was fatal within 48 h. A single i.p. injection of 3 x 10^8 PFU of the phage strain, administered 45 min after the bacterial challenge, was sufficient to rescue 100% of the animals. Even when treatment was delayed to the point where all animals were moribund, approximately 50% of them were rescued by a single injection of this phage preparation. The ability of this phage to rescue bacteremic mice was demonstrated to be due to the functional capabilities of the phage and not to a nonspecific immune effect. The rescue of bacteremic mice could be effected only by phage strains able to grow in vitro on the bacterial host used to infect the animals, and when such strains are heat inactivated they lose their ability to rescue the infected mice

3. **Phage genomics: Small is beautiful. Brussow, H., Hendrix, W. (2002). *Cell* 108:13-16.** The Age of Genomics dawned only gradually for bacteriophages. It was 1977 when the genome of phage phiX174 was published and 1983 when the "large" genome of phage lambda hit the streets. More recently, the pace has quickened, so that we now have over 100 complete phage genomes and can expect thousands in a very few years. These sequences have been marvelously informative for the biology of the individual phages, but with the advent of high volume sequencing technology, the real excitement for phage biology is that it is now possible to analyze the sequences together and thereby address-for the first time at whole genome resolution-a set of fundamental biological questions related to populations: What is the structure of the global phage population? What are its dynamics? How do phages evolve? This is Comparative Genomics with a capital "C"

4. **Evaluation of the international phage typing set and some experimental phages for typing of *Listeria monocytogenes* from poultry in Spain. Capita, R., Alonso-Calleja, C., Mereghetti, L., Moreno, B., del Camino, G. (2002). *Journal of Applied Microbiology* 92:90-96.** Aims: The validity of the international phage set and 13 experimental phages for subtyping *Listeria monocytogenes* strains isolated from poultry in Spain was investigated. Methods and Results: Ninety-six *L. monocytogenes* strains (52 from serogroup 1/2 and 44 from serogroup 4) were phage-typed using the international phage set, 10 experimental phages for typing serogroup 1/2 strains (seven isolated in France: 1313, 9425, 1807, 351, 881, 717 and 586-, and three from Denmark: 5775, 12682 and 6223-) and three experimental phages isolated in France for typing serogroup 4 strains (2425 A, 4286 and 197). Percentages of serogroup 1/2, serogroup 4 and total phage-typeable strains were 57.7%, 52.3% and 55.2%, respectively. Important differences in the behaviour of the phages tested were found. The typeability rate, the specificity index and the percentage of strong reactions were greater in the phages of international set than in the experimental phages. The number of phage typeable strains and the number of phage types (42) were not modified by the use of experimental phages. Conclusions: The phage set used was not effective for typing *L. monocytogenes* strains from poultry in Spain, because a low typeability rate was found. Significance and Impact of the Study: Our results suggest the importance of the availability of new phages specific to a geographical area in order to improve the typeability of the system

5. **A conductance method for the identification of *Escherichia coli* O157:H7 using bacteriophage AR1. Chang, T. C., Ding, H. C., Chen, S. (2002). *Journal of Food Protection* 65:12-17.** The feasibility of using a specific phage (AR1) in conjunction with a conductance method for the identification of *Escherichia coli* O157:H7 was evaluated. The multiplication of strains of *E. coli* O157:H7 was inhibited by AR1; therefore, a time point (detection time, DT) at which an accelerating change in conductance in the culture broth was not obtained. Bacterial strains were subcultured on sorbitol-MacConkey agar and incubated at 35°C for 24 h, and the ability of the bacteria to ferment sorbitol was recorded. An aliquot of 0.5 ml of the bacterial suspension (10^7 CFU/ml) and 0.5 ml of the phage suspension (108 PFU/ml) were added to the conductance tube of a Malthus analyzer containing 5 ml of culture broth. The tubes were incubated at 35°C, and conductance changes in the tubes were continuously monitored at 6-min intervals for 24 h by the instrument. A positive reaction was defined as an *E. coli* strain that could not utilize sorbitol and caused no conductance change (i.e., no DT) within an incubation period of 24 h. Of the 41 strains of *E. coli* O157:H7 tested, all produced positive reactions. When a total of 155 strains of non-O157:H7 *E. coli* were tested, 14 did not have a DT within 24 h. However, among these 14 strains, 13 were sorbitol fermenters, and the remaining one was a nonfermenter. Therefore, by definition, only one strain produced a false-positive reaction. The sensitivity and specificity of the present method were 100% (41 of 41) and 99.4% (154 of 155), respectively. The present method incorporating conductimetric measurement and phage AR1 for the identification of *E. coli* O157:H7 was simple and capable of automation

6. **Removal and inactivation of indicator bacteriophages in fresh waters. Duran, A. E., Muniesa, M., Mendez, X, Valero, F., Lucena, F., Jofre, J. (2002). *Journal of Applied Microbiology* 92:338-347.** Aims: The removal and inactivation of faecal coliform (FC) bacteria, enterococci (ENT), sulphite-reducing clostridia (SRC), somatic coliphages, F-specific RNA bacteriophages and bacteriophages infecting *Bacteroides fragilis* in fresh waters. Methods and Results: Removal was studied in two areas of a river. The results showed different removal of each group of microbes. Faecal coliform bacteria were removed faster than any other, whereas SRC and bacteriophages infecting *Bact. fragilis* were the most persistent. Inactivation was

measured by 'in situ' experiments, which showed significant differences in survival of the different groups of bacterial and bacteriophage indicators. The SRC and bacteriophages were more resistant than faecal coliforms and enterococci, with the exception of F-specific RNA bacteriophages in the summer. Inactivation experiments with pure cultures of bacteriophages confirmed that phage B40-8 of *Bact. fragilis* was the most resistant. Conclusions: Bacteria and bacteriophages show different resistance to natural inactivation. The use of phages allows information to be obtained in addition to that provided by bacterial indicators. Somatic coliphages and phages infecting *Bact. fragilis* might supply that indicator function. Significance and Impact of the Study: Confirmation was obtained that bacteriophages provided additional information to that provided by bacterial indicators to monitor the natural inactivation of viruses and/or pathogens

7. **Enteric bacteriophages as potential fecal indicators in ground beef and poultry meat. Hsu, F. C., Shieh, Y. S. C., Sobsey, M. D. (2002). *Journal of Food Protection* 65:93-99.** Recovery efficiencies of enteric bacteriophages (F^+ RNA coliphages, somatic coliphages, and *Salmonella* phages) as alternative fecal indicators were determined from ground beef and chicken breast meat using amino acid eluants (glycine and threonine) and a complex eluant (3% beef extract). Levels of F^+ RNA coliphages (MS2, GA, Qbeta, FI, and SP), the somatic coliphage PHIX174, and three environmental isolates of *Salmonella* phages (isolated from raw sewage) were assayed using three respective hosts: *Escherichia coli* Famp, *E. coli* C, and *Salmonella typhimurium*. When 8% polyethylene glycol and 0.1 M NaCl were used to precipitate bacteriophages eluted with five different eluants, the highest recoveries of the three phage groups were with 0.5 M threonine and 0.25 M glycine-threonine. The average recoveries of F^+ RNA coliphages, somatic coliphages, and the *Salmonella* phages from ground beef and chicken meat were 100, 69, and 65%, respectively, with threonine (0.5 M, pH 9.0) as the eluate. Of eight market food samples tested, F^+ RNA coliphages were detected in five (63%) and somatic coliphages were detected in seven (88%). The overall detection sensitivity of the method was 3 PFU/100 g of ground beef or chicken meat. Levels of bacteriophages and bacterial indicators on chicken carcass surfaces were determined at identified critical control points at a poultry plant. Through the processing steps of evisceration, washing, and chilling, the levels of F^+ RNA coliphages and fecal coliforms were reduced by 1.6 and 1.9 $\log_{10}$ PFU or CFU/100 g, respectively. F^+ RNA coliphages and perhaps other enteric bacteriophages may be effective candidate indicators for monitoring the microbiological quality of meat, poultry, and perhaps other foods during processing. The bacteriophage concentration method developed provides a simple, rapid, and practical tool for the evaluation of fecal contamination levels in ground beef and processed chicken meat

8. **Identification and characterization of phage-resistance genes in temperate lactococcal bacteriophages. McGrath, S., Fitzgerald, G. F., van Sinderen, D. (2002). *Molecular Microbiology* 43:509-520.** The sie2009 gene, which is situated between the genes encoding the repressor and integrase, on the lysogeny module of the temperate lactococcal bacteriophage Tuc2009, was shown to mediate a phage-resistance phenotype in *Lactococcus lactis* against a number of bacteriophages. The Sie2009 protein is associated with the cell membrane and its expression leaves phage adsorption, transfection and plasmid transformation unaffected, but interferes with plasmid transduction, as well as phage replication. These observations indicate that this resistance is as a result of DNA injection blocking, thus representing a novel superinfection exclusion system. A polymerase chain reaction (PCR)-based strategy was used to screen a number of lactococcal strains for the presence of other prophage-encoded phage-resistance systems. This screening resulted in the identification of two such systems, without homology to sie2009, which were shown to mediate a phage-resistance phenotype similar to that conferred by sie2009. To our knowledge, this is the first description of a phage-encoded superinfection exclusion/injection blocking mechanism in the genus *Lactococcus*

9. **Bacteriophage therapy of infectious disease in aquaculture. Nakai, T., Park, S. C. (2002). *Research in Microbiology* 153:13-18.** Bacteriophages may be candidates as therapeutic agents in bacterial infections. Here we describe the protective effects of phages against experimentally induced bacterial infections of cultured fish and discuss the potential for phage therapy in aquaculture.

10. **The nucleotide sequence of shiga toxin (Stx) 2e-encoding phage fP27 is not related to other Stx phage genomes, but the modular genetic structure is conserved. Recktenwald, J., Schmidt, H. (2002). *Infection and Immunity* 70:1896-1908.** In this study we determined the complete nucleotide sequence of Shiga toxin 2e-encoding bacteriophage fP27, isolated from the Shiga toxin-producing *Escherichia coli* patient isolate 2771/97. fP27 is integrated as a prophage in the chromosomal *yecE* gene. This integration generates identity segments of *attL* and *attR* sites with lengths of 11 nucleotides. The integrated prophage genome has a size of 42,575 bp. We identified 58 open reading frames (ORFs), each with a length of >150 nucleotides. The deduced proteins of 44 ORFs showed significant homologies to other proteins present in sequence databases, whereas 14 putative proteins did not. For 29 proteins, we could deduce a putative function. Most of these are related to the basic phage propagation cycle. The fP27 genome represents a mosaic composed of genetic elements which are obviously derived from related and unrelated phages. We identified five short linker sequences of 22 to 151 bp in the fP27 sequence which have also been detected in a couple of other lambdoid phages. These linkers are located between functional modules in the phage genome and are thought to play a role in genetic recombination. Although the overall DNA sequence of fP27 is not highly related to other known phages, the data obtained demonstrate a typical lambdoid genome structure.

11. **Expression of antisense RNA targeted against *Streptococcus thermophilus* bacteriophages. Sturino, J. M., Klaenhammer, T. R. (2002). *Applied and Environmental Microbiology* 68:588-596.** Antisense RNA complementary to a putative helicase gene (hel3.1) of a cos-type *Streptococcus thermophilus* bacteriophage was used to impede the proliferation of a number of cos-type *S. thermophilus* bacteriophages and one pac-type bacteriophage. The putative helicase gene is a component of the Sfi21-type DNA replication module, which is found in a majority of the *S. thermophilus* bacteriophages of industrial importance. All bacteriophages that strongly hybridized a 689-bp internal hel3.1 probe were sensitive to the expression of antisense hel3.1 RNA. A 40 to 70% reduction in efficiency of plaquing (EOP) was consistently observed, with a concomitant decrease in plaque size relative to that of the *S. thermophilus* parental strain. When progeny were released, the burst size was reduced. Growth curves of *S. thermophilus* NCK1125, in the presence of variable levels of bacteriophage kappa3, showed that antisense hel3.1 conferred protection, even at a multiplicity of infection of approximately 1.0. When the hel3.1 antisense RNA cassette was expressed in cis from the kappa3-derived phage-encoded resistance (PER) plasmid pTRK690::ori3.1, the EOP for bacteriophages sensitive to PER and antisense targeting was reduced to between 10^{-7} and 10^{-8}, beyond the resistance conferred by the PER element alone (less than 10^{-6}). These results illustrate the first successful applications of antisense RNA and explosive delivery of antisense RNA to inhibit the proliferation of *S. thermophilus* bacteriophages

12. **Viruses stop antibiotic-resistant bacteria. Travis, J. (2002). *Science News* 161:???-???** Nearly a century ago, biologists discovered viruses that prey upon bacteria. When penicillin and other antibiotics emerged a few decades later, however,

physicians largely abandoned their efforts to use these bacteriophages, or phages, to thwart infectious diseases. ¶ As more bacteria develop resistance to antibiotics, there's renewed interest in phages (SN: 6/3/00, p. 358). Scientists now report that these viruses can prevent mice from dying after being infected with an antibiotic-resistant bacterium.

13. **Isolation and characterization of bacteriophages from fermenting sauerkraut. Yoon, S. S., Barrangou-Poueys, R., Breidt, F., Jr., Klaenhammer, T. R., Fleming, H. P. (2002). *Applied and Environmental Microbiology* 68:973-976.** This paper presents the first report of bacteriophage isolated from commercial vegetable fermentations. Nine phages were isolated from two 90-ton commercial sauerkraut fermentations. These phages were active against fermentation isolates and selected *Leuconostoc mesenteroides* and *Lactobacillus plantarum* strains, including a starter culture. Phages were characterized as members of the Siphoviridae and Myoviridae families. All *Leuconostoc* phages reported previously, primarily of dairy origin, belonged to the Siphoviridae family

14. **Validation of the use of gamma phage for identifying *Bacillus anthracis*. Abshire, T. G., Brown, J. E., Allan, C. M., Redus, S. L., Teska, J. D., Ezzell, J. W. (2001). *Abstracts of the General Meeting of the American Society for Microbiology* 101:176.** In 1999, the CDC/APHL Laboratory Response Network to Bioterrorism selected gamma phage lysis of *Bacillus anthracis* as a specific method for identification of the bacterium. In the 1950s, Brown and Cherry originally isolated gamma phage and showed it to lyse specifically vegetative *B. anthracis*. That property has been used as the basis for an identification assay on culture plates at USAMRIID and elsewhere. We report here our results of a study to validate the assay for routine use. The primary intent of the assay is to test non-hemolytic, ground-glass-appearing bacterial colonies arising from culture of clinical or non-clinical samples on 5% sheep blood agar. Specifically, the assay was designed to demonstrate clear or partially clear circular zones of lysis on bacterial lawns at the site of gamma phage inoculation following incubation at 35+-2°C for 20 hours. The validation study tested the assay for specificity, precision, and ruggedness. When tested with 50 *B. anthracis* strains and 50 similar non-anthracis *Bacillus* species, whose identity was confirmed using whole-cell fatty acid analysis (MIDI, Inc., Newark DE), the analytical specificity was >95%. This value was intentionally low because our study design included two rare refractory *B. anthracis* strains as well as two rare susceptible non-anthracis strains, *B. cereus* ATCC 4342 and B. mycoides CDC 680. Repeatability, day-to-day precision, and analyst-to-analyst precision were superior. The assay is rugged to variations between phage lots, phage concentration, amounts of bacterial inoculum, and incubation times (as short as 8 h). The data show that the assay is satisfactory for routine use

15. ***Vibrio cholerae* VPIPHI/CTXPHI/TCP: Interactions of phage-phage-bacterium. Ai, Y.-C., Meng, F. (2001). *Weishengwu Xuebao* 41:510-512.**

16. **Isolation and characterisation of *Campylobacter*-specific bacteriophage from retail poultry. Atterbury, R., Connerton, P., Dodd, C., Rees, C., Connerton, I (2001). *International Journal of Medical Microbiology* 291:79-80.**

17. **Persistence of viral pathogens and bacteriophages during sewage treatment: Lack of correlation with indicator bacteria. Baggi, F., Demarta, A., Peduzzi, R. (2001). *Research in Microbiology* 152:743-751.** The effects of different sewage treatments on the viral contamination in rivers which receive water from treatment plants without a final sand filtration step were investigated. They were all heavily contaminated with bacteriophages and human enteric viruses (detected by single step reverse transcription amplification followed by a nested polymerase chain reaction). Bacteriophages, but not faecal indicator organisms, were correlated with viral contamination

18. **Luciferase reporter mycobacteriophages for detection, identification, and antibiotic susceptibility testing of *Mycobacterium tuberculosis* in Mexico. Banaiee, N., Bobadilla-del-Valle, M., Bardarov, S., Jr., Riska, P. F., Small, P. M., Ponce-de-Leon, A., Jacobs, W. R., Jr., Hatfull, G. F., Sifuentes-Osornio, J. (2001). *Journal of Clinical Microbiology* 39:3883-3888.** The utility of luciferase reporter mycobacteriophages (LRPs) for detection, identification, and antibiotic susceptibility testing of *Mycobacterium tuberculosis* was prospectively evaluated in a clinical microbiology laboratory in Mexico City, Mexico. Five hundred twenty-three consecutive sputum samples submitted to the laboratory during a 5-month period were included in this study. These specimens were cultivated in Middle-brook 7H9 (MADC), MGIT, and Lowenstein-Jensen (LJ) media. Of the 71 mycobacterial isolates recovered with any of the three media, 76% were detected with the LRPs, 97% were detected with the MGIT 960 method, and 90% were detected with LJ medium. When contaminated specimens were excluded from the analysis, the LRPs detected 92% (54 of 59) of the cultures. The median time to detection of bacteria was 7 days with both the LRPs and the MGIT 960 method. LRP detection of growth in the presence of p-nitro-alpha-acetylamino-beta-hydroxypropiophenone (NAP) was used for selective identification of M. tuberculosis complex (MTC) and compared to identification with BACTEC 460. Using the LRP NAP test, 47 (94%) out of 50 isolates were correctly identified as tuberculosis complex. The accuracy and speed of LRP antibiotic susceptibility testing with rifampin, streptomycin, isoniazid, and ethambutol were compared to those of the BACTEC 460 method, and discrepant results were checked by the conventional proportion method. In total, 50 MTC isolates were tested. The overall agreement between the LRP and BACTEC 460 results was 98.5%. The median LRP-based susceptibility turnaround time was 2 days (range, 2 to 4 days) compared to 10.5 days (range, 7 to 16 days) by the BACTEC 460 method. Phage resistance was not detected in any of the 243 MTC isolates tested. Mycobacteriophage-based approaches to tuberculosis diagnostics can be implemented in clinical laboratories with sensitivity, specificity, and rapidity that compare favorably with those of the MGIT 960 and BACTEC 460 methods. The phages currently provide the fastest phenotypic assay for susceptibility testing

19. **Proteins PblA and PblB of *Streptococcus mitis*, which promote binding to human platelets, are encoded within a lysogenic bacteriophage. Bensing, B. A., Siboo, I. R., Sullam, P. M. (2001). *Infection and Immunity* 69:6186-6192.** The binding of platelets by bacteria is a proposed central mechanism in the pathogenesis of infective endocarditis. Platelet binding by *Streptococcus mitis* strain SF100 (an endocarditis isolate) was recently shown to be mediated in part by the surface proteins PblA and PblB. The genes encoding PblA and PblB are clustered with genes nearly identical to those of streptococcal phages r1t, 01205, and Dp-1, suggesting that pblA and pblB might reside within a prophage. To address this possibility, cultures of SF100 were exposed to either mitomycin C or UV light, both of which are known to induce the lytic cycle of many temperate phages. Both treatments caused a significant increase in the transcription of pblA. Treatment with mitomycin C or UV light also caused a substantial increase in the expression of PblA and PblB, as detected by Western blot analysis of proteins in the SF100 cell wall. By electron microscopy, phage particles were readily visible in the supernatants from induced cultures of SF100. The phage, designated SM1, had a double-stranded DNA genome of approximately 35 kb. Southern blot analysis of phage DNA indicated that pblA and pblB were contained within the SM1 genome. Furthermore, Western blot analysis of phage proteins revealed that both PblA and PblB were present in the phage particles. These findings indicate that PblA and PblB are encoded by a lysogenic bacteriophage, which could facilitate the dissemination of these potential virulence determinants to other bacterial

pathogens

20. **Bacteriophages transducing antibiotic resistance from a cluster of lysogenic strains of *Pseudomonas aeruginosa* isolated from patients. Blahova, J., Kralikova, K., Krcmery, V., Sr., Schafer, V (2001). *Journal of Chemotherapy* 13:331-333.**

21. **Faecal bacteria and bacteriophage inactivation in a full-scale UV disinfection system used for wastewater reclamation. Bourrouet, A., Garcia, J., Mujeriego, R., Penuelas, G. (2001). *Water Science and Technology* 43:187-194.** A study was carried out to compare the inactivation of faecal bacteria and one type of bacteriophage in a full-scale UV disinfection system. The system is part of a water reclamation facility for effluent reuse in golf course and agricultural irrigation. Influent and effluent samples were taken over two sampling periods (three consecutive days in July and one day in August), with three different UV doses applied each day (ranging from 10 to 40 mWcntdots/cm2 and 20 to 80 mWcntdots/cm2 in July and August, respectively). Effluent samples were also taken from a chlorine disinfection channel (5 mg Cl2/L dose) operating in parallel to the UV system. Total coliforms (TC), faecal coliforms (FC), faecal streptoccoci (FS) and somatic coliphages (SC) were measured in each sample. F-specific RNA bacteriophages and bacteriophages of *Bacteroides fragilis* were also measured one day in July. The decay ratio observed for all the microorganisms was similar when UV doses applied were low (July), ranging from 1.15 to 1.25 log-units. This suggests that bacterial indicators may be suitable for virus inactivation control when low UV doses are applied; however, such low doses are inadequate to achieve effluent quality requirements for unrestricted irrigation. At higher UV doses (August), decay ratios for TC and FC were 3.1 and 2.8 log-units respectively, indicating that they were more susceptible to UV exposure than SC and FS, with decay ratios of 2.6 and 1.0 log-units, respectively. Nevertheless, these higher doses were also inadequate to achieve water quality requirements for unrestricted irrigation. The decay ratio of SC during chlorine disinfection was clearly lower than that of the other microorganisms. Bacteriophages of Bacteroides fragilis were more resistant to UV disinfection than SC and F-specific RNA. In fact, bacteriophages of *Bacteroides fragilis* were not affected during UV exposure. A UV dose ranging from 40 to 80 mWcntdots/cm2 marks the borderline beyond which inactivation rates of SC are clearly lower than those of bacterial indicators

22. **Collective action in an RNA virus. Brown, S. P. (2001). *Journal of Evolutionary Biology* 14:821-828.** A recent empirical study by Turner and Chao on the evolution of competitive interactions among phage virus strains revealed that a strain grown at high rates of co-infection evolved towards lowered fitness relative to an ancestral strain. The authors went on to show that the fitness pay-off matrix between the evolved and ancestral strain conforms to the prisoners' dilemma. In this paper, I use Turner and Chao's data to parameterize a simple model of parasite collective action. The prisoners' dilemma is based on pairwise interactions of a discrete cooperate/defect nature. In contrast, the collective action model explicitly deals with individual-group interactions where the extent of cooperation is a continuous variable. I argue here that the 'collective action' modelling approach is more appropriate than the prisoners' dilemma for the biology of virus evolution, and hence better able to form a predictive framework for further work on related strains of virus, linking mixing ecology, cooperative phenotype and fitness. Furthermore, the collective action model is used to motivate discussion on the evolutionary ecology of viruses, with a focus on the 'levels of selection' debate and the evolution of virulence

23. **A general mechanism for viral resistance to suicide gene expression. Bull, J. J., Badgett, M. R., Molineux, I. J. (2001). *Journal of Molecular Evolution* 53:47-54.** Bacteriophage T7 was challenged with either of two toxic genes expressed from plasmids. Each plasmid contained a different gene downstream of a T7 promoter; cells harboring each plasmid caused an infection by wild-type T7 to abort. T7 evolved resistance to both inhibitors by avoidance of the plasmid expression system rather than by blocking or bypassing the effects of the specific toxic gene product. Resistance was due to a combination of mutations in the T7 RNA polymerase and other genes expressed at the same time as the polymerase. Mutations mapped to sites that are unlikely to alter polymerase specificity for its cognate promoter but the basis for discrimination between phage and plasmid promoters *in vivo* was not resolved. A reporter assay indicated that, relative to wild-type phage, gene expression from the plasmid was diminished several-fold in cells infected by the evolved phages. A recombinant phage, derived from the original mutant but lacking a mutation in the gene for RNA polymerase, exhibited in-termediate activity in the reporter assay and intermediate resistance to the toxic gene cassettes. Alterations in both RNA polymerase and a second gene are thus responsible for resistance. These findings have broad evolutionary parallels to other systems in which viral inhibition is activated by viral regulatory signals such as defective-interfering particles, and they may have mechanistic parallels to the general phenomena of position effects and gene silencing.

24. **Water quality improvement of treated wastewater by intermittent soil percolation. Castillo, G., Mena, M. P., Dibarrart, F., Honeyman, G. (2001). *Water Science and Technology* 43:187-190.** Our research aimed to evaluate intermittent soil infiltration of treated sewage for reuse in the north of Chile. Aerated lagoon effluent was infiltrated in columns packed with native soils (sandy-lime, lime-gravel and limey-sand). Columns were operated for more than a year under different cycles of filling and drying, depths and load pressures depending on soil characteristics. The efficiency of the system was determined through influent-effluent microbiological indicators level (faecal coliforms, *E. coli*, *Salmonella* spp, MS2 phage, and protozoan cysts), physicochemical characterisation (TOC, COD, BOD, nitrogen), and hydraulic flow measurement. Results showed: (a) high reduction of enteric bacteria (5-7 log10), some inactivation of phage (2-4 $\log_{10}$) and complete removal of intestinal cyst; (b) stable removal of organic matter (80-90% reduction of TOC, COD, BOD); and (c) partial ammonia reduction through adsorption and nitrification with denitrification mainly occurring in sandy soil. Preliminary data from pilot plant working in the field showed better results that those obtained in the laboratory especially removal of microbiological indicators. Microbiological quality of effluent met Class A regulations for agricultural reuse (WHO, 1989) and the system looks like an attractive alternative to cope with water shortage in the region

25. **Rapid Identification of *Escherichia coli* O157:H7. Chang, T. C., Chen, S., Ding, H. C. (2001). *Official Gazette of the United States Patent and Trademark Office Patents* 1245:No.** A method of determining whether a test microorganism is a known microorganism, involving use of an agent that specifically affects the growth of the known microorganism. The invention also features a method of identifying E. coli O157:H7 that are based on the following criteria: a test microorganism is *E. coli* O157:H7 if the microorganism is (i) *E. coli*, (ii) incapable of fermenting sorbitol, and (iii) susceptible to infection by AR1 phage

26. **Bacteriophage T4 multiplication in a glucose-limited *Escherichia coli* biofilm. Corbin, B. D., McLean, R. J. C., Aron, G. M. (2001). *Canadian Journal of Microbiology* 47:680-684.** An *Escherichia coli* K-12 biofilm was grown at a dilution rate of 0.028 h-1 for 48 h in a glucose-limited chemostat coupled to a modified Robbins' device to determine its susceptibility to infection by bacteriophage T4. Bacteriophage T4 at a multiplicity of infection (MOI) of 10 caused a log reduction in biofilm density (expressed as colony forming units (CFU) per cm2) at 90 min postinfection. After 6 h, a net decrease and equilibrium in viral titer

was seen. When biofilms were exposed to T4 phage at a MOI of 100, viral titer doubled after 90 min. After 6 h, viral titers (expressed as plaque forming units (PFU) per cm^2) stabilized at levels approximately one order of magnitude higher than seen at a MOI of 10. Scanning confocal laser microscopy images also indicated disruption of biofilm morphology following T4 infection with the effects being more pronounced at a MOI of 100 than at a MOI of 10. These results imply that biofilms under carbon limitation can act as natural reservoirs for bacteriophage and that bacteriophage can have some influence on biofilm morphology

27. **Clinical and environmental isolates of *Vibrio cholerae* serogroup O141 carry the CTX phage and the genes encoding the toxin-coregulated pili. Dalsgaard, A., Serichantalergs, O., Forslund, A., Lin, W., Mekalanos, J., Mintz, E., Shimada, T., Wells, J. G. (2001). *Journal of Clinical Microbiology* 39:4086-4092.** We report sporadic cases of a severe gastroenteritis associated with *Vibrio cholerae* serogroup O141. Like O1 and O139 serogroup strains of *V. cholerae* isolated from cholera cases, the O141 clinical isolates carry DNA sequences that hybridize to cholera toxin (CT) gene probes. The CT genes of O1 and O139 strains are carried by a filamentous bacteriophage (termed CTX phage) which is known to use toxin-coregulated pili (TCP) as its receptor. In an effort to understand the mechanism of emergence of toxigenic O141 *V. cholerae*, we probed a collection of O141 clinical and environmental isolates for genes involved in TCP production, toxigenicity, virulence regulation, and other phylogenetic markers. The collection included strains isolated between 1964 and 1995 from diverse geographical locations, including eight countries and five U.S. states. Information collected about the clinical and environmental sources of these isolates suggests that they had no epidemiological association. All clinical O141 isolates hybridized to probes specific for genes encoding CT (ctx), zonula occludens toxin (zot), repetitive sequence 1 (RS1), RTX toxin (rtxA), the major subunit of TCP (tcpA), and the essential regulatory gene that controls expression of both CT and TCP (toxR). In contrast, all but one of the nonclinical O141 isolates were negative for ctx, zot, RS1, and tcpA, although these strains were positive for rtxA and toxR. The one toxigenic environmental O141 isolate was also positive for tcpA. Ribotyping and CT typing showed that the O141 clinical isolates were indistinguishable or closely related, while a toxigenic water isolate from Louisiana showed a distantly related ribotype. Nonclinical O141 isolates displayed a variety of unrelated ribotypes. These data support a model for emergence of toxigenic O141 that involves acquisition of the CTX phage sometime after these strains had acquired the pathogenicity island encoding TCP. The clonal nature of toxigenic O141 strains isolated from diverse geographical locations suggests that the emergence is a rare event but that once it occurs, toxigenic O141 strains are capable of regional and perhaps even global dissemination. This study stresses the importance of monitoring *V. cholerae* non-O1, non-O139 serogroup strains for their virulence gene content as a means of assessing their epidemic potential

28. **Comparative genomics of lactococcal phages: insight from the complete genome sequence of *Lactococcus lactis* phage BK5-T. Desiere, F., Mahanivong, C., Hillier, A. J., Chandry, P. S., Davidson, B. E., Brussow, H. (2001). *Virology* 283:240-252.** *Lactococcus lactis* phage BK5-T and *Streptococcus thermophilus* phage Sfi21, two cos-site temperate Siphoviridae with 40-kb genomes, share an identical genome organization, sequence similarity at the amino acid level over about half of their genomes, and nucleotide sequence identity of 60% over the DNA packaging and head morphogenesis modules. Siphoviridae with similarly organized genomes and substantial protein sequence similarity were identified in several genera of low-GC-content Gram-positive bacteria. These phages demonstrated a gradient of relatedness ranging from nucleotide sequence similarity to protein sequence similarity to gene map similarity over the DNA packaging and head morphogenesis modules. Interestingly, the degree of relatedness was correlated with the evolutionary distance separating their bacterial hosts. These observations suggest elements of vertical evolution in phages. The structural genes from BK5-T shared no sequence relationships with corresponding genes/proteins from lactococcal phages belonging to distinct lactococcal phage species, including phage sk1 (phage species 936) that showed a closely related gene map. Despite a clearly distinct genome organization, lactococcal phages sk1 and c2 showed nine sequence-related proteins. Over the early gene cluster phage BK5-T shared nine regions of high nucleotide sequence similarity, covering at most two adjacent genes, with lactococcal phage r1t (phage species P335). Over the structural genes, the closest relatives of phage r1t were not lactococcal phages belonging to other phage species, but Siphoviridae from Mycobacteria (high-GC-content Gram-positive bacteria). Evidence for recent horizontal gene transfer between distinct phage species was obtained for dairy phages, but these transfers were limited to phages infecting the same bacterial host species.

29. **Bacteriophages of *Borrelia burgdorferi* and other spirochetes. Eggers, Christian H., Casjens, Sherwood, Samuels, D. S., Saier, Milton H., Jr., Garcia-Lara, Jorge Eds (2001). *JMMB Symposium Series. The spirochetes: Molecular and cellular biology.* 35-44.**

30. **Adsorption and survival of faecal coliforms, somatic coliphages and F-specific RNA phages in soil irrigated with wastewater. Gantzer, C., Gillerman, L., Kuznetsov, M., Oron, G. (2001). *Water Science and Technology* 43:117-124.** This study was carried out to compare the adsorption and survival of faecal coliforms, somatic coliphages and F-specific RNA phages in soil irrigated with wastewater. Adsorption isotherms showed that 3-10X more faecal coliforms than somatic coliphages were adsorbed from wastewater onto soil. The adsorption behavior of F-specific RNA phages was intermediate between those of these two microorganisms. In wastewater, the inactivation factor of somatic coliphages at 8-22°C was 5-7 lower than those of faecal coliforms. F-specific RNA phages have a decrease close to faecal coliforms. In soil, at temperatures of 8-22°C and at moistures of 15-35%, somatic coliphages survived longer than the two other microorganisms. These results seemed to be confirmed by the soil column experiments. The rate of inactivation of all microorganisms was lower in soil than in wastewater and depended extensively on soil temperature and moisture content. Survival was optimal at low temperature (8°C) and low moisture content (15%). Thus, somatic coliphages seemed to be a better indicator of faecal contamination than faecal coliforms under our experimental conditions and based only on the two criteria tested (survival and adsorption). Somatic coliphages were able to contaminate the soil over greater distances and survive better in both wastewater and soil than faecal coliforms. These results need to be confirmed by studies on several soil columns using different kinds of soil and different kinds of wastewater

31. **Bacteriophages: Update on application as models for viruses in water. Grabow, W. O. K. (2001). *Water SA (Pretoria)* 27:251-268.** Phages are valuable models or surrogates for enteric viruses because they share many fundamental properties and features. Among these are structure, composition, morphology, size and site of replication. Even though they use different host cells, coliphages and *Bacteroides fragilis* phages predominantly replicate in the gastro-intestinal tract of humans and warm-blooded animals where enteric viruses also replicate. A major advantage of phages is that, compared to viruses, they are detectable by simple, inexpensive and rapid techniques. In view of these features, phages are particularly useful as models to assess the behaviour and survival of enteric viruses in the environment, and as surrogates to assess the resistance of human viruses to water treatment and disinfection processes. Since there is no direct correlation between numbers of phages and viruses, phages cannot to a meaningful extent be used to indicate numbers of viruses in polluted water. The presence of phages typically associated with human and animal excreta indicates the potential presence of enteric viruses. However, the absence of these phages from water environments is generally a meaningful indication of the absence of enteric viruses. This is because

phages such as somatic coliphages, F-RNA coliphages and *B. fragilis* phages generally outnumber enteric viruses in water environments, and they are at least as resistant to unfavourable conditions including those in water treatment and disinfection processes. However, using highly sensitive molecular techniques viruses have been detected in drinking water supplies which yielded negative results in conventional tests for phages. Initially, data on phages were rather confusing because a wide variety of techniques was used. However, techniques for the detection of phages are being standardised internationally. This applies in particular to somatic and F-RNA coliphages, and *B. fragilis* phages, which are most commonly used in water quality assessment. Reliable and practical techniques now available include direct quantitative plaque assays on samples of water up to 100 ml, and qualitative tests on 500 ml or more using highly sensitive enrichment procedures

32. **Cheese making with bacteriophage resistant bacteria. Hicks, C. L. (2001). *Official Gazette of the United States Patent and Trademark Office Patents* 1251:No.** A method is provided for reducing or preventing bacteriophage attack on bacteria used in a cheese making process. The method includes (a) treating a blocker peptide precursor with a protease enzyme that hydrolyzes the precursor to produce blocker peptides; (b) collecting the blocker peptides so produced; (c) formulating a starter media with the blocker peptides; (d) growing bulk cultures of cheese making bacteria in the inoculated starter media; and (e) adding bacteria grown in the inoculated starter media to a fermentation medium for producing cheese. The present invention also includes a method of making cheese and cheese produced by the method

33. **Seasonal dynamics of viruses in an alpine lake: Importance of filamentous forms. Hofer, J. S., Sommaruga, R. (2001). *Aquatic Microbial Ecology* 26:1-11.** Viruses are an important component of the planktonic food web in freshwater and marine systems, but most studies have been done in the ocean and in lowland lakes. In this work, the seasonal dynamics and structure of the virioplankton as well as their impact on bacteria during a day/night cycle were studied in an alpine lake located 2417 m above sea level. The abundance of virus-like particles (VLP) was determined at 5 discrete depths (0.5 to 8 m) by direct counts with a TEM in samples collected from May to November 1998 at weekly to bi-weekly intervals. Viruses reached the highest abundances under ice (4.6 X 10^6 VLP ml-1) with a second maximum in autumn. After ice-break, the VLP abundance decreased to undetectable values (<2 X 10^4 VLP ml-1) probably because of the negative effect of solar radiation that was negatively correlated with the viral abundance in the upper 2 m of the water column (Spearman rank correlation, rs = -0.773, $p < 0.01$). The virioplankton was morphologically diverse, consisting of forms commonly found in other aquatic systems, but unlike other studies, we found filamentous VLP (FVLP) 450 to 730 nm long that attained abundances of up to 1.3 X 10^6 ml-1 and accounted for 7 to 100% of the total viral abundance. These FVLP were found occasionally inside filamentous heterotrophic bacteria (> 10 mum) and their respective abundances were positively correlated (rs = 0.728, $p < 0.01$). The absence of these conspicuous forms in other aquatic ecosystems suggests that FVLP are well adapted to the harsh environmental conditions or are specific to bacterial hosts found in alpine lakes. Finally, between 5 and 28% of the newly produced bacteria were killed by non-filamentous viruses, which therefore are a modest cause of bacterial mortality in this lake

34. **Profiles of adaptation in two similar viruses. Holder, K. K., Bull, J. J. (2001). *Genetics* 159:1393-1404.** The related bacteriophages variant phiX174 and G4 were adapted to the inhibitory temperature of 44° and monitored for nucleotide changes throughout the genome. Phage were evolved by serial transfer at low multiplicity of infection on rapidly dividing bacteria to select genotypes with the fastest rates of reproduction. Both phage showed overall greater fitness effects per substitution during the early stages of adaptation. The fitness of variant phiX174 improved from -0.7 to 5.6 doublings of phage concentration per generation. Five missense mutations were observed. The earliest two mutations accounted for 85% of the ultimate fitness gain. In contrast, G4 required adaptation to the intermediate temperature of 41.5degree before it could be maintained at 44°. Its fitness at 44° increased from -2.7 to 3.2, nearly the same net gain as in variant phiX174, but with three times the opportunity for adaptation. Seventeen mutations were observed in G4: 14 missense, 2 silent, and 1 intergenic. The first 3 missense substitutions accounted for over half the ultimate fitness increase. Although the expected pattern of periodic selective sweeps was the most common one for both phage, some mutations were lost after becoming frequent, and long-term polymorphism was observed. This study provides the greatest detail yet in combining fitness profiles with the underlying pattern of genetic changes, and the results support recent theories on the range of fitness effects of substitutions fixed during adaptation

35. **Application of rapid detection for *Mycobacterium tuberculosis* with phage splitting assay. Hu, Z., Pang, M., Jin, A. (2001). *Zhonghua Jiehe He Huxi Zazhi* 24:611-613.** Objective: To study the significance of rapid identification for *Mycobacterium tuberculosis* with phage splitting assay. Methods: Strains of *Mycobacterium tuberculosis*, non-tuberculosis mycobacterium, non-mycobacterium and samples of sputum with pulmonary tuberculosis were rapidly detected by phage spot technique. Results: The strains of *Mycobacterium tuberculosis* H37Rv, bovis and africanum were all positive. The results of 10 strains of non-tuberculosis mycobacterium and 7 strains of non-mycobacterium were negative. All of 30 clinical isolates from the patients of the pulmonary tuberculosis were positive. 19 of 20 sputum specimen of pulmonary tuberculosis, which were all positive detected by smear and culture, were positive. There were 15 specimen positive in 21 sputum with negative tested by smear and positive by culture. Besides, 5 of 19 sputum specimen with negative by smear and culture were positive detected by this method. Conclusion: The phage splitting assay can be used for rapid identification of *Mycobacterium tuberculosis*, which possesses high specificity and sensitivity for detection of *Mycobacterium tuberculosis* in sputum specimen

36. **Lytic and lysogenic infection of diverse *Escherichia coli* and *Shigella* strains with a verocytotoxigenic bacteriophage. James, C. E., Stanley, K. N., Allison, H. E., Flint, H. J., Stewart, C. S., Sharp, R. J., Saunders, J. R., McCarthy, A. J. (2001). *Applied and Environmental Microbiology* 67:4335-4337.** A verocytotoxigenic bacteriophage isolated from a strain of enterohemorrhagic *Escherichia coli* O157, into which a kanamycin resistance gene (aph3) had been inserted to inactivate the verocytotoxin gene (vt2), was used to infect Enterobacteriaceae strains. A number of *Shigella* and *E. coli* strains were susceptible to lysogenic infection, and a smooth *E. coli* isolate (O107) was also susceptible to lytic infection. The lysogenized strains included different smooth *E. coli* serotypes of both human and animal origin, indicating that this bacteriophage has a substantial capacity to disseminate verocytotoxin genes. A novel indirect plaque assay utilizing an *E. coli* recA441 mutant in which phage-infected cells can enter only the lytic cycle, enabling detection of all infective phage, was developed

37. **Effects of disinfectants on Shiga-like toxin converting phage from enterohemorrhagic *Escherichia coli* O157 : H7. Kajiura, T, Tanaka, M., Wada, H., Ito, K., Koyama, Y., Kato, F. (2001). *Journal of Health Science* 47:203-207.** Inactivation of free phage carrying slt2 from enterohemorrhagic *Escherichia coli* (*E. coli*) O157 : H7 by four kinds of common disinfectants in Japan was examined under conditions with (dirty) and without (clean) interfering substance. Ethanol (EtOH) inactivated the phage within one minute under both conditions. The effect of sodium hypochlorite (NaOCl) on this phage decreased under the dirty condition, but was potentiated by increasing the concentration and contact time to the degree that could be sufficient for practical use. Use of benzalkonium chloride (BAC) at a high concentration: 0.2%, would be effective. Alkyldiaminoethylglycine hydrochloride (DAG) was not effective on this phage

38. **Phage conversion of staphylococcal bi-component toxin. Kaneko, J. (2001). *Nippon Nogeikagaku Kaishi* 75:939-947.**

39. **Behavior of restriction-modification systems as selfish mobile elements and their impact on genome evolution. Kobayashi, I (2001). *Nucleic Acids Research* 29:3742-3756.** Restriction-modification (RM) systems are composed of genes that encode a restriction enzyme and a modification methylase. RM systems sometimes behave as discrete units of life, like viruses and transposons. RM complexes attack invading DNA that has not been properly modified and thus may serve as a tool of defense for bacterial cells. However, any threat to their maintenance, such as a challenge by a competing genetic element (an incompatible plasmid or an allelic homologous stretch of DNA, for example) can lead to cell death through restriction breakage in the genome. This post-segregational or post-disturbance cell killing may provide the RM complexes (and any DNA linked with them) with a competitive advantage. There is evidence that they have undergone extensive horizontal transfer between genomes, as inferred from their sequence homology, codon usage bias and GC content difference. They are often linked with mobile genetic elements such as plasmids, viruses, transposons and integrons. The comparison of closely related bacterial genomes also suggests that, at times, RM genes themselves behave as mobile elements and cause genome rearrangements. Indeed some bacterial genomes that survived post-disturbance attack by an RM gene complex in the laboratory have experienced genome rearrangements. The avoidance of some restriction sites by bacterial genomes may result from selection by past restriction attacks. Both bacteriophages and bacteria also appear to use homologous recombination to cope with the selfish behavior of RM systems. RM systems compete with each other in several ways. One is competition for recognition sequences in post-segregational killing. Another is super-infection exclusion, that is, the killing of the cell carrying an RM system when it is infected with another RM system of the same regulatory specificity but of a different sequence specificity. The capacity of RM systems to act as selfish, mobile genetic elements may underlie the structure and function of RM enzymes

40. **The bacteriophage lambda attachment site in wild strains of *Escherichia coli*. Kuhn, J., Campbell, A. (2001). *Journal of Molecular Evolution* 53:607-614.** The attachment site (attlambda) of bacteriophage lambda was examined in wild strains of *Escherichia coli*. Although the att region is non-coding, the DNA sequence was invariant in the 13 strains examined. Two other non-coding regions showed nine changes, all associated with a single strain. In four of 33 strains, sequences were inserted in or near the attlambda site and in two of these the insert was related to lambda. Among strains that can be lysogenized by lambda, integration was via the attlambda site in all cases. Some resistant strains can be lysogenized, and these have been termed "lenient". Most of these fail to give normal phage yield after induction. In some cases rare lysogens have been formed in cells that belong to a mutant sub-population

41. **Vibrio detection by 6 species of bacteriophages of Vibrionaceae. Lin, Y., Chen, K., Ou, J. (2001). *Zhonghua Weishengwuxue He Mianyixue Zazhi* 21:108-110.** Objective: To predigest the detection procedure of 6 species of pathogenic bacteria of Vibrionaceae. Methods: Six species of specific bacteriophages were isolated and filtered to identify and type corresponding pathogenic bacteria. Results: 440 strains of bacteriophages, among which 54 strains were selected to form the typing bacteriophage groups, were isolated from 1400 environmental specimens. The typing bacteriophage groups could identify and type 80%-90% of bacteria in laboratory or those from environment. Conclusion: The application of specific bacteriophages to identify the corresponding pathogenic bacteria is fast, economic and easy to operate, so bacteriophage typing is an effective method in epidemiology investigation

42. **The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary. Lipp, E. K., Kurz, R., Vincent, R., Rodriguez-Palacios, C., Farrah, S. R., Rose, J. B. (2001). *Estuaries* 24:266-276.** The Charlotte Harbor estuary in southwest Florida was sampled monthly for one year at twelve stations, in the lower reaches of the Myakka and Peace Rivers. The objectives of the study were to address the distribution and seasonal changes in microbial indicators and human pathogen levels in Charlotte Harbor shellfish and recreational waters, and to determine those factors that may be important in the transport and survival of pathogens. Monthly water samples and quarterly sediment samples were analyzed for fecal coliform bacteria, enterococci, *Clostridium perfringens*, and coliphage. Quarterly samples also were analyzed for the enteric human pathogens, *Cryptosporidium* spp., *Giardia* spp., and enteroviruses. Fecal indicator organisms were generally concentrated in areas of low salinity and high densities of septic systems; however, pollution became widespread during wet weather in the late fall and winter of 1997-1998, coincident with a strong El Nino event. Between December 1997 and February 1998, enteroviruses were detected at 75% of the sampling stations; none were detected in other months. Enteric protozoa were detected infrequently and were not related to seasonal influences. Fecal indicators and enteroviruses were each significantly associated with rainfall, streamflow, and temperature. Regression models suggest that temperature and rainfall can predict the occurrence of enteroviruses in 93.7% of the cases. Based on findings in this watershed, factors such as variability in precipitation, streamflow, and temperature show promise in modeling and forecasting periods of poor coastal water quality

43. **Isolation and characterisation of *Campylobacter* bacteriophage from free-range chicken farm. Loc-Carrillo, C. M., Connerton, P., Dodd, C., Rees, C., Connerton, I (2001). *International Journal of Medical Microbiology* 291:79.**

44. **Production and release of Shiga toxin from *Shigella dysenteriae* 1. McDonough, M. A., Butterton, J. R. (2001). *Abstracts of the General Meeting of the American Society for Microbiology* 101:102.** Phage biology is critical to the regulation and release of Shiga toxin (Stx) from Stx-producing *E. coli* (STEC) lysogens. In the STEC phages 933W and H19B, the stx operon lies downstream of the Q gene, whose protein product acts as a transcriptional activator of late phage genes by antiterminating transcription that initiates at the late promoter pR'. Putative pR' and transcription terminator tR' sites are found upstream of the toxin genes in 933W and H19B. Q-stimulated transcription from pR' through the toxin genes to the lysis genes following prophage induction has been proposed to link phage induction and subsequent bacterial lysis of strains with enhanced toxin production and release. However, stx expression from the defective Stx-encoding prophage in *S. dysenteriae* 1 is different. Our prior work has demonstrated that the Q, pR' and tR' regions have been deleted in *S. dysenteriae* 1, but the sequences from the toxin genes through the lysis genes have been conserved, suggesting that there has been selective pressure for maintaining the linkage of the toxin to the lysis cassette. To investigate if the the lysis genes are functional in *S. dysenteriae* 1, the lysis gene S and endolysin gene R were placed under the inducible araBAD promoter, and the resulting plasmid pMAM29 was placed into *S. dysenteriae* 1 strain 3818T. After induction with arabinose, the OD600 of a 3818T(pMAM29) culture rapidly decreased and thereafter did not increase further. In the absence of arabinose, the growth of the strain was unimpeded, confirming that arabinose induction had elicited cell lysis. Additionally, strain 3818T carrying control plasmid pBAD neither lysed nor showed any growth inhibition in the presence of arabinose. To examine if deletion of the lysis genes decreases lysis of *S. dysenteriae* 1 during in vitro growth, or alters the location or release of Stx, a defined deletion of the S and R genes in the S. dysenteriae 1 genome was made using in vivo marker exchange. These studies may allow a better understanding of the molecular mechanisms underlying toxin-mediated mucosal injury following *S. dysenteriae* 1 infection

45. **Novel in vivo use of a polyvalent *Streptomyces* phage to disinfest *Streptomyces* scabies-infected seed potatoes. McKenna, F., El-Tarabily, K. A., Hardy, G. E. S. T., Dell, B. (2001). *Plant Pathology (Oxford)* 50:666-675.** A highly virulent and polyvalent Streptomyces phage was isolated from a potato field near Albany, Western Australia. The efficacy of the isolated phage to disinfest seed potato tubers artificially inoculated with a common scab-causing streptomycete was evaluated. The phage suspension was prepared in a mini-bioreactor. Diseased potatoes were bathed in a phage suspension ($1X10^9$ plaque-forming units per mL) for 24 h. The suspension was constantly circulated within a novel 25 L phage bath by means of an air-sparging pipe driven from an air compressor. Phage-treated scab-affected seed potatoes planted into free-draining polystyrene boxes containing steam-pasteurized field soil produced tuber progeny with significantly ($P<0.05$) reduced levels of surface lesions of scab (1.2%) compared with tubers harvested from nonphage-treated tubers (23%). The number of scab lesions was also significantly reduced ($P<0.05$) by phage treatment of mother tubers. No significant differences were recorded in weight, size or number of harvested tubers from phage-treated or nontreated mother tubers. This is the first in vivo study that has used Streptomyces phage to significantly disinfest seed potatoes of Streptomyces scabies and thereby reduce contamination of soil from seed-tuber-borne inoculum and reduce infection of daughter tubers

46. **Evidence for bacteriophages within gram-negative cocci: Obligate endoparasitic bacteria of *Naegleria* sp. Michel, R., Schmid, E. N., Gmeiner, G., Mueller, K. D., Hauroeder, B. (2001). *Acta Protozoologica* 40:229-232.** Gram-negative cocci observed as endocytobionts within the cytoplasm of a *Naegleria* strain isolated from a garden pond harboured small hexagonal particles of about 70 nm identified as bacteriophages called "Neo-Ph/2". These phages resembled the recently described phages; strain "Neo-Ph/1" observed for the first time within the Chlamydia-like endocytobiont *Neochlamydia hartmannellae* (Parachlamydiaceae) multiplying within *Hartmannella vermiformis* (Schmid et al. 2001). The possible reasons for this obvious similarity are object for discussion in this article

47. **Effects of bacteriophages on the population dynamics of four strains of pelagic marine bacteria. Middelboe, M., Hagstrom, A., Blackburn, N., Sinn, B., Fischer, U., Borch, N. H., Pinhassi, J., Simu, K., Lorenz, M. G. (2001). *Microbial Ecology* 42:395-406.** Viral lysis of specific bacterial populations has been suggested to be an important factor for structuring marine bacterioplankton communities. In the present study, the influence of bacteriophages on the diversity and population dynamics of four marine bacterial phage-host systems was studied experimentally in continuous cultures and theoretically by a mathematical model. By use of whole genome DNA hybridization toward community DNA, we analyzed the dynamics of individual bacterial host populations in response to the addition of their specific phage in continuous cultures of mixed bacterial assemblages. In these experiments, viral lysis had only temporary effects on the dynamics and diversity of the individual bacterial host species. Following the initial lysis of sensitive host cells, growth of phage-resistant clones of the added bacteria resulted in a distribution of bacterial strains in the phage-enriched culture that was similar to that in the control culture without phages after about 50-60 h incubation. Consequently, after a time frame of 5-10 generations after lysis, it was the interspecies competition rather than viral lysis of specific bacterial strains that was the driving force in the regulation of bacterial species composition in these experiments. The clonal diversity, on the other hand, was strongly influenced by viral activity, since the clonal composition of the four species in the phage-enriched culture changed completely from phage-sensitive to phage-resistant clones. The model simulation predicted that viral lysis had a strong impact on the population dynamics, the species composition, and the clonal composition of the bacterial community over longer time scales (weeks). However, according to the model, the overall density of bacteria in the system was not affected by phages, since resistant clones complemented the fluctuations caused by viral lysis. Based on the model analysis, we therefore suggest that viral lysis can have a strong influence on the dynamics of bacterial populations in planktonic marine systems

48. **Environmental bacteriophage-host interactions: Factors contribution to natural transduction. Miller, R. V. (2001). *Antonie van Leeuwenhoek* 79:141-147.** Over the past two decades the potential for the exchange of bacterial genes in natural environments through transduction (bacteriophage-mediated gene transfer) has been well established. Studies carried out by various laboratories throughout the world have demonstrated that both chromosomal and plasmid DNA can be successfully transduced in natural environments ranging from sewer plants to rivers and lakes. Transduction has been shown to take place in the gills of oysters and the kidneys of mice. Model studies have demonstrated the ability of transduction to maintain genetic material in bacterial gene pools that would otherwise be lost because of negative fitness. Thus, transduction may affect the course of bacterial evolution. Identification of natural transduction has led to the investigation of the dynamics of bacteriophage host interactions in natural aquatic environments and to the exploration of various environmental factors that affect virus-host interactions. Two important environmental factors which affect virus-host interactions are the metabolic state of the host and the exposure of the host to DNA-damaging stresses such as solar UV light. Recent researches on these two areas of virus-host relationships are reviewed

49. **Bacteriophage biology and Kenneth Schaffner's rendition of developmentalism. Morgan, G. J. (2001). *Biology & Philosophy* 16:85-92.** In this paper I consider Kenneth Schaffner's (1998) rendition of "developmentalism" from the point of view of bacteriophage biology. I argue that the fact that a viable phage can be produced from purified DNA and host cellular components lends some support to the anti-developmentalist, if they first show that one can draw a principled distinction between genetic and environmental effects. The existence of host-controlled phage host range restriction supports the developmentalist's insistence on the parity of DNA and environment. However, in the case of bacteriophage, the developmentalist stands on less firm ground than when organisms with nervous systems, such as Schaffner's *C. elegans*, are considered

50. **A visualization method of filamentous phage infection and phage-derived proteins in *Escherichia coli* using biotinylated phages. Nakamura, M., Tsumoto, K., Ishimura, K., Kumagai, I (2001). *Biochemical & Biophysical Research Communications* 289:252-256.** Direct visualization of filamentous phage infection in *Escherichia coli* (*E. coli*) was attempted using biotinylated phages (BIO-phages). The biotinylation of the phages did not influence their infectivity into *E. coli*. *E. coli* infected with BIO-phages could be detected by using fluorescein-conjugated avidin with confocal laser scanning microscopy, and BIO-phages and BIO-phage-derived proteins in *E. coli* could be directly observed by using the avidin-biotin-peroxidase complex method with electron microscopy. This is the first report of direct visualization of phage infection and phage-derived proteins in the host cell using a biotin-avidin interaction. This simple and powerful method is applicable to the study of infection by various viruses

51. **Presence of an inducible phage in an avian pathogenic *Escherichia coli* (APEC) strain. Nassar, A., Schouler, C., Dho-Moulin, M. (2001). *Abstracts of the General Meeting of the American Society for Microbiology* 101:114.** Avian pathogenic *Escherichia coli* (APEC) are one of the most important world wide bacterial pathogens causing extra-intestinal disease in chickens, turkeys, and other avian species. Even various potential virulence factors have been identified, the pathogenic mechanism of colibacillosis induced by APEC strains has not yet been elucidated. To identify nucleic acid sequences and

possible ORFs from the APEC strain MT512 (O2:K1) that are absent in the non pathogenic strain EC79 (O2:K-), a genomic subtraction was performed. Subtracted fragments specific for the pathogenic strain were obtained, of which one showed homology to phage 21 which is present in some strains of the ECOR collection. Mitomycin C induction experiments showed that a prophage could be induced from the APEC strain MT512 as well as from the pathogenic ECOR strains EC32 and EC53 harboring phage 21. Electron microscopy of purified phages revealed that they could belong to the Siphoviridae family. Restriction analysis of genomic phage DNA of the APEC strain and of the pathogenic ECOR strains showed that the molecular size of the double-stranded phage genome is approximately 40 kb. Restriction profile and sequence analysis of phages DNAs showed identity between phages of the APEC strain and those of the ECOR strains. The possible role of this phage in acquisition of virulence factors by the APEC strain MT512 is under investigation

52. **DNA inversion in the tail fiber gene alters the host range specificity of carotovoricin Er, a phage-tail-like bacteriocin of phytopathogenic *Erwinia carotovora* subsp. carotovora Er. Nguyen, H. A., Tomita, T., Hirota, M., Kaneko, J., Hayashi, T., Kamio, Y. (2001). *Journal of Bacteriology* 183:6274-6281.** Carotovoricin Er is a phage-tail-like bacteriocin produced by *Erwinia carotovora* subsp. carotovora strain Er, a causative agent for soft rot disease in plants. Here we studied binding and killing spectra of carotovoricin Er preparations for various strains of the bacterium (strains 645Ar, EC-2, N786, and P7) and found that the preparations contain two types of carotovoricin Er with different host specificities; carotovoricin Era possessing a tail fiber protein of 68 kDa killed strains 645Ar and EC-2, while carotovoricin Erb with a tail fiber protein of 76 kDa killed strains N786 and P7. The tail fiber proteins of 68 and 76 kDa had identical N-terminal amino acid sequences for at least 11 residues. A search of the carotovoricin Er region in the chromosome of strain Er indicated the occurrence of a DNA inversion system for the tail fiber protein consisting of (i) two 26-bp inverted repeats inside and downstream of the tail fiber gene that flank a 790-bp fragment and (ii) a putative DNA invertase gene with a 90-bp recombinational enhancer sequence. In fact, when a 1,400-bp region containing the 790-bp fragment was amplified by a PCR using the chromosomal DNA of strain Er as the template, both the forward and the reverse nucleotide sequences of the 790-bp fragment were detected. DNA inversion of the 790-bp fragment also occurred in *Escherichia coli* DH5alpha when two compatible plasmids carrying either the 790-bp fragment or the invertase gene were contrasformed into the bacterium. Furthermore, hybrid carotovoricin CGE possessing the tail fiber protein of 68 or 76 kDa exhibited a host range specificity corresponding to that of carotovoricin Era or Erb, respectively. Thus, a DNA inversion altered the C-terminal part of the tail fiber protein of carotovoricin Er, altering the host range specificity of the bacteriocin

53. **Naturally occurring lactococcal plasmid pAH90 links bacteriophage resistance and mobility functions to a food-grade selectable marker. O'Sullivan, D., Ross, R. P., Twomey, D. P., Fitzgerald, G. F., Hill, C., Coffey, A. (2001). *Applied and Environmental Microbiology* 67:929-937.** The bacteriophage resistance plasmid pAH90 (26,490 bp) is a natural cointegrate plasmid formed via homologous recombination between the type I restriction-modification specificity determinants (hsdS) of two smaller lactococcal plasmids, pAH33 (6,159 bp) and pAH82 (20,331 bp), giving rise to a bacteriophage-insensitive mutant following phage challenge (D. O'Sullivan, D. P. Twomey, A. Coffey, C. Hill, G. F. Fitzgerald, and R. P. Ross, Mol. Microbiol. 36:866-876; 2000). In this communication we provide evidence that the recombination event is favored by phage infection. The entire nucleotide sequence of plasmid pAH90 was determined and found to contain 24 open reading frames (ORFs) responsible for phenotypes which include restriction-modification, phage adsorption inhibition, plasmid replication, cadmium resistance, cobalt transport, and conjugative mobilization. The cadmium resistance property, encoded by the cadA gene, which has an associated regulatory gene (cadC), is of particular interest, as it facilitated the selection of pAH90 in other phage-sensitive lactococci after electroporation. In addition, we report the identification of a group II self-splicing intron bounded by two exons which have the capacity to encode a relaxase implicated in conjugation in gram-positive bacteria. The functionality of this intron was evident by demonstrating splicing in vivo. Given that pAH90 encodes potent phage defense systems which act at different stages in the phage lytic cycle, the linkage of these with a food-grade selectable marker on a replicon that can be mobilized among lactococci has significant potential for natural strain improvement for industrial dairy fermentations which are susceptible to phage inhibition

54. **Filamentous bacteriophage stability in non-aqueous media. Olofsson, L., Ankarloo, J., Andersson, P. O., Nicholls, I. A. (2001). *Chemistry & Biology (London)* 8:661-671.** Background: Filamentous bacteriophage are used as general cloning vectors as well as phage display vectors in order to study ligand-receptor interactions. Exposure to biphasic chloroform-water interface leads to specific contraction of phage, to non-infective I- or S-forms. Results: Upon exposure, phage were inactivated (non-infective) at methanol, ethanol and l-propanol concentrations inversely dependent upon alcohol hydrophobicity. Infectivity loss of phage at certain concentrations of l-propanol or ethanol coincided with changes in the spectral properties of the fl virion in ultraviolet fluorescence and circular dichroism studies. Conclusions: The alcohols inactivate filamentous phage by a general mechanism-solvation of coat protein-thereby disrupting the capsid in a manner quite different from the previously reported I- and S-forms. The infectivity retention of phagemid pG8H6 in 99% acetonitrile and the relatively high general solvent resistance of the phage strains studied here open up the possibility of employing phage display in non-aqueous media

55. **[Stability of *Lactococcus lactis* phages treated with sodium hypochlorite and during storage]. Parada, J. L., de, Fabrizio SV (2001). *Revista Argentina de Microbiologia* 33:89-95.** Survival of lytic bacteriophages active against *Lactococcus lactis* ssp. lactis and ssp. cremoris was determined after treatment with sodium hypochlorite and during storage at 4 degrees C. Three phages were isolated from dairy plants in Argentina (ARG) and the other phages were isolated in the United States of America (US). All of them represent phages that infected cheese manufacture industries and belong to different morphological or serological groups. These phages showed higher survival in M17 broth, buffered with sodium glycerophosphate, than in trypteine soy broth (TSB). Phage populations did not decrease significantly during 14 weeks in M17 broth, whereas in TSB the titers of phage suspensions began to decline around 9 days. In addition, the effect of sodium hypochlorite was more marked in broth than in milk. A higher surviving fraction was obtained in milk, even when tenfold higher concentrations of chlorine were used. The effect of hypochlorite on phages of the same serological group was quite similar and independent of phage morphology. However, phage 137-1, which belongs to other serological group, showed lower resistance to sodium hypochlorite. Comparing the hypochlorite inactivation for ARG and US phages, it was observed that they have their own inactivation values, independently of their origin and morphological group. Long periods of time and high concentrations of chlorine were necessary to reduce the surviving fraction in milk. This indicates that hypochlorite concentrations and times of contact can be critical for the efficiency of the operative sanitization processes

56. **Removal of pathogenic and indicator microorganisms by a constructed wetland receiving untreated domestic wastewater. Quinonez-Diaz, M. de, Karpiscak, M. M., Ellman, E. D., Gerba, C. P. (2001). *Journal of Environmental Science and Health Part A Toxic-Hazardous Substances & Environmental Engineering* A36:1311-1320.** Wetlands containing floating, emergent and submergent aquatic plants, and other water-tolerant species have been found to economically provide a mechanism of enhancing the quality of domestic wastewater. The use of constructed wetlands for the removal of indicator bacteria (total and fecal coliforms), coliphages, protozoan parasites (Giardia and Cryptosporidium) and enteric viruses

was investigated. A pilot scale constructed wetland consisting of two cells, one planted with bulrush and the other unplanted bare sand, were used to compare their efficiency in removing pathogens from raw sewage. Overall more than 90 percent of all microorganisms studied were removed by either of the two systems with a 1 to 2 day retention time. Removal of all mentioned microorganisms was greater from the surface flow in the unplanted cell than in the planted cell, except for Giardia and Cryptosporidium, although the differences were not statistically significant. Enteric viruses, coliphages and indicator bacteria were found to penetrate 2 m below the surface, although concentrations were reduced by greater than 99 percent in both cells. Less virus penetration into the sand occurred in the planted wetland versus the unplanted wetland. Water temperature was found to be the most important factor in the removal of enteric bacteria and viruses, while turbidity reduction was related to Giardia removal. These results demonstrate that significant reductions of pathogenic microorganisms can occur in constructed wetlands receiving untreated domestic wastewater with only a 1-2 day retention time

57. **Phage spread dynamics in clonal bacterial populations is depending on features of the founder cell. Ramirez, E., Carbonell, X, Villaverde, A. (2001). *Microbiological Research* 156:35-40.** Plate-cultured bacterial colonies are intriguing models to study host-parasite interactions in senescent populations. During the growth of bacteriophage-infected colonies there is a synchronous prophage induction episode among lysogenic cells that allows a dramatic but time-restricted amplification of viral particles. We report here that the dynamics of phage spread depends on the history of the lysogenic cell that establishes the clonal population, the duration of the pre-burst period being shorter when the founder, infected cell derives from older colonies. These results offer a physiologic explanation for the self-contained progression of the viral spread in closed environments, that ensures both viral dissemination but also survival of most of the host cells

58. **Gene transfer in bacterial biofilms. Roberts, AP., Mullany, P., Wilson, M., Doyle, R. J. (2001). *Methods in Enzymology* 60-65.**

59. **Independent origins and horizontal transfer of bacterial symbionts of aphids. Sandstrom, J. P., Russell, J. A., White, J. P., Moran, N. A. (2001). *Molecular Ecology* 10:217-228.** Many insect groups have obligate associations with primary endosymbionts: Mutualistic bacteria that are maternally transmitted and derived from an ancient infection. Often, the same insects are hosts to 'secondary' bacterial symbionts which are maternally transmitted but relatively labile within host lineages. To explore the dynamics of secondary symbiont associations in aphids, we characterized bacteria infecting 15 species of macrosiphine aphids using DNA sequencing, diagnostic polymerase chain reaction (PCR), diagnostic restriction digests, phylogenetic analyses, and electron microscopy to examine aphids from nature and from laboratory colonies. Three types of bacteria besides Buchnera were found repeatedly; all three fall within the Enterobacteriaceae. The R-type has a 16S rDNA less than 0.1% different from that of the secondary symbiont previously reported from *Acyrthosiphon pisum* and is related to *Serratia* species. The T-type includes a symbiont previously reported from a whitefly; the U-type comprises a new cluster near the T-type. The T-type was found in every one of 40 Uroleucon ambrosiae clones collected throughout the United States. In contrast, *A. pisum* individuals were infected by any combination of the three symbiont types. Secondary symbionts were maternally transmitted for 11 months within laboratory-reared *A. pisum* clones and were present in sexually produced eggs. PCR screens for a bacteriophage, APSE-1, indicated its presence in both *A. pisum* and *U. ambrosiae* containing secondary symbionts. Electron microscopy of R-type and T-type bacteria in *A. pisum* and in *U. ambrosiae* revealed rod-shaped organisms that attain extremely high densities within a few bacteriocytes

60. **Shiga-toxin-converting bacteriophages. Schmidt, H. (2001). *Research in Microbiology* 152:687-695.** Shiga toxins (Stx) comprise a family of potent cytotoxins that are involved in severe human disease. Stx are mainly produced by *Escherichia coli* isolated from human and nonhuman sources, and by *Shigella dysenteriae* type 1. The genes encoding Stx are thought to be generally encoded in the genome of lambdoid prophages (Stx-converting bacteriophages; Stx phages). They share a unique position in the late region of the phage genome downstream of the late promoter pR'. This location suggests that expression of stx is controlled by a Q-like antiterminator. Therefore, induction of Stx-converting prophages appears to trigger increased production of Stx. Following induction, Stx phages can be transduced in vivo and in vitro other bacteria. Stx phages play an important role in the expression of Stx and in lateral gene transfer and are therefore a contribution to the emergence of new Stx-producing *E. coli* (STEC) variants

61. **Bacteriophages: A rich store of new antibiotics? Senior, K. (2001). *Drug Discovery Today* 6:865-866.**

62. **Isolation of clones sensitive to bacteriophage lambda from the phage resistant *Escherichia coli* strain. Slavchenko, I. Y. (2001). *Biopolimery i Kletka* 17:160-165.** A method of selection of the phage lambda sensitive clones from the population of the bacteriophage lambda resistant cells using the phage lambdacI857ApRTcR has been theoretically formulated and experimentally approved. The isolation was realized as follows. The phage lambda resistant *E. coli* strain was infected by phage lambda, which introduces antibiotic-resistance markers into the cell. The sensitive to phage lambda vir and lysing at 43°C clones were isolated on the selective medium. The frequency of occurrence of such cells in the population was 10^{-6}. The presence of ts-mutation in the phage cI gene has allowed to cure the cells from phage lambdacI857ApRTcR during cultivation of these cells under non-selective conditions at increased temperature and to obtain the phage lambda sensitive clones originated from resistant *E. coli* strain. The method can also be used for the estimation of the *E. coli* populations heterogeneity by a given feature

63. **The ability of acceptance of bacteriophages carried genes for verotoxin production by bacili of Enterobacteriaceae family. Sobieszczanska, B., Gryko, R. (2001). *Medycyna Doswiadczalna i Mikrobiologia* 53:269-276.** Lysogenised verotoxigenic strains are the source of structural genes of verocytotoxins (stx-1 and stx-2) for the others intestinal bacili. The aim of the study was to estimate the ability of transfer of bacteriophages induced with UV irradiation from reference verotoxigenic strains of *E. coli* O157:H7 (CB571 and EDL933) into 125 wild-strains of bacili of Enterobacteriaceae family. None of tested recipient strains showed the production of cytotoxin on Vero and HeLa cell lines, what was acknowledged as the lack of stx genes. Contrary to the laboratory strain of *E. coli* C600 none of 125 tested recipient strains accepted the phages. Obtained lysogenised laboratory strains of *E. coli* C600/CB571 and *E. coli* C600/EDL933, besides of the ability to produce verotoxins (with the presence of stx-1 and stx-2 genes), did not differ phenotypically and genotypically from parent strain of *E. coli* C600. The estimation of the ability to transfer of phages carried stx-1 and/or stx-2 genes was impossible because of too small number of tested wild strain of bacili or because of really low frequency of acceptation of phages by wild strains of intestinal bacili

64. **Kinetics of T7 phage neutralization in the blood of normal and immunodeficient mice. Srivastava, A. S., Kaido, T. J., Carrier, E. (2001). *Blood* 98:407b.** We are attempting to identify and characterize gene delivery vectors engineered to express tissue homing peptides that would help target the vector to the fetal liver or other sites of hematopoeisis. The therapeutic usefulness of such a vector depends upon sufficient amounts reaching the target tissue for subsequent gene expression. Host-

mediated immune responses may neutralize the vector such that its therapeutic effect would be reduced. We used bacteriophage vector T7Select415 from Novagen ($T7^+$) engineered to display random nonamer peptides (Cys-X7-Cys) on phage coat protein 10B. After amplifying and purifying the phage peptide library as well as T7 phage without the peptide library ($T7^-$), the phage were injected intravenously into female Balb/c mice. We found that phage were eliminated from the blood as determined by measuring pfu in blood samples drawn at different time points following injection. Specifically, at 5, 15, 30, and 60 minutes after injection, we recovered 87%, 26%, 2.5%, and 0.04% of the total $T7^-$ phage injected, respectively, which was similar to the amounts of $T7^+$ recovered which was 63%, 28%, 9%, and 5%, respectively. Nearly 100% of total phage injected was recovered from the blood of severe combined immunodeficient (SCID) mice at all time points tested. We also found that the kinetics of T7 inactivation in vitro in Balb/c or SCID serum was similar to those observed in vivo. These data suggest that expression of Cys-X7-Cys peptides on T7 phage coat protein does not affect the rate of clearance of the phage in mouse blood. Additionally, host immune factors may play a role in the neutralization of T7 phage in blood as SCID mice were unable to eliminate the virus at any time point tested. We are currently isolating and characterizing clones from the library that target the fetal liver by in vivo panning techniques, and we are characterizing the specific host factors involved in T7 bacteriophage neutralization

65. **Persistence of two model enteric viruses (B40-8 and MS-2 bacteriophages) in water distribution pipe biofilms. Storey, M. V., Ashbolt, N. J. (2001). *Water Science and Technology* 43:133-138.** The persistence of two model enteric virions (*Bacteroides fragilis* phage B40-8 and coliphage MS-2) within pipe biofilms was investigated in situ in an urban distribution system. Biofilms were allowed to develop on uPVC and stainless steel (SS) coupons in a modified Robbins' device for 70 d within a 150 mm uPVC reticulation main. Coupons were then placed in annular reactors and slug dosed with B40-8 and MS-2 phages (10^8 pfu/mL). Pipe water velocity, pH and free chlorine were recorded during the experimental period. Biofilms on uPVC were generally more abundant (based on total bacterial counts, HPCs, total protein and total carbohydrate). Both B40-8 and MS-2 were incorporated into biofilms formed on uPVC and SS coupons (>10^4 and >10^3 pfu/mug protein respectively) and persisted for >30 d and 6 d respectively, reflecting biofilm biomass on the two pipe surfaces. Virion loss/inactivation from biofilm followed an initial rapid phase, followed by a very slow phase representing approximately 0.01% of the original virion population. Virions, therefore, have the potential to accumulate within distribution biofilm and problems could arise when clusters of biofilm-associated enteric virions become detached from the substrata by hydrodynamic forces or sudden changes in disinfection regime

66. **Bacteriophages as therapeutic agents. Sulakvelidze, A., Morris, J. G. (2001). *Annals of Medicine* 33:507-509.** The emergence of antibiotic resistance among pathogenic bacteria is one of the most critical problems of modern medicine, and novel, effective approaches for treating infections caused by multidrug-resistant bacteria are urgently required. In this context, one intriguing approach is to use bacteriophages (viruses that kill bacteria) to eliminate specific bacterial pathogens. Bacteriophage therapy was widely used around the world in the 1930s and 1940s, and it is still used in Eastern Europe and the former Soviet Union. However, phage therapy was all but abandoned in the West after antibiotics became widely available. Promising results from recent animal studies using phages to treat bacterial infections, together with the urgent need for novel and effective antimicrobials, should prompt additional rigorous studies to determine the value of this therapeutic approach

67. **Use of bioluminescent *Salmonella* for assessing the efficiency of constructed phage-based biosorbent. Sun, W., Brovko, L., Griffiths, M. (2001). *Journal of Industrial Microbiology & Biotechnology* 27:126-128.** A bacteriophage-based biosorbent for *Salmonella enteritidis* was constructed, and bacterial bioluminescence was used for assessment of the efficiency of cell capture. A strain of *S. enteritidis* with bioluminescent phenotype was constructed by transformation with plasmid pT7 carrying the entire lux operon from *Photorhabdus luminescens*. The relation between relative light output (RLU) and colony-forming units (CFU/ml) of the bioluminescent strain was established. The bacteriophage specific to *S. enteritidis* was biotinylated, and the biotinylation procedure was optimized based on the maximum retention of phage infectivity. The biotinylated phages were then coated onto streptavidin-labeled magnetic beads, and were used to capture the bioluminescent *S. enteritidis* cells. Our preliminary results showed that the number of cells captured by constructed biosorbent was five times higher than that of the control, magnetic beads coated with nonbiotinylated phage, indicating the capture is specific

68. **Bacteriophages, method for screening same and bactericidal compositions using same, and detection kits using same. Takahashi, S. (2001). *Official Gazette of the United States Patent and Trademark Office Patents* 1252:No.** The bacteriophage has a high level of specificity to a certain specific pathogenic bacterium so that the bacteriophage can surely kill the pathogenic bacterium as a host through phogocytic action. The bio-bactericidal material containing the bacteriophage can be applied to food such as fresh food, etc., and to places, etc. or to even persons for cooking food material such as restaurants, school kitchens, etc., or any other thing which requires disinfection from pathogenic bacteria, and it can kill pathogenic bacteria. The bio-bactericidal material containing a cocktail of two or more different kinds of the bacteriophages can kill corresponding kinds of pathogenic bacteria concurrently. Further, the phage can infect only the pathogenic bacterium as a host bacterium, and does not infect persons, making it very safe and useful

69. **Association of the activatable Shiga toxin type 2 variant, Stx2d, with an inducible bacteriophage. Teel, L. D., Schmitt, C. K., Melton-Celsa, A. R., O'Brien, A. D. (2001). *Abstracts of the General Meeting of the American Society for Microbiology* 101:90-91.** The gene for Shiga toxin type 2 (Stx2) in *Escherichia coli* O157:H7 strains is typically borne on a readily inducible, toxin-converting lambdoid-like bacteriophage. However, the genes for variants of Stx2 present in Shiga toxin-producing *Escherichia coli* (STEC), with few exceptions, have not been demonstrated to be phage-borne. In this study, we examined the O91:H21 STEC isolate B2F1 that encodes two Stx2 variant toxins that are activatable by intestinal mucus for the presence of Stx2d-converting bacteriophages. First, we analyzed the DNA sequence of cosmids that encoded stx2d1 or stx2d2 and found that both toxin genes were flanked by similar sequences that resembled those published for Stx2 toxin-converting phages. Next, mutants of B2F1 were constructed that produced one or the other Stx2d toxin. Each mutant was then treated with an agent known to induce bacteriophages (mitomycin C). Toxin gene copy number increased in both mutants after mitomycin C treatment. However, toxin levels (as determined by Vero cell cytotoxicity) were substantially increased in sonically-disrupted extracts of the Stx2d1-producing mutant but not the Stx2d2-producing mutant. Furthermore, small turbid plaques were visible on a lawn of *E. coli* K-12 strain DH5a after induction of the Stx2d1-producing mutant and a putative stx2d1-containing lysogen was isolated. Induction of this lysogen that had been transformed with a RecA-expressing plasmid resulted in plaque formation and enhanced toxin production, indicators of the presence of an Stx2d1-converting phage. Finally, the pathogencity of the Stx2d1-producing mutant in the orally infected streptomycin-treated mouse model increased when animals were given subinhibitory doses of ciprofloxacin, a result which suggests increased toxin production by induction of the Stx2d1-converting bacteriophage lytic cycle in vivo. We conclude that only stx2d1 appears to be borne on an inducible toxin-converting bacteriophage and that

stx2d2 expression is not influenced by bacteriophage induction

70. **Evolution: Towards a genetical theory of adaptation. Travisano, M. (2001). *Current Biology* 11:R440-R442.** The population genetic basis for adaptation has remained obscure despite a longstanding body of theory. Microbial selection experiments are beginning to provide some answers

71. **The use of cadmium resistance on the phage-resistance plasmid pNP40 facilitates selection for its horizontal transfer to industrial dairy starter lactococci. Trotter, M., Mills, S., Ross, R. P., Fitzgerald, G. F., Coffey, A. (2001). *Letters in Applied Microbiology* 33:409-414.** Aims: To facilitate the horizontal transfer and selection of phage-resistance plasmids in industrial lactococci. Methods and Results: Cadmium-resistance properties similar to those previously identified in Lactococcus were linked to the well-known phage-resistance plasmid pNP40. This finding was exploited to facilitate delivery of the plasmid to an industrial cheese starter *Lactococcus lactis* DPC4268. Additionally, 25 different cadmium-sensitive cheese starter lactococci were also identified as potential recipients for the phage-resistance plasmid pNP40, and also the plasmids pAH90/pAH82 which also encode cadmium resistance. All three plasmids were successfully conjugated to strain DPC4268. Cheddar cheese was manufactured in industry with the pNP40 phage-resistant transconjugant. Significance and Impact of the Study: Food-grade enhancement of phage resistance in industrial starter strains has been made simpler by the use of this selection, especially since the majority of potential recipient starter strains analysed were cadmium sensitive

72. **Investigation of expression of a phage encoded pertussis toxin operon in *Bordetella avium* lysogens. van Horne, S. J., Bjornsen, D., Carpentier, P., Temple, L. M. (2001). *Abstracts of the General Meeting of the American Society for Microbiology* 101:64-65.** We have isolated a lambda-like phage (Ba1) from *Bordetella avium* that possesses the structural genes for pertussis toxin. Although the three major Bordetella species have chromosomal copies of the pertussis toxin operon in their chromosomes, only in *B. pertussis* are the genes expressed. We have sequenced a 3,010 bp fragment that has 99% identity to the pertussis toxin operon, which encodes part of the S1, S2, all of S4 and S5 and beginning of S3 subunits. PCR amplification has not revealed sequences homologous to the Ptl secretion genes in the Ba1 genome. Pertussis toxin is a member of the AB5 family of toxins, which includes Shiga toxin, cholera toxin, and the *E. coli* heat labile enterotoxin. While pertussis toxin and cholera toxin are actively secreted, Shiga toxin is phage encoded and appears to be released during the lytic cycle. By analogy, the pertussis toxin genes on phage Ba1 may represent a form of pertussis toxin that is encoded as a late gene product and secreted by bacterial lysis, similar to Shiga toxin. To test pertussis toxin expression in *B. avium* lysogens, biological assays and western blot were performed, and no pertussis toxin expression was detected. These results suggest that the pertussis toxin operon may be silent. However, given the possibility that the phage encoded toxin may be expressed as a late gene product, we are currently utilizing RT-PCR to determine if indeed the Ba1 encoded pertussis toxin genes are transcribed during a lytic infection of *B. avium*. In a survey of 14 *B. avium* strains from commercial turkeys as well as wild ducks, geese, and turkeys, we have detected phage and toxin sequences. Preliminary PCR and Southern blotting results indicate that phage-specific and toxin-specific sequences are not always coincident in a particular strain. Furthermore, there appears to be no correlation between the presence of phage or toxin DNA and whether the strain in question came from sick turkeys or apparently healthy wild birds. Further characterization of the phage encoded pertussis toxin operon will hopefully provide insight into the role, if any, the pertussis toxin operon plays in *B. avium* pathogenesis

73. **Phage therapy of *Campylobacter jejuni* colonization in broilers. Wagenaar, J. A., Van Bergen, M. A. P., Mueller, M. A., Monie, K., Carlton, R. M. (2001). *International Journal of Medical Microbiology* 291:92-93.**

74. **Transducing phages of Actinomycetales. Westpheling, J., Burke, J. A. (2001). *Official Gazette of the United States Patent and Trademark Office Patents* 1247:No.** The present invention is directed to isolated transducing phages, methods of isolating transducing phages, and methods of using transducing phages including, for instance, transferring at least one nucleic acid fragment from a donor microbe to a recipient microbe, and producing a secondary metabolite from a microbe. The transducing phages typically have a broad host range, and transduce microbes in the Order Actinomycetales, in particular in the Family Streptomycetaceae, including *Streptomyces coelicolor*, *Streptomyces lividans*, *Streptomyces venezuelae*, *Streptomyces avermitilis*, and *Saccharopolyspora erythraea*. The transducing phages can be specialized transducing phages or generalized transducing phages

75. **Bacteriophage-based transgenic fish for mutation detection. Winn, R. N. (2001). *Official Gazette of the United States Patent and Trademark Office Patents* 1251:No.** The present invention provides transgenic fish whose somatic and germ cells contain a genomically integrated bacteriophage lambda-derived transgene construct. The transgene construct can include an excisable test nucleic acid sequence containing a heterologous mutation target nucleic acid sequence that is detectable via bioassay in a bacterial cell into which the test nucleic acid has been introduced. The frequency of mutations in the mutation target nucleic acid sequence following exposure of the transgenic fish to one or more potentially mutagenic agents can thus be evaluated

76. **Efficient and predictable recovery of viruses from water by small scale ultrafiltration systems. Winona, L. J., Ommani, A. W., Olszewski, J., Nuzzo, J. B., Oshima, K. H. (2001). *Canadian Journal of Microbiology* 47:1033-1041.** Current methods to concentrate viruses from large volumes of water are prone to inconsistent results and are costly and complex procedurally. Ultrafiltration can utilize size exclusion rather than adsorption and (or) elution to concentrate viruses and, therefore, may offer greater flexibility in developing methods that can provide more consistent recoveries among different viruses and widely varying water conditions. Two small scale ultrafiltration systems (hollow fiber and tangential flow) were tested with a virus suspended in 2 L of reagent grade, tap, ground, or surface water. Three model viruses were used (bacteriophages PP7 and T1 and poliovirus) to compare and characterize the recovery of viruses with the two ultrafiltration systems. Pretreatment of the ultrafilters with blocking agents and the use of elution agents can serve to prevent viral adsorption to the filter surface or to elute bound virus and keep viral agents suspended in the retentate. The use of a blocking and elution step concentrated viruses (>60% recovery) from widely varying water qualities, including surface water, such that a single method can be used to efficiently concentrate viruses from all of the water types tested. Both ultrafiltration systems appear to be able to efficiently recover viruses; however, the hollow fiber systems provided slightly better results in the 2-L volumes tested

77. **Influence of phage population on the phage-mediated bioluminescent adenylate kinase (AK) assay for detection of bacteria. Wu, Y., Brovko, L., Griffiths, M. W. (2001). *Letters in Applied Microbiology* 33:311-315.** Aims: The effect of phage concentration on the activity of adenylate kinase (AK) released from the cells lysed during infection was investigated in order to optimize a bioluminescent phage-mediated method for bacterial enumeration. Methods and Results: The number of bacteria lysed by phages specific to *Salmonella enteritidis* and *E. coli* was determined using a bioluminescent method for the detection

of AK released. In order to optimize the assay, the effect of phage concentration and time of infection on the amount of AK released was investigated. The release of AK was greatest at a multiplicity of infection (moi) of 10-100. Conclusions: The amount of AK released from *Salmonella enteritidis* and *E. coli* G2-2 cells by specific phages, SJ2 and AT20, respectively, depended on the type of bacteria, the stage of growth, the nature of phage, moi and time. Significance and Impact of the Study: An assay is described which allows detection of *E. coli* and *Salmonella enteritidis* within 2 h at levels of 10^3 cfu ml-1

78. **Dynamic bacterial and viral response to an algal bloom at subzero temperatures. Yager, P. L., Connelly, T. L., Mortazavi, B., Wommack, K. E., Bano, N., Bauer, J. E., Opsahl, S., Hollibaugh, J. T. (2001). *Limnology and Oceanography* 46:790-801.** New evidence suggests that cold-loving (psychrophilic) bacteria may be a dynamic component of the episodic bloom events of high-latitude ecosystems. Here we report the results of an unusually early springtime study of pelagic microbial activity in the coastal Alaskan Arctic. Heterotrophic bacterioplankton clearly responded to an algal bloom by doubling cell size, increasing the fraction of actively respiring cells (up to an unprecedented 84% metabolically active using redox dye CTC), shifting substrate-uptake capabilities from kinetic parameters better adapted to lower substrate concentrations to those more suited for higher concentrations, and more than doubling cell abundance. Community composition (determined by polymerase chain reaction/DGGE and nucleotide sequence analysis) also shifted over the bloom. Results support, for the first time with modern molecular methods, previous culture-based observations of bacterial community succession during Arctic algal blooms and confirm that previously observed variability in pelagic microbial activity can be linked to changes in community structure. During early bloom stages, virioplankton and bacterial abundance were comparable, suggesting that mortality due to phage infection was low at that time. The virus-to-bacteria ratio (VBR) increased 10-fold at the height of the bloom, however, suggesting an increased potential for bacterioplankton mortality resulting from viral infection. The peak in VBR coincided with observed shifts in both microbial activity and community structure. These early-season data suggest that substrate and virioplankton interactions may control the active microbial carbon cycling of this region

79. **Role of ciliates, flagellates and bacteriophages on the mortality of marine bacteria and on dissolved-DNA concentration in laboratory experimental systems. Alonso, M. C., Rodriguez, V, Rodriguez, J., Borrego, J. J. (2000). *Journal of Experimental Marine Biology and Ecology* 244:239-252.** Several marine bacteriophages, a ciliate (*Uronema* sp.), and a flagellate (*Pseudobodo* sp.) were used to study comparatively the grazing and clearance rates of four marine bacteria. Bacteria were fluorescently labelled using rhodamine isothiocyanate. The results obtained indicate that bacteriophages and flagellates caused a significantly higher ($P < 0.025$) decrease in the number of marine bacteria compared to the ciliate *Uronema* sp. The role of bacteriophages, ciliates and flagellates in the production of dissolved-DNA (D-DNA) was also studied. The interaction between both bacteriophages and protists with marine bacteria are responsible for an increase of the D-DNA concentration, although a direct relationship between D-DNA concentration and predator number was only obtained from experiments performed with *Uronema* sp

80. **Viruses in Trichomonas. Benchimol, M., Alderete, John F. (2000). *XXVII Annual Meeting on Basic Research in Chagas Disease and the XVI Annual Meeting of Brazilian Society of Protozoology* 95:62-63.**

81. **Genome plasticity in Enterobacteriaceae. Brunder, W., Karch, H. (2000). *International Journal of Medical Microbiology* 290:153-165.** The comparative analysis of multiple representatives of the genomes of particular species are leading us away from a view of bacterial genomes as static, monolithic structures towards the view that they are relatively variable, fluid structures. This plasticity is mainly the result of the rearrangement of genes within the genome and the acquisition of novel genes by horizontal transfer systems, e.g. plasmids, bacteriophages, transposons or gene cassettes. These mechanisms often act in concert thus generating a complex genetic structure. Genomic variations are not a phenomenon at the DNA level alone, they influence the phenotype of a bacterium as well and can render a formerly harmless organism into a hazardous pathogen. This review deals not only with the mechanisms of genome rearrangements and the horizontal transfer of genes in Enterobacteriaceae but also points out that mobile genetic elements themselves are subjected to variation

82. **The temperate nature of aquatic and soil bacteriophage. Cook, H. A., Gallucci, C. M., Hale, A. B. (2000). *76th Annual Meeting of the Pennsylvania Academy of Science* 73:153.**

83. **Pulsed-field gel electrophoresis analysis of virus assemblages present in a hypersaline environment. Diez, B., Anton, J., Guixa-Boixereu, N., Pedros-Alio, C., Rodriguez-Valera, F. (2000). *International Microbiology* 3:159-164.** A method for analyzing virus assemblages in aquatic environments was developed and used for studying the highest-salinity ponds (from 13.4 to 35% salinity) from a multi-pond solar saltern in Alicante, Spain. The protocol consisted of a series of concentration and purification steps including tangential flow filtration and ultracentrifugation, followed by the preparation of total viral nucleic acids that were subsequently separated by pulsed-field gel electrophoresis. For every sample analyzed, a characteristic DNA pattern was obtained, whose complexity was related to viral diversity. The comparison of our results with a similar analysis carried out with marine virus assemblages shows that, as expected, the viral diversity corresponding to the analyzed hypersaline environment is considerably lower than that of a marine environment

84. **Compositions containing bacteriophages and methods of using bacteriophages to treat infections. Ghanbari, H. A., Averback, P. (2000). *Official Gazette of the United States Patent and Trademark Office Patents* 1238:No.** Purified, host-specific, non-toxic, wide host range and virulent bacteriophage preparations that are effective in killing bacterial organisms in vivo are disclosed. Also disclosed are compositions containing these bacteriophages, methods of making the bacteriophage preparations and methods of treating bacterial infections using the compositions. Methods of treating bacterial infections using the compositions containing the bacteriophages in combination with conventional antibiotics also are disclosed

85. **A procedure for detection of coliphages in the drinking water. Kashkarova, G. P., Dorodnikov, A. I. (2000). *Gigiena i Sanitariya* 66-68.**

86. **[Vibrio cholerae O139 bacteriophages]. Kudriakova, T. A., Makedonova, L. D., Kachkina, G. V., Saiamov, S. R. (2000). *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* 28-30.** Cholera bacteriophages have been isolated from 27 lysogenic cultures of *V. cholerae* O139. As shown the pages [sic] under study belong to two morphological groups A1 and F1 and serological types II and XII. The use of prophage typing and the sensitivity test to specific phage made it possible to differentiate *V. cholerae* strains, serogroup O139

87. **Development and qualification of a novel virus removal filter for cell culture applications. Liu, S., Carroll, M., Iverson, R., Valera, C., Vennari, J., Turco, K., Piper, R., Kiss, R., utz, H. (2000). *Biotechnology Progress* 16:425-434.** Commercial

bioreactors employing mammalian cell cultures to express biological or pharmaceutical products can become contaminated with adventitious viruses. The high expense of such a contamination can be reduced by passing all gases and fluids feeding the bioreactor through virus inactivation or removal steps, which act as viral barriers around the bioreactor. A novel virus barrier filter has been developed for removing viruses from serum-free cell culture media. This filter removes the 20 nm minute virus of mice by >3 log reduction value (LRV), the 28 nm bacteriophage PHIX174 by >4.5 LRV, the mycoplasma *Acholeplasma laidlawii* by >=8.8 LRV, and the bacteria *Brevundimonas diminuta* by >=9.2 LRV. Robust removal occurs primarily by size exclusion as demonstrated over a wide range of feedstocks and operating conditions. The filtered media are indistinguishable from unfiltered media in growth of cells to high densities, maintenance of cell viability, and productivity in expressing protein product. Insulin and transferrin show high passage through the filter. The virus barrier filter can be autoclaved. The relatively high membrane permeability enables the use of a moderate filtration area

88. **Bacteriophages active against *Helicobacter pylori* in UK sewage: Natural born killers? Morton, D., Bardhan, K. D. (2000). *Gastroenterology* 118:AGA.**

89. **The presence of bacteriophages active against *Helicobacter pylori* in UK sewage: Natures eradicator? Morton, D., Bardhan, K. D. (2000). *Gut* 46:A69.**

90. **Detection of bacterial pathogens by phage antibody display. Pai, N., Topping, K. P., Greenman, J., Paget, T. A. (2000). *Disease Markers* 16:99-100.**

91. **Phage sensitivities of lactococci isolated from raw milk and whey. Sanlibaba, P., Akcelik, M. (2000). *Turkish Journal of Biology* 24:425-435.** In this study, lactococcal strains isolated from raw milk and whey samples of different types of cheese produced without using industrial starter cultures in different regions of Turkey were tested for sensitivity to 22 lactic phages. In addition to the strain isolation materials, virulent lactic phages were also isolated from samples obtained from dairy plants using industrial starter cultures. 18 L lactis subsp. lactis strains out of 73 lactococcal strains were found to be resistant to all the phages. It was determined that the total phage resistance levels of the other 55 strains were between 18.18 and 90.90%

92. **Studies on phage control of pustule disease in abalone *Haliotis discus hannai*. Tai-wu, L., Xiang, J., Liu, R. (2000). *Journal of Shellfish Research* 19:535.**

93. **Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Thingstad, T. F. (2000). *Limnology and Oceanography* 45:1320-1328.** Mechanisms controlling virus abundance and partitioning of loss of bacterial production between viral lysis and protozoan predation are discussed within the framework of an idealized Lotka-Volterra-type model. This combines nonselective protozoan predation with host-selective viral lysis of bacteria. The analysis leads to a reciprocal relationship between bacterial diversity and viruses, in which coexistence of competing bacterial species is ensured by the presence of viruses that "kill the winner," whereas the differences in substrate affinity between the coexisting bacterial species determine viral abundance. The ability of the model to reproduce published observations, such as an approximate 1:10 ratio between bacterial and viral abundance, and the ability of viral lysis to account for 10-50% of bacterial loss are discussed.

94. **Method and device for detecting bacteriophage using contrast-coloring and precipitable dyes. Wicks, J. H., Krejcarek, G. E., Williams, M. G. (2000). *Official Gazette of the United States Patent and Trademark Office Patents* 1236:No.** The use of a precipitable dye and a contrast-coloring dye together enhance visualization of plaques in confluent lawns of bacteria in bacteriophage and bacteria assays. A test sample suspected of containing a bacteriophage is combined with bacteria capable of replicating the bacteriophage, and applied to a water-proof surface to form a support for bacterial growth. The support is provided with the contrast-coloring dye and precipitable dye, and nutrients and salts capable of supporting growth of the bacteria. A lawn of bacteria is formed on the support, and plaques detected on the lawn indicate presence of the bacteriophage. The plaques contain a precipitate formed by enzymatic cleavage of the precipitable dye by an enzyme of the bacterial lawn. A similar procedure is used for detecting bacteria, except that a test sample suspected of containing a bacteria is combined with bacteriophage capable of replicating in the bacteria, and plaques detected indicate presence of the bacteria. The bacteriophage and bacteria assays are carried out with a disposable device containing at least one well having a water-proof surface and a depth of about at least 5 millimeters. A hydratable material containing the contrast-coloring dye and precipitable dye is positioned on the surface. The well may contain substantially vertical sides with a removable cover resting on top of the sides

95. **Detection and occurrence of indicator organisms and pathogens. Baker, K. H., Herson, D. S. (1999). *Water Environment Research* 71:530-551.**

96. **Distribution of the human faecal bacterium *Bacteroides fragilis*, its bacteriophages and their relationship to current sewage pollution indicators in bathing water. Bradley, G., Carter, J., Gaudie, D., King, C. (1999). *1998 Meeting of the Society for Applied Microbiology on Aquatic Microbiology* 90S-100S.**

97. **History of the discovery and study of brucellar bacteriophages. Lyapustina, L. V., Lyamkin, G. I., Taran, I. F. (1999). *Zhurnal Mikrobiologii Epidemiologii i Immunobiologii* 123-124.**

98. **Enzyme for phage resistance. Moineau, S., Walker, S. A., Vedamuthu, E. R., Vandenbergh, P. A. (1999). *Official Gazette of the United States Patent and Trademark Office Patents* 1227:No.** An isolated DNA of a *Lactococcus lactis* showing a SEQ ID NO:1 encoding a restriction and two modification enzymes (R/M SEQ ID NO: 2, 3 and 4). The isolated DNA is used to transform sensitive dairy cultures, such as *Lactococcus lactis* and *Streptococcus thermophilus*, to provide phage resistance. *Escherichia coli* can be used to produce endonucleases

99. **Method and test kits for detection of bacteriophage. Sanders, M. F. (1999). *Official Gazette of the United States Patent and Trademark Office Patents* 1223:NO.**

100. **Effect of bacteriophages on TNF-alpha, IL-6 and IFN production by human peripheral blood cells (PBC). Weber-Dabrowska, B., Czarny, A., Mulczyk, M. (1999). *Seventh Annual Conference of the International Cytokine Society* 11:922.**

101. **Method to detect bacteria. Wilson, S. M. (1999). *Official Gazette of the United States Patent and Trademark Office Patents* 1228.** The present invention relates to a method for enhancing the time of response of an assay for a first bacterium, wherein: a) the first bacterium is exposed to infection by phage particles to which the first bacterium is permissive; b) the infected bacterium is treated to inactivate exogenous phage particles; c) the treated bacterium is cultivated in the presence of a second bacterium which is permissive to infection by the phage or its replicand and which has a doubling rate greater than the effective doubling rate of the first bacterium; and d) assessing the extent of plaque formation and/or of second bacterium growth in the cultivated second bacterium cells. The method can be used to assess the presence of first bacterium in a sample, notably where the first bacterium is a slow growing bacterium, such as *Mycobacterium tuberculosis*, where the method enables an operator to detect the presence of low amounts of the bacterium in sample within days instead of weeks as required by conventional cultivation techniques. The invention can also be used to assess the effect of a drug or other treatment on a bacterium or on a virus. The invention also provides a diagnostic kit for use in the method of the invention

102. **Bacteriophage of *Chlamydia psittaci*. Bavoil, P. M., Hsia, R. C. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1209:2328.**

103. **Method of selecting specific bacteriophages. Borrebaeck, C. A. K., Duenas, M. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1206:2910.**

104. **Bacteriophage-mediated gene transfer systems capable of transfecting eukaryotic cells. Chada, S., Dubensky, T. W., Jr. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1209:459.**

105. **Bacteriophage-triggered cell suicide systems and fermentation methods employing the same. Klaenhammer, T. R., Conkling, M. A., O'Sullivan, D., Djordjevic, G., Walker, S. A., Taylor, C. G. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1213:1727.**

106. **Bacteriophage genotypically modified to delay inactivations by the host defense system. Merril, C. R., Carlton, R. M., Adhya, S. L. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1214:4125.**

107. **Antibacterial therapy with bacteriophage genotypically modified to delay inactivation by the host defense system together with an antibiotic. Merril, C. R., Carlton, R. M., Adhya, S. L. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1211:2866-2867.**

108. **Phage-resistant streptococcus. Mollet, B., Pridmore, D., Zwahien, M. C. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1211:2869.**

109. **Nucleic acid sequence and plasmids comprising at least one phage resistence mechanism, bacteria containing them and their use. Prevots, F., Tolou, S., Daloyau, M. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1206:2923.**

110. **Genetically engineered reporter bacteria for the detection of bacteriophage. Rees, C. E. D., Rostas-Mulligan, K., Park, S. F., Denyer, S. P., Anderson, G. S., Stewart, B., Jassim, S. A. A. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1208:410.**

111. **Detection of listeria by means of recombinant bacteriophages. Scherer, S., Loessner, M. (1998). *Official Gazette of the United States Patent and Trademark Office Patents* 1215:2969.**

112. **A theoretical approach to structuring mechanisms in the pelagic food web. Thingstad, T. F. (1998). *Hydrobioligia* 363:59-72.** In the literature there is a commonly used idealized concept of the food web structure in the pelagic photic zone food web, based to a large extent on size dependent relationships. An outline is here given of how the elementary size-related physical laws of diffusion and sinking, combined with the assumption of predators being size selective in their choice of prey, give a theoretical foundation for this type of structure. It is shown how such a theoretical fundament makes it possible to relate a broad specter of phenomena within one generic and consistent framework. Phenomena such as Hutchinson's and Goldman's paradoxes, the influence of nutrients and water column stability on the balance between microbial and classical food webs, bacterial carbon consumption, new production and export of DOC and POC to the aphotic zone, eutrophication and diversity, can all be approached from this perspective. By including host-specific viruses, this approach gives a hierarchical structure to the control of diversity with nutrient content controlling the maximum size of the photic zone community, size selectivity of predators regulating how the nutrient is distributed between size-groups of osmotrophic and phagotrophic organisms, and viral host specificity regulating how the nutrients within a size group is distributed between host groups. I also briefly discuss how some biological strategies may be successful by not conforming to the normal rules of such a framework. Analyzing the behavior of these idealized systems is thus claimed to facilitate our understanding of the behavior of complex natural food webs.

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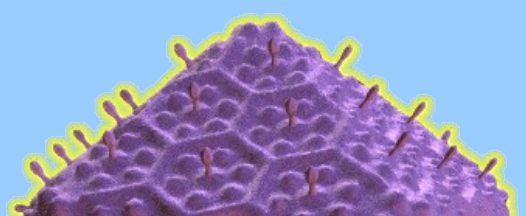

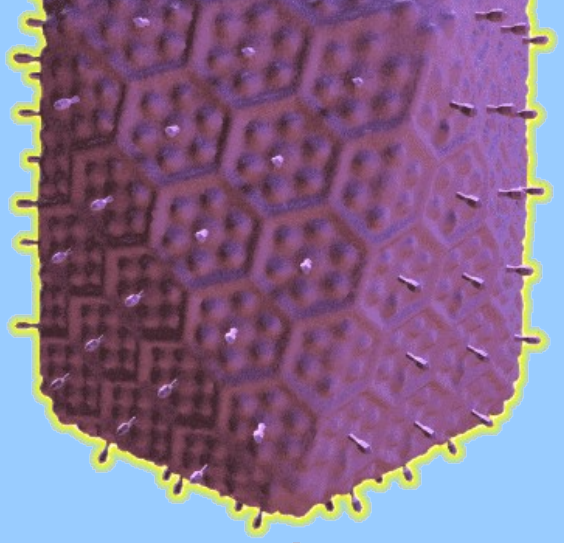

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