Bacteriophage Ecology Group (BEG) News

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

July 1, 2003 issue (volume 17)

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Editorial

Phage T4 Meets Microbial Diversity

by Jim D. Karam

Bacteriophages are extremely diverse in the range of microbial hosts that they infect and the chemical nature, size and geometry of their genetic materials. As the most abundant organisms on Earth [1], they contribute substantially to overall diversity of the gene pool in the microbial world. It is no surprise then that they have become prominent in the ongoing discussion on microbial diversity [2-4]. The interactions of phages with their hosts are not only important for maintenance of the ecological balance, they may also constitute a major component of the network for lateral transfer of genes among microorganisms. This notion is based on studies with all types of archived phages, which of course represent only a minute fraction of the estimated number of unique phage genomes in nature. Nevertheless, the extent to which phage may contribute to microbial diversity is becoming better appreciated because of an ongoing expansion of sequence databases for all types of organisms, including phage. In the US, funding agencies have teamed together to coordinate a significant increase in support for microbial genome sequencing projects, with a great deal of emphasis being placed on speed and economy of generating, assembling, annotating, and sharing sequence data. It is anticipated that the microbial genome database will continue to expand at a fast pace in the foreseeable future.

The most commonly used approach for sequencing a microbial genome involves genomic library construction (usually from randomly sheared genomic DNA), sequencing a large number of library clones (high-throughput sequencing), computer-assisted assembly of sequence data into contiguous segments (contigs), and carrying out more sequencing and data assembly to close gaps between contigs. The high-throughput stage is expected to yield data from overlapping clones for better accuracy of sequence reads and length of assembled contigs. In principle, the approach is straightforward and has the clearly defined goal of producing an accurate single contiguous sequence of the genome. In practice, it is riddled with bottlenecks that can be different for different genomes, depending on their sizes, states of modification, content of sequences that cannot be cloned and other factors. Personnel training and quality of operations in general are also critical factors to consider in such projects. It is very common for projects to use commercial outfits or collaborations with well-funded research institutes for operations that are impractical to support locally. Usually progress is very rapid during the early stages of a project, but gets bogged down in later stages. Some genomes remain 80-90% finished for months or years, but can still be mined for useful information, provided that this information is released to the scientific community. The technology continues to improve on several fronts, and we can expect that alternate approaches, e.g., circumventing cloning and more powerful computer programming, will cut down the time and expense required to produce a finished genome sequence and allow the sequencing of several genomes concurrently by the same team.

Phage oriented projects have so far completed the sequences of ~150 genomes (GenBank). In some cases, several members of the same phage family (Siphoviridae, Myoviridae or Podoviridae; ICTV nonenclature) are included in databases. Collectively, the data suggest that despite their vast differences in genetic composition, all dsDNA phages share similar genome architecture. The typical dsDNA phage genome consist of a mosaic of gene sets that are shared with other members of the same phage "genus" and gene sets that are unique to each genome and interspersed with the genus-specific sets. That is, dsDNA phage genomes seem to evolve by gathering genes from different sources, including genes that qualify the phages for membership in their particular genera. In some instances, lateral DNA transfer (by homologous or nonhomologous recombination) is suspected to be responsible for mosaic patterns that appear inside some phage genes. Since gene evolution by mutation (vertical change) and genome evolution by lateral DNA transfer probably occur independently of each other, it is difficult to relate whole genomes belonging to the same genus to one another in chronological order. Such timelines are more meaningful when sequences of shared (homologous) genes or gene clusters (or their protein products) are compared, e.g., divergence of an essential gene/protein within a phage genus. The framework represented by genomes of the T4-like phages is an excellent example of how vertical and horizontal evolution may drive diversity in a dsDNA phage genome type. The T4 genome type is large by viral standards and carries many genes that one usually finds in cellular rather than viral genomes. Among these are genes for some enzymes of intermediary metabolism, a multi-component DNA replisome, extensive machinery for genetic recombination, and certain types of mobile DNA elements (including homing endonuclease genes) that can move themselves and flanking DNA unidirectionally [5, 6]. There is also a well-studied prototype, phage T4 [7, 8], than can be used as reference when comparing nucleotide sequences and genome organization of different T4-like phages.

In a collaborative project with Henry Krisch (CNRS, Toulouse, France), we have been sequencing the genomes of a number of T4-like Myoviridae that diverge in host range and/or other characteristics, as determined by preliminary genetic and genomic scanning. Thanks to the efforts of Hans Ackermann, a number of these phages that infect bacterial hosts other than E. coli have been archived at LaValle University (Quebec, CA) and made available for our studies. The sequences of 2 *Aeromonas* phages, Aeh1 (*A. hydrophila*) and 44RR2.8t (*A. salmonicida*) and 2 coliphages RB69 and RB49 are now posted on a publicly accessible web site (http://phage.bioc.tulane.edu) and are in the process of being submitted to GenBank. Although the available data probably represent only a very tiny sampling of what must exist in nature for this type of phage genome, certain predictions can already be made with regards to the kind of diversity one may encounter if a much more extensive collection of T4-like phages is analyzed. For example, whereas genome size appears to be rather fixed for some dsDNA phages, T4-like genomes can vary in length over a wide range. Currently, the observed range is ~164Kbp (for phage RB49) to ~233Kbp (for phage Aeh1). So it appears that genomes of the T4 kind can recruit variable amounts of DNA to go with a certain core that is common to all. Reversible gain and loss of genes and homologues may occur depending on composition of the gene pool where exchanges take place. Based on what we know from T4 studies, the highly recombinogenic character of this genetic system may allow it to be an effective scavenger of DNA from microbial hosts. . The Aeh1 genome carries 23 tRNA genes (19 amino-acid specificities), which is one indication of DNA acquisition from cellular sources. Matches to bacterial sequences in databases account for 2-5% of the predicted ORFs for any of the genomes sequenced so far. This is probably a vast underestimate of the contribution of bacterial DNA to T4-like genomes. More likely, much of the other nonT4-like DNA we observe for these phages has its matches in microorganisms that have yet to be discovered. The combinatorial potential of the genome framework acquired by the T4-like phages might underlie a potential for these phages to cross species barriers between bacteria. If this is happening in nature, then the T4-like population and unrelated phage populations with similar potential [4] could be dynamically affecting microbial diversity on a global scale.

It is still unclear what constitutes the "core" DNA of a T4-like genome. It could be >100 ORFs. Because morphological criteria have figured significantly in the classification of phages into families and genera, it has not been surprising to find homologues of the T4 morphogenesis genes in all the genomes examined so far in the "T4-Like Genome Project" (http://phage.bioc.tulane.edu). On the other hand, homologues of the T4 DNA replication/recombination gene clusters are consistently being observed to coexist with the morphogenesis clusters. Functional coupling between replication and morphogenesis has been documented in T4 studies, and could conceivably be required for natural selection of this type of phage genome. It remains to be seen if phages of the T4 morphotype exist in nature which utilize a different mode of replication from the T4 paradigm, or vice versa. To find out, one would have to utilize specific probes to access a much larger set of genomes than exists today in laboratory archives. It is particularly important to be able to screen environmental sources for genomes of phages that cannot be isolated through traditional plaque assays. T4-like phages that have significantly larger genomes than T4 and those that grow on bacterial hosts other than E. coli (or the enterobacteria in general) are underrepresented in laboratory collections [9]. Also, no phages of this genus have been reported whose heads/genomes are much smaller than T4. Finding more of T4's relatives in a variety of environmental niches and sequencing them would boost our understanding of the pathways leading to microbial diversity. In addition, such phages/genomes would constitute a treasure chest of genes and proteins for all types of studies in basic and applied molecular biology. I beg the BEG to undertake the search for more T4-like phages.

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9. Ackermann, H. W., and Krisch, H. M. (1997). A catalogue of T4-type bacteriophages. Arch Virol 142, 2329-2345.

Editorial Archive

- BEG: What we are, Where we are, Where we're going by Stephen T. Abedon
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- Phage T4 Meets Microbial Diversity by Jim D. Karam

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.

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New BEG Members

Please welcome our newest members

name (home page links)	status	e-mail	address
Knut Yngve Børsheim	PI	borsheim @chembio.ntnu.no	Norwegian University of Science and Technology, Department of Biotechnology, N-7491 Trondheim, Norway
	interests:	Aquatic microbial ecology (contents | BEG members | top of page)	
Donna H. Duckworth	PI	duckwort @mgm.ufl.edu	Box 100266, HSC, University of Florida, Coll. Medicine, Gainesville, Fl.32615
	interests:	Phage biology, the interaction of phages with each other and with plasmids, phage therapy, and use of phage for decontamination. (contents | BEG members | top of page)	
Sabarinathan K. Gopalasubramaniam	---	kannankrishnan2002 @yahoo.co.in	Tamil Nadu Agricultural University ,Coimbatore,Tamil nadu, INDIA
	interests:	Identification of bacteriophages infecting cyanobacteria, particularly *Spirulina* spp., plus controlling cyanobacterial blooms using bacteriophages. (contents | BEG members | top of page)	
Lee H. Lee	PI	leel @mail.montclair.edu	Dept. of Biology & Molecular Biology, Montclair State University, Upper Montclair, NJ 07043
	interests:	1. Microorganisms as environmental indicators to study heavy metal contamination; 2. Cyanophage AS-1 capsid proteins; 3. Isolation of novel bacteria associated with marine algae and screening for possible antibiotics/antiviral substances; 4. Genome project of cyanophage AS-1 and cyanobacteria *Anacystis nidulans*. (contents | BEG members | top of page)	

Saligrama V. Manjunath	---	svmanju @indiatimes.com	Dept. of Plant pathology UAS, GKVK, Bangalore, India-560065
	interests:	Phages of *Ralstonia solanacearum* and their efficacy in the control bacterial wilt of solanaceous crops. (contents | BEG members | top of page)	
Mathias Middelboe	PI	mmiddelboe @zi.ku.dk	Marine Biological Laboratory, University of Copenhagen Strandpromenaden 5, DK-3000 Helsingor, Denmark
	interests:	Phage-host dynamics, effects of viruses on bacterial and algal population dynamics, role of viruses for pelagic nutient cycling, benthic viruses (contents | BEG members | top of page)	
Frederic Ropiquet	PI	frederic.ropiquet @biopole.fr	LBP Agro-Pharma, Toulouse, France
	interests:	Phage therapy (contents | BEG members | top of page)	
William R. Wikoff	PI	wikoff @biochem.wustl.edu	Dept. of Biochemistry and Molecular Biophysics, Washington University Medical School, Box 8231, 660 S. Euclid Ave., St. Louis, MO 63110
	interests:	Virus and bacteriophage structure and assembly. Primary approaches are x-ray crystallography, x-ray solution scattering and electron microscopy. Particularly interested in the physical stability of viral capsids and how enhanced stability may convey an evolutionary advantage. (contents | BEG members | top of page)	

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

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Meetings

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

Evergreen International Phage Meeting

Next Summer's phage meeting has been scheduled for July 23-27, 2003. Information pertaining to the meeting may be found at http://www.evergreen.edu/phage/. This meeting will bring together phage people with the widest possible array of interests - from the ecological to the molecular - in a setting of rain forest spender. Click here for a tour of The Evergreen State College.

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.

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Submissions

Some Recent Phage and Phage-Related U.S. Patents (1976-present)

1. **Anti-viral acoustically transparent earphone cover. Ullrich, K. A. (1996). Patent number 5,545,859.** An earphone cover, for attachment to an operative region of an audiometric testing device includes a body formed from a substantially antiviral, acoustically-transparent material, and is constructed for covering such operative region. The body is preferably formed as a polyethylene film with a thickness of about 1-mil. Anti-viral testing shows that the earphone cover is an effective barrier throughout a 60-minute exposure time to a viral organism described as fX174 bacteriophage ATCC# 13706-B1. Acoustic transparency testing shows the earphone cover exhibits acceptable % total harmonic distortion and attenuation. A method of preventing patient cross-contamination associated with audiometric testing is also described. Both the structure and method are usable without affecting calibration of the audiometric testing device.

2. **Antibacterial therapy with bacteriophage genotypically modified to delay inactivation by the host defense system. Merril, C. R., Carlton, R. M., Adhya, S. L. (2003). Patent number 5,660,812 (see also 5,688,501; 5,660,812).** The present invention is directed to bacteriophage therapy, using methods that enable the bacteriophage to delay inactivation by any and all parts of the host defense system (HDS) against foreign objects that would tend to reduce the numbers of bacteriophage and/or the efficiency of those phage at killing the host bacteria in an infection. Disclosed is a method of producing bacteriophage modified for anti-HDS purposes, one method being selection by serial passaging, and the other method being genetic engineering of a bacteriophage, so that the modified bacteriophage will remain active in the body for longer periods of time than the wild-type phage.

3. **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). Patent number 5,766,892.** The present invention is directed to bacteriophage therapy, using methods that enable the bacteriophage to delay inactivation by any and all parts of the host defense system (HDS) against foreign objects that would tend to reduce the numbers of bacteriophage and/or the efficiency of those phage at killing the host bacteria in an infection. Disclosed is a method of producing bacteriophage modified for anti-HDS purposes, one method being selection by serial passaging, and the other method being genetic engineering of a bacteriophage, so that the modified bacteriophage will remain active in the body for longer periods of time than the wild-type phage.

4. **Bacteria and bacteriophage detection using immobilized enzyme substrates. Adams, C. A., Krejcarek, G. E., Wicks, J. H. (2002). Patent number 6,436,661.** Methods of detecting bacteria including the use of an immobilized enzyme substrate, and the immobilized enzyme substrate.

5. **Bacterial detection by phage transduction of ice nucleation and other phenotypes. Wolber, P. K., Green, R. L. (1995). Patent number 5,447,836.** Viable bacteria may be detected in biological samples by exposing bacterial cultures obtained from the samples to transducing particles having a known host range. Such transducing particles carry a heterologous gene capable of altering the phenotype of the bacteria in a readily detectable manner. For example, the transducing particles may carry an ice nucleation gene and the alteration of phenotype may be detected using an ice nucleation assay. By employing a panel of phage, unknown bacteria may be typed based on the pattern of reactivity observed. The method is particularly useful for detecting viable bacteria which may have been debilitated by exposure to sterilizing conditions, such as in food processing. The method is also useful for tracking a target bacteria in the ambient environment.

6. **Bacterial phage associated lysing enzymes for treating dermatological infections. Fischetti, V., Loomis, L. (2001). Patent number 6,248,324.** The present invention discloses a composition for dermatological infections by the use of a lytic enzyme in a carrier suitable for topical application to dermal tissues. The method for the treatment of dermatological infections comprises administering a composition comprising effective amount of a therapeutic agent, with the therapeutic agent comprising a lytic enzyme produced by infecting a bacteria with phage specific for that bacteria.

7. **Bacteriophage-based transgenic fish for mutation detection. Winn, R. N. (2001). Patent number 6,307,121.** 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.

8. **Bacteriophage-mediated gene transfer systems capable of transfecting eukaryotic cells. Chada, S., Dubensky, T. W., Jr. (1998). Patent number 5,736,388.** Lamboid bacteriophage capable of specifically interacting with and delivering nucleic acid molecules to eukaryotic cells are disclosed. Such bacteriophage-derived gene transfer systems target one or more specific receptors on eukaryotic cells, for instance by incorporating mutant tail fiber proteins or by incorporating known ligands for specific eukaryotic receptors into lambda phage. Also disclosed are methods for identifying and producing modified bacteriophage tail fiber polypeptides capable of specifically interacting with eukaryotic transmembrane proteins. Methods of treating diseases using such gene transfer systems are also disclosed.

9. **Bacteriophage-resistant plant growth promoting rhizobacteria. Suslow, T. V. (1986). Patent number 4,584,274.** Bacteriophage-resistant plant growth promoting rhizobacteria are employed for enhancing the yield of root crops, such as potatoes, sugar beets, radishes and the like, grown in soils which are infected by bacteriophage which limit the root colonization by the corresponding wild-type rhizobacteria. The strain SH5 PR3 was deposited at the ATCC for patent purposes on Jan. 20, 1983, and granted Accession No. 39270.

10. **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). Patent number 5,792,625.** Described herein is a bacterial cell containing a recombinant bacteriophage defense mechanism. The defense mechanism comprises a bacteriophage promoter (e.g., a phage f31 promoter; a T7 promoter) operatively associated with a heterologous DNA encoding a product lethal to the bacterial cell. The bacterial cell is susceptible to infection by a bacteriophage, and the promoter is activated upon the infection of said bacterial cell by that bacteriophage. Bacteria useful in carrying out the invention include both gram negative and gram positive bacteria (e.g., *Lactococcus lactis*; *Escherichia coli*); the heterologous DNA may encode an enzyme that degrades nucleic acid (e.g., the products of the LlaI restriction cassette; barnase). Recombinant DNAs useful for making the foregoing cells, cultures prepared from such cells, and fermentation methods carried out with such cells are also disclosed.

11. **Bacteriophage assay. Hyman, L. J., Toth, I. K. (2003). Patent number 6,555,331.** There is provided an assay suitable for the typing of bacterial strains. In the assay a predetermined amount of phage is combined with a bacterial isolate of unknown strain, the mixture being located in a suitable container. The mixture of phage and bacteria is conveniently held in a liquid or semi-liquid medium facilitating interaction of the two species. The extent of bacterial growth in the presence of the phage is measured by conventional means, preferably by means of an OD reading. Desirably the phage is retained in the selected container, which is conveniently a micro-titer plate, through use of a fixant such as 5% gelatin.

12. **Bacteriophage composition useful in treating food products to prevent bacterial contamination. Averback, P., Gemmell, J. (2002). Patent number 6,461,608.** The present invention is directed to novel bacteriophage compositions useful in treating food products to prevent bacterial contamination.

13. **Bacteriophage genotypically modified to delay inactivations by the host defense system. Merril, C. R., Carlton, R. M., Adhya, S. L. (1998). Patent number 5,811,093.** The present invention is directed to bacteriophage therapy, using methods that enable the bacteriophage to delay inactivation by any and all parts of the host defense system (HDS) against foreign objects that would tend to reduce the numbers of bacteriophage and/or the efficiency of those phage at killing the host bacteria in an infection. Disclosed is a method of producing bacteriophage modified for anti-HDS purposes, one method being selection by serial passaging of a bacteriophage, and the other method being genetic engineering of a bacteriophage, so that the modified bacteriophage will remain active in the body for longer periods of time than the wild-type phage.

14. **Bacteriophage of *Chlamydia psittaci*. Bavoil, P. M., Hsia, R. C. (1998). Patent number 5,741,697.** The present invention is directed to an isolated bacteriophage designated fCPG1. The invention is further directed to an isolated DNA molecule encoding bacteriophage fCPG1, or a fragment thereof, a DNA molecule comprising DNA encoding bacteriophage fCPG1 with heterologous DNA inserted therein, or a fragment thereof, and to oligonucleotides consisting essentially of a portion of the DNA molecule encoding fCPG1. The bacteriophage fCPG1 was isolated from *Chlamydia psittaci* strain Guinea Pig inclusion Conjunctivitis.

15. **Bacteriophage prevention and control of harmful plant bacteria US PATENT-4828999. MAY 9 1989. Jackson, L. E. (1989). Patent number 4,828,999.** For preventing or controlling bacterial harm to plants, as by disease or ice nucleation, a bateriophage composition of matter containing one or more viral h mutants specific to amutant of the bacteria concerned is produced and applied to seed, soil or soil supplements, plants, or plant materials that have been exposed to or are contaiminated with or infected by bacerial disease, or to growing plants subject to ice nucleation or other bacterial harm. The invention is concerned with the composition and with the method of producing and using same.

16. **Bacteriophage resistant recombinant bacteria. Hill, C. J., Klaehammer, T. R. (1996). Patent number 5,538,864.** Recombinant bacteria containing phage-encoded resistance ("Per") and methods of making and using the same are disclosed. Such bacteria are made by (a) conducting a fermentation of a substrate in a medium containing a defined bacterial culture until bacteriophage are detected in the medium, the bacteriophage being specific to at least one bacteria in the medium; (b) isolating the bacteriophage; (c) digesting DNA of the bacteriophage to produce a library of DNA fragments; (d) transforming the bacteria susceptible to said bacteriophage with the library of DNA fragments to provide transformed bacteria; (e) selecting from among the transformed bacteria, a bacteriophage-resistant transformed bacteria; (f) adding bacteriophage resistant transformed bacteria to the medium; and (g) recommencing step (a). Also disclosed are bacterial cells which contain a first bacteriophage defense mechanism (Per), wherein Per comprises a bacteriophage origin of replication (ori) operatively associated with a DNA sequence incapable of producing live bacteriophage. The bacterial cell is capable of being infected by a bacteriophage, the DNA of which, once injected into the bacterial cell, competes with Per for binding to DNA polymerase.

17. **Bacteriophage RM 378 of a thermophilic host organism. Hjorleifsdottir, S., Hreggvidsson, G. O., Fridjonsson, O. H., Aevarsson, A., Kristjansson, J. K. (2002). Patent number 6,492,161.** A novel bacteriophage RM 378 of *Rhodothermus marinus*, the nucleic acids of its genome, nucleic acids comprising nucleotide sequences of open reading frames (ORFs) of its genome, and polypeptides encoded by the nucleic acids, are described.

18. **Bacteriophage, a process for the isolation thereof, and a universal growth medium useful in the process thereof. Agrawal, P., Soni, V. (2002). Patent number 6,482,632.** The present invention provides a isolated bacteriophage useful as a tool for studying biological, biochemical, physiological and genetic properties of actinomycetes and other organisms which comprises a novel strain of *Saccharomonospora* having certain specified characteristics. The invention also relates to a process for the isolation of the said bacteriophage and/or DNA phage and to a novel universal growth medium which is particularly useful in the said process. Another embodiment of the process relates to a cloning vector which comprises a plasmid or bacteriophage comprising the phage DNA of the invention.

19. **Bacteriophages as recognition and identification agents. Teodorescu, M. C., Gaspar, A. M. (1989). Patent**

number 4,797,363. Bacteriophages are employed as agents for recognition and identification of molecules and cellular materials, using their ability to recognize their bacterial host, by coating them with antibodies or by selecting them to perform in a manner analogous to antibodies. Visibility for identification is effected by incorporating a fluorescent agent, a radioisotope, a metal, an enzyme, or other staining material. The bacteriophage are prepared so as to bind to the molecular or cellular material through either the tail or head segment of the bacteriophage.

20. **Bacteriophages, method for screening same and bactericidal compositions using same, and detection kits using same. Takahashi, S. (2001). Patent number 6,322,783.** 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 [*sic?*] 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.

21. **Bioluminescent biosensor device. Sayler, G. S., Ripp, S. A., Applegate, B. (2003). Patent number 6,544,729.** Disclosed are methods and devices for detection of bacteria based on recognition and infection of one or more selected strains of bacteria with bacteriophage genetically modified to cause production of an inducer molecule in the bacterium following phage infection. The inducer molecule is released from the infected bacterium and is detected by genetically modified bacterial bioreporter cells designed to emit bioluminescence upon stimulation by the inducer. Autoamplification of the bioluminescent signal permits detection of low levels of bacteria without sample enrichment. Also disclosed are methods of detection for select bacteria, and kits for detection of select bacteria based on the described technology.

22. **Breathable non-woven composite viral penetration barrier fabric and fabrication process. Langley, J. D., Hinkle, B. S. (1998). Patent number 5,728,451.** A breathable non-woven fabric having barrier capabilities to biological liquids comprised of at least one non-woven layer bonded to at least one surface of a thermoplastic microporous film, the non-woven composite fabric providing a barrier to passage: (a) of biological liquid when the composite fabric is subjected to contact with synthetic blood under the dictates of testing procedure ASTM ES21-92; and (b) to viral penetration when the composite fabric is subject to contact with fX174 bacteriophage suspension at a titer of 10^3 PFU/mL for 5 minutes with no applied pressure, 1 minute at 13.8 kPa (2.0 PSIG), and 54 minutes with no applied pressure while maintaining a moisture of vapor transmission rate of greater than about 450 grams per square meter for 24 hours at about 75º F. and about 65% relative humidity, the non-woven composite fabric which has been thermally bonded by unwinding and contacting at least one continuous thermoplastic non-woven web to at least one side of a continuous thermoplastic microporous film, continuously transporting said contacted webs and film through a thermal bonding zone and thermally bonding the webs and film at multiple spaced-apart locations, said bonding having a dwell time sufficient to thermally bond said composite while avoiding burn-through degradation of the film and webs.

23. **Cheese making with bacteriophage resistant bacteria. Hicks, C. L. (2001). Patent number 6,297,042 .** 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.

24. **Composition for treating dental caries caused by *Streptococcus mutans*. Fischetti, V., Loomis, L. (2002). Patent number 6,399,098.** The present invention discloses a method for treating baterial dental caries caused by *Streptococcus mutans*, comprising administering a composition comprising an effective amount of at least one lytic enzyme produced by a bacteriophage specific for *Streptococcus mutans*, with the lytic enzyme having the ability to exclusively digest a cell wall of the *Streptococcus mutans* infecting all or part of a mouth or teeth, and a toothpaste for delivering the enzyme to the mouth and teeth.

25. **Composition for treatment of a bacterial infection of the digestive tract. Fischetti, V., Loomis, L. (2002). Patent number 6,399,097.** An enteric coated pill for treating bacterial infections of the digestive tract, wherein the bacteria to be treated are selected from the group consisting of *Listeria*, *Salmonella*, *E. coli*, *Campylobacter* and combinations thereof, said pill comprising an effective amount of at least one lytic enzyme genetically coded by a bacteriophage specific for said bacteria of the digestive tract, whereby said enzyme has the ability to digest the cell wall of said bacteria; and a carrier for said enzyme.

26. **Composition for treatment of a bacterial infection of an upper respiratory tract. Fischetti, V., Loomis, L. (2003). Patent number 6,423,299.** An aerosol composition for treating bacterial infections of the respiratory tract by delivering said aerosol to the mouth, throat or nasal passage, wherein the bacteria to be treated is selected from the group consisting of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus* Group A, and combinations thereof, said aerosol comprising an effective amount of at least one lytic enzyme genetically coded by a bacteriophage specific for a specific said bacteria of the respiratory tract, whereby said at least one lytic enzyme has the ability to digest the cell wall of said specific bacteria; and a carrier for delivering said enzyme.

27. **Composition incorporating bacterial phage associated lysing enzymes for treating dermatological infections. Fischetti, V., Loomis, L. (2001). Patent number 6,277,399.** A composition for treatment of bacterial infections of burns and wounds of the skin comprises an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria, and a carrier for delivering said at least one lytic enzyme to the skin. The carrier may be, but is not limited to, a liquid solution applied to a bandage.

28. **Compositions and methods for phage resistance in dairy fermentations. Broadbent, J. R., Oberg, C. J., Caldwell, S. (1997). Patent number 5,677,166.** A latococcal- and streptococcal-phage-resistant starter culture for

fermenting milk comprises a food-grade bacterium from the genera *Pediococcus*, *Leuconostoc*, *Lactococcus*, *Streptococcus*, or *Lactobacillus* transformed with a genetic element containing genes for a lactose fermentation phenotype. A method of making a lactococcal-phage-resistant starter culture comprises transforming a non-lactose fermenting, food-grade bacterium with a genetic element carrying determinants for a lactose fermentation phenotype. A method of making cheese with lactococcal-phage-resistant starter culture is also disclosed.

29. **Compositions containing bacteriophages and methods of using bacteriophages to treat infections. Ghanbari, H. A., Averback, P. (2000). Patent number 6,121,036.** 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.

30. **Conferred susceptibility to lambda phage in non-coliform procaryotic hosts. Ludwig, R. A., de Vries, G. E. (1988). Patent number 4,784,952.** Vectors suitable for effecting expression of lamB protein in a desired procaryotic host, and methods for their construction, are disclosed. Transformation with these vectors results in the ability of the procaryotic host to sustain infection by lambda phage.

31. **Detection of *Listeria* by means of recombinant bacteriophages. Scherer, S., Loessner, M. (1998). Patent number 5,824,468.** The invention relates to a detection procedure for bacteria of the genus *Listeria*, comprising the steps: (a) provision of a DNA vector prepared by means of recombination techniques, comprising a genetic system comprising DNA which encodes the expression of one or more proteins, the proteins not being a gene product of bacteria of the genus *Listeria* and it being possible to determine the presence of the proteins by a detection reaction, and the DNA vector infecting the bacteria of the genus Listeria and it being possible in this way to transfer the genetic system to the bacteria; (b) mixing of the sample with said DNA vector under conditions which allow an infection of bacteria of the genus *Listeria* by the DNA vector; (c) expression of the detectable proteins in the bacteria of the genus *Listeria*; (d) detection of the detectable proteins, the presence of bacteria of the genus *Listeria* being detected, and to recombinant DNA vectors and reagent compositions suitable for this detection procedure.

32. **Device and method for phage-based antibiotic susceptibility testing. Cottingham, H. V. (1999). Patent number 5,858,693.** A phage-based antibiotic susceptibility test is carried out by maintaining a patient sample in a sealed sample well during addition of the phage and Luciferin substrate used in the test, in order to prevent contamination of the laboratory environment. The phage is adhered in dried form to a metal carrier disk which is retained beneath the cap of the sealed sample well by means of an external magnet, and is mixed with the patient sample by removing the external magnet and allowing the carrier disk to fall to the bottom of the sample well. The Luciferin substrate is adhered to the underside of the cap and is mixed with the patient sample by shaking or inverting the sealed sample well after the metal carrier disk has separated from the underside of the cap. A row of connected sample wells and caps may be employed to allow the same patient sample to be tested with multiple antibiotics.

33. **DNA encoding phage abortive infection protein from *Lactococcus lactis* and method of use thereof. Moineau, S., Holler, B. J., Emond, E., Vandenbergh, P. A., Vedamuthu, E. R., Kondo, J. K. (1999). Patent number 5,928,688 (see also 5,910,571) .** DNA encoding phage resistance protein which aborts infection by the phage, designated as AbiE. The DNA which is contained in a *Lactococcus lactis* deposited as NRRL-B-21443 and described in SEQ ID NO:1, is incorporated into a bacterium to encode the AbiE and provide phage resistance. *Lactococcus* and other bacteria encoding the AbiE are useful in industrial fermentations wherein phage are a problem.

34. **DNA encoding phage resistance protein. Moineau, S., Emond, E., Walker, S., Vedamuthu, E. R., Kondo, J. K. (1999). Patent number 5,994,118.** A novel protein (Abi900, 183 amino acids) and its gene were isolated from a 11-kb natural plasmid (pSRQ900) of *Lactococcus lactis*. When pSRQ900 is introduced into dairy starter cultures, the Abi900 protein confers strong resistance to bacteriophage infection.

35. **DNA encodoing phage abortive infection protein from *Lactococcus lactis* and method of use thereof. Moineau, S., Holler, B. J., Vandenbergh, P. A., Vedamuthu, E. R., Kondo, J. K. (1998). Patent number 5,814,499.** DNA encoding phage resistance protein which aborts infection by the phage, designated as AbiE. The DNA which is contained in a *Lactococcus lactis* deposited as NRRL-B-21443 and described in SEQ ID NO: 1, is incorporated into a bacterium to encode the AbiE and provide phage resistance. *Lactococcus* and other bacteria encoding the AbiE are useful in industrial fermentations wherein phage are a problem.

36. **DNA fragments coding for a bacteriophage-resistant mechanism. Chopin, M.-C., Cluzel, P. J. (1997). Patent number 5,629,182.** The invention relates to DNA fragments encoding an Abi-type mechanism of resistance to bacteriophages, which fragments are capable of being obtained by cloning of chromosomal or plasmid DNA of a bacteriophage-resistant lactic acid bacterial strain, as well as to a polypeptide involved in an Abi-type resistance mechanism, encoded by one of the said fragments. The invention also encompasses recombinant vectors and transformed bacterial strains comprising the said DNA fragments.

37. **Enzyme for phage resistance. Moineau, S., Walker, S. A., Vedamuthu, E. R., Vandenbergh, P. A. (1999). Patent number 5,972,673.** 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.

38. **Externalization of products of bacteria. Auerbach, J. I., Rosenberg, M. (1987). Patent number 4,637,980.** A bacterial product is made by transforming a temperature sensitive lysogen with a DNA molecule which codes, directly or indirectly, for the product, culturing the transformant under permissive conditions and externalizing the product by raising the temperature to induce phage encoded functions.

39. **Filtration medium. Degen, P. J., Bilich, M. H., Staff, T. A., Gerringer, J., Salinaro, R. F. (1998). Patent number 5,788,862.** The present invention provides a filtration medium comprising an ultrafiltration membrane and a monomer surface coating thereon of an acrylic or methacrylic acid monomer having alcohol functional groups, wherein the filtration medium after having been fully dried is characterized by having a titer reduction of at least about 10^3 with respect to PP7 bacteriophage and a critical wetting surface tension of at least about 70 mN/m. The filtration medium preferably further comprises a fibrous nonwoven web embedded in the membrane. The present invention also provides a method of filtering a fluid through the present inventive filtration medium, as well as a method of preparing such a filtration medium.

40. **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). Patent number 5,723,330.** A method for testing for target bacteria involves adding bacteriophage to a sample to infect the bacteria in the sample; killing extracellular bacteriophage without killing phage-infected bacteria; amplifying bacteriophage remaining in the sample; and causing the bacteriophage to infect reporter bacteria and thereby produce an observable signal. The reporter bacteria are genetically engineered to have an indicator gene which on expression gives rise to a detectable signal, wherein expression of the indicator gene is initiated on bacteriophage infection of the bacteria.

41. **Inviable phages, their production and DNA thereof. Mattson, T. L., Epstein, R. (1998). Patent number 5,834,291 (see also 5,559,018).** Inviable T4 phage-like particles capable of directing the expression of large non-T4 DNA fragments from T4 expression control sequences are produced. Thus, *E. coli* harboring pBR322 derivatives containing cloned T4 gene 23 DNA sequences were infected with T4 phage carrying a deletion of the denB gene. Homology-dependent recombination results in the production of inviable phage-like particles containing DNA molecules composed of multiple, tandemly repeated copies of entire plasmid molecules covalently linked to single copies of normal phage genes. The yield of these inviable particles, intially low, was increased by means of a reiterated infection process that involves the use of a cloned T4 origin of replication. When T4 gene 32 expression control sequences linked in proper orientation to a DNA sequence coding for the non-T4 protein b-galactosidase were also cloned in one such pBR322 derivative (pVH773), inviable phage particles capable of directing the synthesis of enzymatically active b-galactosidase were produced. The present process is applicable to other T-even bacterio-phages.

42. **Isolated DNA encoding enzyme for phage resistance. Moineau, S., Walker, S., Vedamuthu, E. R., Vandenbergh, P. A. (2003). Patent number 5,925,388 (see also 5,824,523).** An isolated DNA of a *Lactococcus lactis* showing a SEQ ID NO:1 encoding a restriction and twp 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.

43. **Lysogenic bacteriophage isolated from acidophilium. Ward, T. W., Bruhn, D. F., Bulmer, D. K. (1992). Patent number 5,132,221.** A bacteriophage identified as fAc1 capable of infecting acidophilic heterotropic bacteria (such as *Acidiphilium* sp.) and processes for genetically engineering acidophilic bacteria for biomining or sulfur removal from coal are disclosed. The bacteriophage is capable of growth in cells existing at pH at or below 3.0. Lytic forms of the phage introduced into areas experiencing acid drainage kill the bacteria causing such drainage. Lysogenic forms of the phase having genes for selective removal of metallic or nonmetallic elements can be introduced into acidophilic bacteria to effect removal of the desired element form ore or coal.

44. **Matrices with memories and uses thereof. Nova, M. P., Senyei, A. E., Potash, H. (2003). Patent number 6,100,026 (see also 5,961,923; 6,017,496; 6,340,588).** Combinations, called matrices with memories, of matrix materials that are encoded with an optically readable code are provided. The matrix materials are those that are used in as supports in solid phase chemical and biochemical syntheses, immunoassays and hybridization reactions. The matrix materials may additionally include fluophors or other luminescent moieties to produce luminescing matrices with memories. The memories include electronic and optical storage media and also include optical memories, such as bar codes and other machine-readable codes. By virtue of this combination, molecules and biological particles, such as phage and viral particles and cells, that are in proximity or in physical contact with the matrix combination can be labeled by programming the memory with identifying information and can be identified by retrieving the stored information. Combinations of matrix materials, memories, and linked molecules and biological materials are also provided. The combinations have a multiplicity of applications, including combinatorial chemistry, isolation and purification of target macromolecules, capture and detection of macromolecules for analytical purposes, selective removal of contaminants, enzymatic catalysis, cell sorting, drug delivery, chemical modification and other uses. Methods for tagging molecules, biological particles and matrix support materials, immunoassays, receptor binding assays, scintillation proximity assays, non-radioactive proximity assays, and other methods are also provided.

45. **Method and buffered bulk starter media for propagation of useful bacteria. Sandine, W. E., Huggins, A. R. (1988). Patent number 4,766,076.** The invention comprises a novel starter medium for the commercial propagation of acid producing bacteria, such as those used in food fermentation processes. The compositions are unique in that they contain a highly effective buffering ingredient which is a sodium, potassium, or ammonium salt or double salt of a linear aliphatic dibasic acid having from three to seven carbon atoms. The salts are present in an amount sufficient to maintain the growth medium at pH levels of about 5.0 or above during the time in which the bacteria are multiplying in the culture medium. Disodium or diammonium succinate, glutarate, or adipate are materials which have been found to be particularly effective. These may be used in combination with nutrients such as whey, whey permeate, nonfat dried milk, yeast extract, and diammonium phosphate. The addition of trace quantities of certain metals promotes the growth and activity of the acid producing bacteria. Small quantities of ferrous, manganous, or manganese ions are particularly useful. A combination of iron and manganese with the other ingredients in the media produced results better than either of these materials standing by itself. In a commercial test, the cheese produced using an inoculant based on one of the present formulations was of excellent quality. Most of the formulations containing the bibasic acid salts appear to be highly resistant to bacteriophage infection.

46. **Method and compositions for use in the treatment of fireblight. Vedamuthu, E. R., Vidaver, A. K. (1988). Patent number 4,783,406 (see also 4,678,750).** A method and compositions for the treatment of fireblight disease in plants are described. The compositions include a phage for *Erwinia amylovora* which produces fireblight and an enzyme produced by the phage which depolymerizes a polysaccharide produced by *Erwinia amylovora* which is the cause of the fireblight disease. Purified enzyme preparations are described.

47. **Method and device for detecting bacteriophage using contrast-coloring and precipitable dyes. Wicks, J. H., Krejcarek, G. E., Williams, M. G. (2000). Patent number 6,090,541.** 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.

48. **Method and test kits for detection of bacteriophage. Sanders, M. F. (1999). Patent number 5,914,240 .** A method for detection, identification and/or quantification of bacteriophage of bacterial host specificity for bacterial genus, species or serotype, based upon the occurrence of release of cell contents, particularly nucleotides e.g. ATP, on lysis of bacterial cell walls on incubation with bacterial host cells. When new phage particles are released at the end of the phage replication cycle nucleotide levels are measured and compared with controls. The method provides for the detection of specific phages which is faster and more sensitive than known techniques. The method is only limited by the availability of host bacteria/target phage pairings.

49. **Method for detecting bacteria using bacteriophage, contrast-coloring dye and precipitable dye. Wicks, J. H., Krejcarek, G. E., Williams, M. G. (1999). Patent number 5,958,675.** Bacteria are detected in a test sample by contacting the test sample with a bacteriophage that is capable of replicating in the bacteria, adding the resultant sample to a water-proof surface of a support for bacterial growth that contains a contrast-coloring dye and a precipitable dye, forming a bacterial lawn of a bacteria in which the bacteriophage can replicate on the support and detecting plaques on the bacterial lawn as an indication of the presence of the bacteria. The combination of precipitable dye and contrast-coloring dye improves visualization of plaques. A precipitate is formed in plaques by enzymatic cleavage of the precipitable dye by an enzyme of the bacterial lawn. A procedure for detecting bacteriophage is similar to that for detecting bacteria, except that a test sample suspected of containing bacteriophage is combined with bacteria in which the bacteriophage can replicate, and plaques detected indicate presence of the bacteriophage. The bacteria and bacteriophage detections are carried out with a disposable device containing at least one well having a water-proof surface and substantially vertical sides that extend at least 5 millimeters in height from the surface. A hydratable material containing the precipitable dye and the contrast-coloring dye is positioned on the water-proof surface. A removable cover rests on top of the sides of the well.

50. **Method for detecting bacteria with bacteriaphage [*sic*]. Nakayama, , H. (2003). Patent number 6,555,312.** A method for detecting a bacterium for measurement, including the steps of: allowing a bacteriophage to bind to the bacterium, the bacteriophage being capable of specifically binding to the bacterium and growing in the bacterium, whereby a gene within the bacteriophage which expresses a light-emission protein is introduced into the bacterium so that a protein is produced within the bacterium as a product of the gene; and providing an external factor in a non-invasive manner from outside of the bacterium, thereby causing only the actually-present bacterium to emit light in a specific manner.

51. **Method for facilitating externalization of proteins synthesized in bacteria. Zinder, M. D., Model, P., Boeke, J. D. (1986). Patent number 4,595,658.** A method of externalizing proteins from the periplasmic space of gram-negative bacteria and in particular, *E. coli* and its relatives, comprising utilizing bacteria which have a phage gene, coding for a protein (such as gene III protein) or a mutant bacterial gene (such as a gene coding for a membrane function) which causes perturbation of the outer bacterial membrane resulting in leakage of proteins in the periplasmic space from the cell.

52. **Method for forming an array of biological particles. Raybuck, M. (1998). Patent number 5,763,170.** A method for forming and using an array of, e.g., bacteria, yeast or bacteriophage for the purpose of identifying particular constituents thereof. The array is formed by directing a stream of droplets, each containing on average about 1 or a few biological particles, at spaced locations in an array on a surface, e.g., a nylon membrane or agar gel.

53. **Method for identifying target bacteria. Sanders, M. F. (1999). Patent number 5,888,725.** A method for detection, identification and/or quantification of target organisms of specific bacterial genus, species or serotype, based upon the occurence of release of cell contents, particularly nucleotides, e.g., ATP, on lysis of bacterial cell walls on incubation with bacteriophages (phages) specific for them. When new phase particles are released at the end of the phage replication cycle nucleotide levels are measured and compared with controls. The method provides for the detection of specific bacteria which does not require insertion of the lux gene into the phage genome yet is faster and more sensitive than known non-modified phage utilizing techniques. The method is only limited by the availability of phage types suitable for selective attack of the target bacterial to be detected and can detect a single *Salmonella* in a sample of milk in under 12 hours.

54. **Method for producing single and/or mixed strain concentrates of bacteria. Sandine, W. E., Huggins, A. R. (1985). Patent number 4,528,269 .** An improved method which differentiates or separates heterogeneous populations of fast and slow acid producing strains of bacteria by growth of the strains under closely controlled unique conditions so as to allow the selection of a colony of one or the other strains is described. Preferably a gelled, solid growth medium containing in admixture: (1) milk protein, a milk protein derivative, or a milk protein substitute;

(2) an acid pH sensitive color change indicator; and, (3) a buffering agent is used. The colonies have a contrasting color within and around them because of the effect of the acid produced by the bacteria on the indicator. The growth of the bacteria is under anaerobic or near anaerobic conditions in order to achieve certainty in the colony selection for fast or slow acid production. The bacteria can also be mixed with phage which inhibit or kill the members of a heterogeneous or homogeneous population of bacteria on the medium and grown to produce phage resistant colonies. The relatively large colonies which exhibit enhanced acid production and proteolysis of the milk protein on the plating container are selected for commercial use in preparing fermented products, particularly fermented foods.

55. **Method for producing mucoid and phage resistant group N streptococcus strains from non-mucoid and phage sensitive parent strains. Vedamuthu, E. R. (1990). Patent number 4,918,014 (see also 4,874,616).** A method for imparting phage resistance to phage sensitive strands of *Streptococcus* group N is described. The method involves transferring plasmid encoding for production of a mucoid substance (Muc^+) into the phage sensitive strain. Even if the Muc^+ plasmid is removed by curing at elevated temperatures the strains remain resistant to phage. The resulting resistant strains are novel and are used for fermentations, particularly milk fermentations.

56. **Method of conferring bacteriophage resistance to bacteria. Hershberger, C. L., Rosteck, P. R. (1985). Patent number 4,530,904.** A novel method for protecting a bacterium from a naturally occurring bacteriophage and the cloning vectors and transformants for carrying out the aforementioned method are disclosed.

57. **Method of detecting compounds utilizing genetically modified lambdoid bacteriophage. Ray, B. L., Lin, E. C. C., Crea, R. (1997). Patent number 5,650,267.** Disclosed is an infective lambdoid bacteriophage which includes a protein construct comprising a genetically modified major tail protein truncated at its carboxy terminus, and a target molecule peptide bonded to the carboxy terminus of the tail protein. Also disclosed are nucleic acids encoding the construct and methods of detecting a molecule-of-interest in a solution and of detecting a cell which produces a molecule-of-interest.

58. **Method of detecting a pathogen using a virus. Cherwonogrodzky, J. W., Lotfali, K. (2002). Patent number 6,436,652 (see also 6,355,445).** A bacteriophage linked to an enzyme can replace an antibody in a system for detecting the presence of a bacteria in a sample. Specifically *Brucella abortus* (a pathogen which causes brucellosis in cattle) can be detected using *Brucella* bacteriophage for the virus, urease for the enzyme linked to the bacteriophage, m-maleimidobenzoyl-N-hydrosysuccimide ester as a coupling reagent, sera from mice immunized with *Brucella* bacteriophage for a detector antibody, urease conjugated to anti-mouse sheep antibody for an indicator, and urea with bromcresol purple as the substrate. The materials can be used in indirect (sandwich) or direct enzyme-linked viral assays (ELVirA).

59. **Method of eliminating genetic routes for bacteriophage evolution and products produced thereby. Klaenhammer, T. R., Moineau, S. (1997). Patent number 5,618,723 (see also 5,580,725).** A process of identifying and disrupting bacterial DNA sequences that contribute to the evolution of new lytic bacteriophages is described. Vectors and recombinant bacteria for use in producing fermentative starter cultures and culture resistant to the appearance of new phages, and methods of producing such vectors and recombinant bacteria, are described.

60. **Method of making cheese using viral enzymes. Gasson, M. J. (1994). Patent number 5,360,617.** The lysin from a Lactococcus (preferably prolate-headed) bacteriophage is used to lyse bacterial starter cultures during cheese-making. Such bacteriophage include fvML3. In addition, the fvML3 lysin has been characterized and a coding sequence for it has been cloned.

61. **Method of preparing cheese starter media. Reddy, M. S. (1986). Patent number 4,621,058.** Low cost, readily dispersible, phage-resistant cheese starter media are described which include milk-derived nutrients (e.g., nonfat milk and whey) along with a minor proportion of preferably free or unbound lecithin. The media also may advantageously include sodium tetraphosphate which assists in the dispersion of whey solids. The media of the invention can be used at significantly lower levels as compared with nonfat dry milk solids (e.g. 7 percent versus 12 percent), while nevertheless obtaining essentially equivalent results in terms of culture growth and final culture properties. A method of producing the media is also disclosed, involving liquid preblending of phosphates and lecithin, followed by addition thereof to milk-derived nutrients and reaction of the phosphates to tie up free calcium ion. Preferably, reaction is carried out for about 1 to 12 hours while agitating. The final step involves drying of the mixture to yield a substantially homogeneous, reconstitutable powder. In other cases the phosphate-lecithin preblend can be dried for later addition to milk-derived nutrients to produce a final starter medium.

62. **Method of preparing food and composition for protecting microorganisms used in the preparation of food. Lembke, A., Deininger, R., Lembke, J. (1989). Patent number 4,834,987.** In the preparation of food with the aid of microorganisms, the latter are directly protected against viral or phage attack by the addition of formic acid or esters of formic acid or salts of formic acid and/or tetrahydrofolic acid. Furthermore, an indirect protection by inactivating the bacterial viruses in the environment is described.

63. **Method of recovering bacteriophage. DeBonville, D. A., Logan, K. A. (1993). Patent number 5,204,257.** A method of recovering nucleic acid-containing particles from a liquid medium by contacting the liquid medium containing the particles with a mixture of hydroxylated silica beads and a salt solution to bind the nucleic acid-containing particles, centrifuging the mixture to pellet the bound particles, and separating the pellet from the liquid.

64. **Method to detect bacteria. Wilson, S. M. (2002). Patent number 6,461,833 (see also 5,985,596).** 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.

65. **Methods for rapid microbial detection. Rees, C. E. D., Rostas-Mulligan, K., Park, S. F., Denyer, S. P., Stewart, G. S. A. B., Jassim, S. A. A. (1996). Patent number 5,498,525.** A method of testing for target bacteria involves adding bacteriophage to a sample to infect the bacteria in the sample; killing extracellular bacteriophage without at the same time killing phage-infected bacteria; amplifying bacteriophage remaining in the sample; and causing the bacteriophage to infect reporter bacteria and thereby produce an observable signal. The reporter bacteria are genetically engineered to have an indicator gene which on expression gives rise to a detectable signal, wherein expression of the indicator gene is initiated on bacteriophage infection of the bacteria.

66. **Methods of detection utilizing modified bacteriophage. Li, M. (2001). Patent number 6,190,856.** Viruses expressing ligands on their surfaces are used as a detection means for the related polypeptide which binds the ligand. Multiple copies of the ligand can be expressed on the viral surface. These viruses may be used to detect polypeptides, cells, receptors and channel proteins.

67. **Mycobacteriophage DSGA specific for the *Mycobacterium tuberculosis* complex. Pearson, R. E., Dickson, J. A., Hamilton, P. T., Little, M. C., Beyer, Jr. W. F. (1995). Patent number 5,476,768.** Mycobacteriophage DS6A has been characterized and found to specifically infect all species of the TB complex, without any detectable infection of mycobacteria species other than those of the TB complex. DNA sequence analysis revealed several potential open reading frames, including one encoding a protein analogous to gp37 of mycobacteriophage L5 and a second encoding a protein with significant homology to the *S. coelicolor* DNA polymerase b subunit. Based on the DNA sequence analysis, cloning sites can be identified for insertion of reporter genes, making DS6A useful as a reporter phage for specific detection and identification of species of the TB complex.

68. **Mycobacteriophage specific for the *Mycobacterium tuberculosis* complex. Pearson, R. E., Dickson, J. A., Hamilton, P. T., Little, M. C., Beyer, Jr. W. F. (1997). Patent number 5,612,182 (see also 5,582,969).** Mycobacteriophage DS6A has been characterized and found to specifically infect all species of the TB complex, without any detectable infection of mycobacteria species other than those of the TB complex. DNA sequence analysis revealed several potential open reading frames, including one encoding a protein analogous to gp37 of mycobacteriophage L5 and a second encoding a protein with significant homology to the *S. coelicolor* DNA polymerase b subunit. Based on the DNA sequence analysis, cloning sites can be identified for insertion of reporter genes, making DS6A useful as a reporter phage for specific detection and identification of species of the TB complex.

69. **Non-isotopic substrate assay employing bacteriolysis products. Young, D. M. (1978). Patent number 4,104,126.** Substrates such as haptens and antigens, and those for receptor proteins and native circulating binding proteins are assayed by determining bacteriolysis products occasioned by bacteriophage infection of host cells, in a modification of the "chemically modified bacteriophage assay." Thus, a substrate such as an antigen is conjugated with bacteriophage and the conjugate competes with antigen in the specimen under assay for a limited number of binding sites on antibody. Phage conjugate surviving antibody inactivation is quantified by determining intracellular constituents of host bacteria subsequently infected by the bacteriophage remaining viable, which latter can be related to the levels of antigen originally present in the specimen. A preferred embodiment involves colorimetric assay for beta galactosidase freed by phage lysis of *E. coli*. Generally, the method is of sensitivity comparable to that of radioimmunoassay, but is attended by substantial advantages not common to the latter technique. The method is far less cumbersome than the plaque-containing techniques hitherto employed in bacteriophage assays.

70. **Novel bacteriophage and method for preparing same. Nakano, E., Saito, N., Fukushima, D. (1982). Patent number 4,332,897.** A novel bacteriophage whose DNA molecule has endonuclease-sensitivity only in the DNA region carrying genetic information for the production of phage coat proteins can be obtained by isolating an endonuclease-resistant mutant from one of the lambdoid bacteriophages and mating the resulting bacteriophage with a lambdoid phage having endonuclease-sensitivity in the DNA region carrying genetic information for the production of coat proteins.

71. **Nucleic acid sequence and plasmids comprising at least one phage resistance mechanism, bacteria in which they are present, and their use. Prevots, F., Remy, E., Ritzenthaler, P. (1997). Patent number 5,658,770.** The invention relates to a DNA sequence of about 1.9 kb comprising at least one phage resistance mechanism, said sequence being obtained from the HindIII--HindIII DNA sequence of 3.3 kb contained in the strain *Lactococcus lactis* ssp *lactis*, deposited in the CNCM under no. I-945, by the PCR method.

72. **Nucleic acid sequences and plasmids comprising at least one phage resistance mechanism, bacteria containing them and their use. Prevots, F., Tolou, S., Daloyau, M. (1999). Patent number 5,955,332 (see also 5,712,150).** The invention relates to polynucleotides of 1345 bp and 3704 bp and the like, which comprise at least one phage resistance mechanism and obtainable from the total DNA contained in the Lactococcus lactis ssp cremoris strain deposited in the CNCM under No. I-941.

73. **Parenteral use of bacterial phage associated lysing enzymes for the therapeutic treatment of bacterial infections. Fischetti, V., Loomis, L. (2001). Patent number 6,264,945 .** The present invention discloses a method and composition for the treatment of bacterial infections by the parenteral introduction of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for that bacteria and an appropriate carrier for delivering the lytic enzyme into a patient. The injection can be done intramuscularly, subcutaneously, or intravenously.

74. **Phage-dependent super-production of biologically active protein and peptides. Kordyum, V. A., Chernykh, S. I., Slavchenko, I. Y., Vozianov, O. F. (2001). Patent number 6,268,178.** This invention relates to a method for enhancing the production of biologically active proteins and peptides in bacterial cells by infecting bacterial cells of the producer strain, which contain a plasmid with one or more targeted genes, with bacteriophage .lambda. with or without the targeted gene(s). The phage increases synthesis of the targeted protein and induces lysis of the producer

strain cells. Super-production is achieved by cultivating the producer strain cells under culture conditions that delay lytic development of the phage. The biologically active proteins and peptides subsequently accumulate in a soluble form in the culture medium as the cells of the producer strain are lysed by the phage.

75. **Phage-resistant streptococcus. Mollet, B., Pridmore, D., Zwahlen, M. C. (1998). Patent number 5,766,904.** DNA fragment of phages which are virulent towards a *Streptococcus*, capable of conferring on a *Streptococcus* containing it resistance to at least one phage, especially a fragment homologous or hybridizing to the 3.6 kb HindIII fragment present in the plasmid CNCM I-1588 or the 6.5 kb EcoRV fragment present in the plasmid CNCM I-1589. Process for making a *Streptococcus* resistant to at least one phage, by cloning into a vector a DNA fragment of a phage which is virulent towards a *Streptococcus*, capable of conferring on a *Streptococcus* resistance to at least one phage and introducing the vector into a *Streptococcus*.

76. **Phage bonded to a nuclear location signal. Nakanishi, M., Nagoshi, E., Akuta, T., Takeda, K., Hasegawa, M. (2001). Patent number 6,235,521 .** A l phage with a nuclear localization signal has been obtained by constructing a vector capable of expressing a fused protein between a gpD protein constituting the head of a l phage and a nuclear localization signal sequence, transforming *Escherichia coli* with this vector, and propagating a mutant l phage which cannot express the gpD protein in E. coli in this transformant. It has been confirmed that the resulting l phage is capable of packaging l phage DNAs of 80% and 100% genome sizes. After further confirming that the nuclear localization signal exposed on the outside of the head of this phage, this phage has been microinjected into cells to analyze its nuclear localization activity. Thus, it has been clarified that this phage has a nuclear localization activity.

77. **Phage defense rotation strategy. Klaenhammer, T. R., Sing, W. D., Hill, C. J. (1997). Patent number 5,593,885.** A phage defense rotation strategy for use in the successive fermentations of a substrate in a fermentation plant is disclosed. The strategy comprises (a) fermenting substrate with a first bacterial culture comprising a bacterial strain capable of fermenting the substrate and, preferably, carrying a first phage defense mechanism; and then (b) fermenting the substrate with a second bacterial culture comprising a second bacterial strain isogenic with the first bacterial strain, wherein the second strain carries a second phage defense mechanism different from the first phage defense mechanism. Also disclosed is a mixed bacterial culture capable of fermenting a substrate. The mixed culture comprises (a) a first bacterial strain carrying a first phage defense mechanism; and (b) a second bacterial strain isogenic with the first strain, wherein the second strain carries a phage defense mechanism different from the phage defense mechanism carried by the first strain.

78. **Phage detection. LaBelle, G. G., Staehler, G. E. (1980). Patent number 4,218,534.** To select a blend of strains not susceptible to the current bacteriophage in the cheesemaking plant the cheesemaker inoculates each of the test tubes in the kit with filtered when obtained from current production. Each tube contains a genetically distinct starter culture strain or a culture blend in a sterile milk medium and contains a dye which will change color in the desired pH range. After incubation for ten hours the cultures resistant to the prevailing phage will exhibit the desired color change and will have developed a firm curd. A starter culture now known to be resistant to the prevailing phage can now be selected. Tests show success closely approaching 100% as opposed to 96% (or less) with the traditional rotation method of selecting culture blends.

79. **Phage with nuclear localization signal. Nakanishi, M., Nagoshi, E., Akuta, T., Takeda, K., Hasegawa, M. (2001). Patent number 6,300,120.** A l phage with a nuclear localization signal has been obtained by constructing a vector capable of expressing a fused protein between a gpD protein constituting the head of a l phage and a nuclear localization signal sequence, transforming *Escherichia coli* with this vector, and propagating a mutant l phage which cannot express the gpD protein in E. coli in this transformant. It has been confirmed that the resulting l phage is capable of packaging l phage DNAs of 80% and 100% genome sizes. After further confirming that the nuclear localization signal exposed on the outside of the head of this phage, this phage has been microinjected into cells to analyze its nuclear localization activity. Thus, it has been clarified that this phage has a nuclear localization activity.

80. **Polyvinylidene fluoride membrane and method for removing viruses from solutions. Degen, P. J., Bilich, M. H., Staff, T. A., Gerringer, J., Salinaro, R. F. (1998). Patent number 5,736,051.** The present invention provides an isotropic, skinless, porous, polyvinylidene fluoride membrane having a K_{UF} of at least about 15 psi (103 kPa), and preferably below about 50 psi (345 kPa), when tested using liquid pairs having an interfacial tension of about 4 dynes/cm (4 mN/m). The present inventive membrane preferably has a titer reduction of at least about 10^8 against T_1 bacteriophage, more preferably also against PR772 coliphage, and even more preferably also against PP7 bacteriophage. The present inventive membrane can have a thickness of about 20 mil (500 mm) or less and even as low as about 5 mil (125 mm) or less. The present invention also provides a method of preparing such a membrane by providing a casting solution comprising polyvinylidene fluoride and a solvent therefor, heating the casting solution to a uniform temperature of about 57° C. to about 60° C., spreading the casting solution onto a substrate to form a film, quenching the film in a quench bath so as to form a porous membrane, and washing and drying the porous membrane.

81. **Process for retarding bacterial growth in cheese. Day, C. A., Holton, B. W. (1991). Patent number 5,006,347.** The present invention discloses the use of bacteriophages for controlling unwanted fermentation of cheese by bacteria.

82. **Production of phage and phage-associated lysin. Yang, H.-H., Hiu, S. F., Harris, J. L. (1989). Patent number 4,859,597.** A method for producing a lysin-free phage inoculum, which comprises: (a) inoculating a growing Streptococcal culture with phage, (b) incubating the culture for a plurality of lytic cycles of phage until the cells are completely lysed to obtain a lysate, and (c) removing cell debris and free lysin from the lysate to form a lysin-free phage suitable for use as an inoculum. A method for producing lysin is also disclosed.

83. **Prophylactic and theraputic treatment of group A streptococcal infection. Fischetti, V., Loomis, L. (1999). Patent number 5,985,271.** The present invention relates to an oral delivery system containing a group c streptococcal phage associated lysin enzyme for the prophylactic and therapeutic treatment of *Streptococcal* A throat infections, commonly known as strep throat

84. **Protection of microorganisms against bacteriophage attacks. Wolf, E., Lembke, A., Deininger, R. (1983). Patent number 4,409,245.** Living cultures of microorganisms used in the preparation of foodstuffs by microbiological processing are protected against attack by bacteriophage viruses by the addition thereto of terpene. The terpene is added in an amount which is effective to obtain viricidal activity but ineffective to cause toxic effects on the microorganisms. The terpene is one obtainable from aromatic plants by steam distillation. Terpenes or mixtures of terpenes which have proved suitable are those obtained from black pepper oil, cinnamon flower oil, cardamon oil, linallyl acetate, cinnamic aldehyde, safrol, carvon and cis/ trans citral, used individually or mixed together. They may added dissolved in a carrier such as 1,2-propanediol. The terpenes demonstrate a viricidal activity in a concentration which is one or more powers of ten lower than the concentration at which the terpenes have toxic effects on the microorganisms.

85. **pTN1060, a conjugal plasmid and derivatives thereof that confer phage resistance to group N streptococci. Klaenhammer, T. R., Sanozky-Dawes, R. B. (1989). Patent number 4,883,756.** The present invention relates to the plasmid pTN1060 and derivatives thereof which confer phage restriction and modification activity to group N streptococci. The invention further relates to microorganisms containing pTN1060 or a derivative thereof and to starter cultures containing the microorganisms.

86. **PTR2030, a conjugal plasmid and derivatives thereof that confer phage resistance to group N streptococci. Klaenhammer, T. R., Sanozky, R. B., Steenson, L. R. (1992). Patent number 5,139,950 (see also 4,931,396).** The present invention relates to the plasmid pTR2030 and derivatives thereof which confer phage resistance to group N streptococci. The invention further relates to microorganisms containing pTR2030 or a derivative thereof and to starter cultures containing the microorganisms.

87. **Quantitative detection of specific nucleic acid sequences using lambdoid bacteriophages linked by oligonucleotides to solid support. Ray, B. L., Lin, E. C. C. (2003). Patent number 5,679,510.** The present invention provided compositions, methods and kits for detection and quantitation of pathogenic organisms. The composition of the invention is an oligonucleotide probe comprising a bacteriophage covalently linked to one site on an oligonucleotide probe complementary to a conserved region of a pathogenic organism. At a second site, the oligonucleotide probe is linked to a matrix. The oligonucleotide probe contains a region complementary to one strand of a restriciton endonuclease recognition site or an oligoribonucleotide moiety. The number of pathogenic organisms present in a biological fluid sample may be quantitated in accordance with the method of the invention by combining the composition of the invention with the sample, allowing hybridization to occur. Hybridization generates a DNA-RNA hybrid, and by adding the appropriate nucleolytic enzyme capable of cleaving DNA-RNA hybrids; bacteriophage will be released for measurement. The kit of the invention provides components which allow the method of the invention to be performed.

88. **Rapid identification of environmental bacillus. Kiel, J. L., Alls, J. L., Weber, R. A., Parker, J. E. (1992). Patent number 5,156,971.** A diagnostic test for environmental bacillus which comprises the steps of inoculating an agar growth medium comprising a nitrate source, luminol and 3-amino-L-tyrosine (3AT) with the sample, incubating the inoculated medium and determining the presence of the bacillus. The novel medium preferably comprises potassium nitrate, luminol, 3-amino-L-tyrosine and trypticase soy agar. Antibiotics and/or a specific bacteriophage may be added to the medium surface in localized areas to show specific bacterial lysis for identification. The novel medium and the methods of this invention are suitable for the identification of *B. anthracis*.

89. **Rapid Identification of *Escherichia coli* O157:H7. Chang, T. C., Chen, S., Ding, H. C. (2001). Patent number 6,210,911.** 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.

90. **Rapid identification of microorganisms. Chang, T. C., Chen, S., Ding, H. C. (2002). Patent number 6,428,976.** 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 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.

91. **Recombinant phages. Mardh, S. (2002). Patent number 6,497,874.** The present invention relates to bacteriophages for use in the treatment or prophylaxis of bacterial infections, especially mucosal bacterial infections such as Heliobacter pylori infections, in particular, it relates to modified filamentous bacteriophages, e.g. M13 phages, for such use, which bacteriophages present at the surface a recombinant protein comprising (i) a first component derived from a bacteriophage surface protein; and (ii) a second component comprising variable region sequences of an antibody to provide a bacterial antigen binding site, said second component rendering said bacteriophage capable of binding to and thereby inhibiting growth of bacterial cells involved in the etiology of said infection.

92. **Remotely programmable matrices with memories. Nova, M. P., Senyei, A. E. (2002). Patent number 6,416,714 (see also 5,741,462; 5,751,629; 5,874,214; 5,925,562; 6,025,129; 6,331,273; 6,352,854).** Combinations, called matrices with memories, of matrix materials with remotely addressable or remotely programmable recording devices that contain at least one data storage unit are provided. The matrix materials are those that are used in as supports in solid phase chemical and biochemical syntheses, immunoassays and hybridization reactions. The data storage units are preferably non-volatile antifuse memories. By virtue of this combination, molecules and biological particles, such as phage and viral particles and cells, that are in proximity or in physical contact with the matrix combination can be labeled by programming the memory with identifying information and can be identified by retrieving the stored information. Combinations of matrix materials, memories, and linked molecules and biological materials are also provided. The combinations have a multiplicity of applications, including combinatorial chemistry, isolation and purification of target macromolecules, capture and detection of macromolecules for analytical purposes, selective removal of contaminants, enzymatic catalysis, chemical modification and other uses.

93. **RNA bacteriophage-based delivery system. Stockley, P. G., Mastico, R. A. (2000). Patent number 6,159,728.** A delivery system, especially for delivery to targeted sites in the human or animal body, comprises capsids of the coat protein amino acid sequence of phage MS-2 or related phage, or a modification thereof which retains capsid-forming capability, and at least some of the capsids enclosing a moiety foreign to the genome of MS-2 or related phage.

94. **Super fast tuberculosis diagnosis and sensitivity testing method. Ledley, R. S. (1999). Patent number 5,922,282.** Very rapid diagnosis and sensitivity testing can significantly stem the growing *Tuberculosis* epidemic in the United States, caused by susceptible AIDs patients and the occurrence of antibiotic resistant mycobacilli. Thus I have invented an automated computerized microscope, the ATBD unit, and slide module to diagnose and test patient's sputum by examining individual living mycobacteria from the patient sample with no culturing required. The diagnosis and sensitivity testing is accomplished in minutes or hours, instead of the current weeks to months. The system inserts a plasmid, specific for M. tuberculosis, carrying the luciferase gene into the mycobacteria by improved electroporesis on the slide. Luminescence indicates tuberculosis. Then the mycobacteria are bathed in antibiotics, and if the luminescence is not turned off, the patient's bacteria are resistant. A phage carrying the luciferase gene can also be used to infect the M.TB. Finally, the invention can be applied to any mycobacteriological infection to do diagnosis sensitivity testing even when the species is not known.

95. **Therapeutic antimicrobial polypeptides, their use and methods for preparation. Jaynes, J. M., Enright, F. M., White, K. L. (2001). Patent number 6,303,568.** A novel class of antimicrobial agents for animal species including cecropins, attacins, lysozymes, phage derived polypeptides, such as those transcribed from gene 13 of phage 22, an S protein from lambda phage, and an E protein from phage PhiXl74, as well as, synthetically derived polypeptides of similar nature. The antimicrobial agents can be used to treat microbial infections and as components of medicinal compositions. The genes encoding for such antimicrobial agents can be used to transform animal cells, especially embryonic cells. The transformed animals including such antimicrobial cells are also included.

96. **Therapeutic treatment of group A streptococcal infections. Fischetti, V., Loomis, L. (2000). Patent number 6,017,528 (see also 5,997,862).** The present invention relates to compositions containing Group C streptococcal phage associated lysin enzyme for the prophylactic and therapeutic treatment of Streptococcal infections, including the infection commonly known as strep throat. Methods for therapeutically and prophylactically treating such infections also are described.

97. **Topical treatment of streptococcal infections. Fischetti, V., Loomis, L. (2000). Patent number 6,056,955.** The present invention discloses a method and composition for the topical treatment of streptococcal infections by the use of a lysin enzyme blended with a carrier suitable for topical application to dermal tissues. The method for the treatment of dermatological streptococcal infections comprises administering a composition comprising effective amount of a therapeutic agent, with the therapeutic agent comprising a lysin enzyme produced by group C streptococcal bacteria infected with a C1 bacteriophage. The therapeutic agent can be in a pharmaceutically acceptable carrier.

98. **Transducing particles and methods for their production. Gutterson, N. I., Tucker, W. T., Wolber, P. K. (1993). Patent number 5,187,061.** Viable bacteria may be detected in biological samples by exposing bacterial cultures obtained from the samples to transducing particles having a known host range. Such transducing particles carry a heterologous gene capable of altering the phenotype of the bacteria in a readily detectable manner. For example, the transducing particles may carry an ice nucleation gene and the alteration of phenotype may be detected using an ice nucleation assay. By employing a panel of phage, unknown bacteria nmay be typed based on the pattern of reactivity observed. The transducing particles may be prepared by introducing a synthetic transposable element carrying the heterologous gene to a host carrying a prophage having the desired host range. After transposition, the host may be induced to a lytic cycle to release the transducing particles carrying the heterologous gene.

99. **Transducing phages of Actinomycetales. Westpheling, J., Burke, J. A. (2001). Patent number 6,245,504.** 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.

100. **Use of bacterial phage associated lysing enzymes for treating various illnesses. Fischetti, V., Loomis, L. (2001). Patent number 6,238,661.** Compositions and methods for the prophylactic and therapeutic treatment of bacterial infections are disclosed which comprise administering to an individual an effective amount of a composition comprising an effective amount of lytic enzyme and a carrier for delivering the lytic enzyme. This method and composition can be used for the treatment of upper respiratory infections, skin infections, wounds, burns, vaginal infections, eye infections, intestinal disorders and dental problems.

101. **Use of bacterial phage associated lysing enzymes for treating dermatological infections. Fischetti, V., Loomis, L. (2002). Patent number 6,432,444.** A bandage for treating a bacterial infection of skin is disclosed wherein the bandage contains a composition produced by the method of obtaining an effective amount of at least one lytic enzyme genetically coded for by a specific bacteriophage specific for a bacteria infecting the skin, wherein the bacteria to be treated is selected from the group consisting of *Staphylococcus*, *Pseudomonas*, *Streptococcus*, and combinations thereof. This lytic enzyme is specific for and has the ability to digest a cell wall of one of the bacteria and is coded for by the same bacteriophage capable of infecting the bacteria being digested. The enzyme produced is mixed with a topical carrier.

102. **Use of bacterial phage associated lysing enzymes for treating bacterial infections of the mouth and teeth. Fischetti, V., Loomis, L. (2002). Patent number 6,335,012.** The present invention discloses a method for treating

dental caries, comprising administering a composition comprising an effective amount of at least one lytic enzyme produced by a bacteriophage specific for said bacteria, the lytic enzyme having the ability to digest a cell wall of the bacteria infecting all or part of a mouth or teeth, and a carrier for delivering the enzyme to the mouth and teeth.

103. **Use of bacterial phage associated lysing enzymes for treating streptococcal infections of the upper respiratory tract. Fischetti, V., Loomis, L. (2002). Patent number 6,326,002.** The present invention discloses a method for treating Streptococcal infections of the upper respiratory tract, comprising administering an effective amount of a lysin enzyme produced by group C Streptococcal bacteria infected with a C1 bacteriophage specific for the bacteria to a mouth, throat, or nasal passage of a mammal, the method providing a concentration of from about 100 to 500,000 active enzyme units per milliliter of fluid in the environment of said nasal or oral passages.

104. **Use of bacterial phage associated lysing enzymers for the prophylactic and therapeutic treatment of various illnesses. Fischetti, V., Loomis, L. (2003). Patent number 6,056,954.** A method for the prophylactic and therapeutic treatment of bacterial infections is disclosed which comprises the treatment of an individual with an effective amount of a lytic enzyme composition specific for the infecting bacteria, with the lytic enzyme comprising an effective amount of lytic enzyme, wherein the lytic enzyme is in an environment having a pH which allows for activity of said lytic enzyme; and a carrier for delivering said lytic enzyme. This method, and composition can be used for the treatment of upper respiratory infections, skin infections, wounds, and burns, vaginal infections, eye infections, intestinal disorders and dental problems.

105. **Use of bacteriophages for control of *Escherichia coli* O157. Waddell, T. E., Mazzocco, A., Pacan, J., Ahmed, R., Johnson, R., Poppe, C., Khakhria, R. (2002). Patent number 6,485,902.** A method of reducing levels of *E. coli* O157 strains within the gastrointestinal tract of a ruminant animal using specific bacteriophage(s) is herein described. Also described is a pharmaceutical composition comprising at least one of said bacteriophages and a method for isolating or selecting bacteriophages useful in reducing *E. coli* O157 levels as described above.

106. **Use of phage associated lytic enzymes for treating bacterial infections of the digestive tract. Fischetti, V., Loomis, L. (2001). Patent number 6,254,866.** A method for treatment of bacterial infections of the digestive tract is disclosed which comprises administering a lytic enzyme specific for the infecting bacteria. The lytic enzyme is preferably in a carrier for delivering said lytic enzyme. The bacteria to be treated is selected from the group consisting of *Listeria*, *Salmonella*, *E. coli*, *Campylobacter*, and combinations thereof. The carrier for delivering at least one lytic enzyme to the digestive tract is selected from the group consisting of suppository enemas, syrups, or enteric coated pills.

107. **Use of phage associated lytic enzymes for the rapid detection of bacterial contaminants. Trudil, D. (2002). Patent number 6,395,504.** A method for the use of a phage associated lysing enzyme for the detecting the presence and determining the quantity of bacteria present in or on a wide variety of substances is described. The total concentration of microbes is determined by adding or incorporating a phage associated lytic agent to a disposable test system device with the luminescent reagents luciferin and luciferase, and introducing the disposable test system into a luminometer that can read the luminescence. Other systems can be used with the lytic enzymes for the quantitative and qualitative determination for the presence of bacteria.

108. **Use of stabilizing agents in culture media for growing acid producing bacteria. Kegel, M. A., Wallace, D. L. (1989). Patent number 4,806,479.** Disclosed is a bulk starter medium for the propagation of a mother culture of an acid producing bacteria which medium contains a carbohydrate source, a nitrogen containing growth stimulant, a phage control agent and an essentially insoluble or temporarily insolubilized neutralizing agent to which has been added a foodgrade hydrocolloid stabilizing agent.

109. **Vaginal suppository for treating group B *Streptococcus* infection. Fischetti, V., Loomis, L. (2002). Patent number 6,428,784.** A composition for treatment of bacterial infections of the vagina is disclosed which comprises a lytic enzyme composition specific for the infecting bacteria, and a carrier for delivering said lytic enzyme. The lytic enzyme is which is specific for the group B *Streptococcus*, is genectically coded for by a specific bacteriophage.

110. **Viral products. Gasson, M. J. (2000). Patent number 6,083,684 (see also 5,763,251).** Bacteriophages of food-contaminating or pathogenic bacteria or the lysins thereof are used to kill such bacteria. Examples include lysins from bacteriophages of *Listeria monocytogenes* and *Clostridium tyrobutyricum*. Tests for bacterial contamination can be made specific for specific bacteria by using the appropriate bacteriophage or lysin thereof and determining whether cells are lysed thereby.

111. **Water system virus detection. Fletcher, J. C., Fraser, A. S., Wells, A. F., Tenoso, H. J. (1978). Patent number 4,118,315.** The performance of a waste-water reclamation system is monitored by introducing a non-pathogenic marker virus, bacteriophage F_2, into the waste-water prior to treatment and, thereafter, testing the reclaimed water for the presence of the marker virus. A test sample is first concentrated by absorbing any marker virus onto a cellulose acetate filter in the presence of a trivalent cation at low pH and then flushing the filter with a limited quantity of a glycine buffer solution to desorb any marker virus present on the filter. Photo-optical detection of indirect passive immune agglutination by polystyrene beads indicates the performance of the water reclamation system in removing the marker virus. A closed system provides for concentrating any marker virus, initiating and monitoring the passive immune agglutination reaction, and then flushing the system to prepare for another sample. Peristaltic pumps are provided for volumetric control and for positive fluid displacement. Solenoid valves direct the output from the pumps in preselected routes to accomplish the process for concentrating and detecting the marker virus.

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- Bacteriophages: A Model System for Human Viruses
- How Big is 10^{30}?
- Selling Phage Candy
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- Rendering Phage Heads
- The Contractile-Tail Sheath, In Three Dimensions
- Eye On The Needle: Phage T4 Puncturing Point May Answer Penetrating Questions
- Pioneering genetic researcher Gisela Mosig dies
- Updated Eiserling T4 Virion
- Some Recent Phage and Phage-Related U.S. Patents (1976-present)

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.

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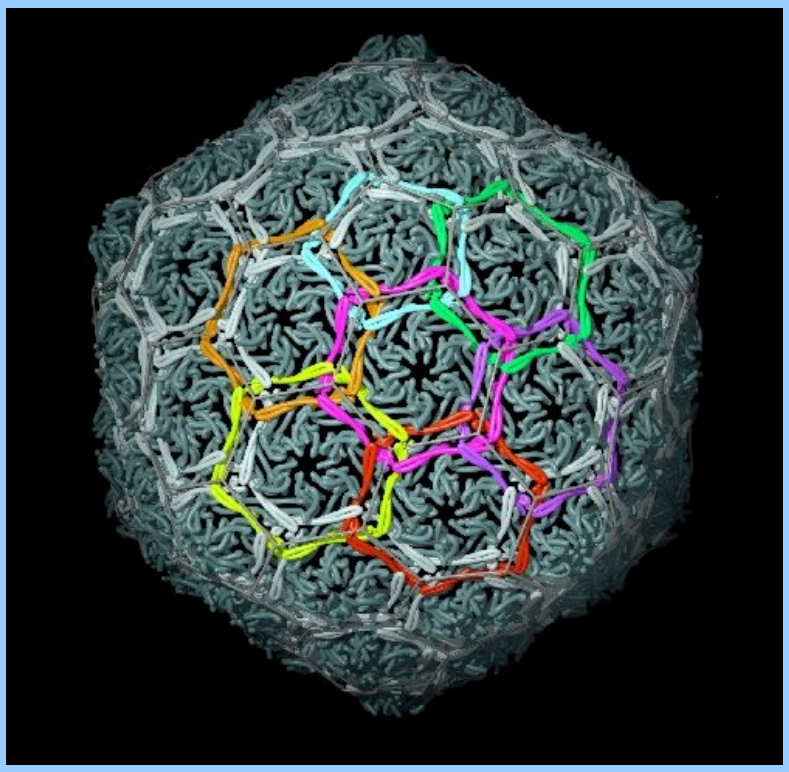
Phage Images



Phage Image Archive

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

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!

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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. Association of a bacteriophage with virulence in *Vibrio harveyi*. Austin, B., Pride, A. C., Rhodie, G. A. (2003). *Journal of Fish Diseases* 26:55-58. [PRESS FOR ABSTRACT]

2. Detection and drug-susceptibility testing of *M. tuberculosis* from sputum samples using luciferase reporter phage: comparison with the Mycobacteria Growth Indicator Tube (MGIT) system. Bardarov, S. Jr., Dou, H., Eisenach, K., Banaiee, N., Ya, S. u., Chan, J., Jacobs, W. R., Jr., Riska, P. F. (2003). *Diagnostic Microbiology and Infectious Disease* 45:53-61. [PRESS FOR ABSTRACT]

3. On the insertion of foreign DNA into mammalian genomes: mechanism and consequences. Doerfler, W., Grtraud, O., Rainer, S., Katja, F., Hilde, H., Petra, W., Jörg, S. (2003). *Gene* 157:241-245. [PRESS FOR ABSTRACT]

4. CTXphi-independent production of the RS1 satellite phage by *Vibrio cholerae*. Faruque, S. M., Kamruzzaman, M., Sack, D. A., Mekalanos, J. J., Nair, G. B. (2003). *Proceedings of the National Academy of Sciences, USA* 100:1280-1285. [PRESS FOR ABSTRACT]

5. Reduction of ß-amyloid plaques in brain of transgenic mouse model of Alzheimer's disease by EFRH-phage immunization. Frenkel, D., Dewachter, I., Van Leuven, F., Solomon, B. (2003). *Vaccine* 21:1060-1065. [PRESS FOR ABSTRACT]

6. New insights into the possible role of bacteriophages in host defense and disease. Gorski, A., Dabrowska, K., Switala-Jele, K., Nowaczyk, M., Weber-Dabrowska, B., Boratynski, J., Wietrzyk, J., Opolski, A. (2003). *Medical Immunology* 2:2. [PRESS FOR ABSTRACT]

7. Evaluation of *Bacillus subtilis* and coliphage MS2 as indicators of advanced water treatment efficiency. Huertas, A., Barbeau, B., Desjardins, C., Galarza, A., Figueroa, M. A., Toranzos, G. A. (2003). *Water Science and Technology* 47:255-259. [PRESS FOR ABSTRACT]

8. Effects of lysogeny of Shiga toxin 2-encoding bacteriophages on pulsed-field gel electrophoresis fragment pattern of *Escherichia coli* K-12. Iguchi, A., Osawa, R., KAWANO, J., Shimizu, A., Terajima, J., Watanabe, H. (2003). *Current Microbiology* 46:224-227. [PRESS FOR ABSTRACT]

9. Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Joerger, R. D. (2003). *Poultry science* 82:647640. [PRESS FOR ABSTRACT]

10. Evidence for horizontal transfer of the EcoT38I restriction-modification gene to chromosomal DNA by the P2 phage and diversity of defective P2 prophages in *Escherichia coli* TH38 strains. Kita, K., Kawakami, H., Tanaka, H. (2003). *Journal of Bacteriology* 185:2296-2305. [PRESS FOR ABSTRACT]

11. Comparative genomic analyses of the vibrio pathogenicity island and cholera toxin prophage regions in nonepidemic serogroup strains of *Vibrio cholerae*. Li, M., Kotetishvili, M., Chen, Y., Sozhamannan, S. (2003). *Applied and Environmental Microbiology* 69:1728-1738. [PRESS FOR ABSTRACT]

12. Reduction of poliovirus 1, bacteriophages, *Salmonella montevideo*, and *Escherichia coli* O157:H7 on strawberries by physical and disinfectant washes. Lukasik, J., Bradley, M. L., Scott, T. M., Dea, M., Koo, A., Hsu, W. Y., Bartz, J. A., Farrah, S. R. (2003). *Journal of Food Protection* 66:188-193. [PRESS FOR ABSTRACT]

13. Use of real-time quantitative PCR for the analysis of phiLC3 prophage stability in lactococci. Lunde, M., Blatny, J. M., Lillehaug, D., Aastveit, A. H., Nes, I. F. (2003). *Applied and Environmental Microbiology* 69:41-48. [PRESS FOR ABSTRACT]

14. The prospect for bacteriophage therapy in Western Medicine. Merril, C. R., Scholl, D., Adhya, S. L. (2003). *Nature Reviews* 2:489-497. [PRESS FOR ABSTRACT]

15. Comparative reduction of Norwalk virus, poliovirus type 1, F+ RNA coliphage MS2 and *Escherichia coli* in miniature soil columns. Meschke, J. S., Sobsey, M. D. (2003). *Water Science and Technology* 47:85-90. [PRESS FOR ABSTRACT]

16. Survival of bacterial indicator species and bacteriophages after thermal treatment of sludge and sewage. Moce-Llivina, L., Muniesa, M., Pimenta-Vale, H., Lucena, F., Jofre, J. (2003). *Applied and Environmental Microbiology* 69:1452-1456. [PRESS

FOR ABSTRACT]

17. Genome organization and molecular analysis of the temperate bacteriophage MM1 of *Streptococcus pneumoniae*. Obregon, V., Garcia, J. L., Garcia, E., Lopez, R., Garcia, P. (2003). *Journal of Bacteriology* 185:2362-2368. [PRESS FOR ABSTRACT]

18. Growth and reduction of microorganisms in sediments collected from a greywater treatment system. Ottosson, J., Stenstrom, T. A. (2003). *Letters in Applied Microbiology* 36:168-172. [PRESS FOR ABSTRACT]

19. Use of a phage-based assay for phenotypic detection of mycobacteria directly from sputum. Park, D. J., Drobniewski, F. A., Meyer, A., Wilson, S. M. (2003). *Journal of Clinical Microbiology* 41:680-688. [PRESS FOR ABSTRACT]

20. Pharmacokinetic principles of bacteriophage therapy. Payne, R. J. H., Jansen, V. A. A. (2003). *Clinical Pharmacokinetics* 42:315-325. [PRESS FOR ABSTRACT]

21. Homogeneity of the morphological groups of bacteriophages infecting *Bacteroides fragilis* strain HSP40 and strain RYC2056. Queralt, N., Jofre, J., Araujo, R., Muniesa, M. (2003). *Current Microbiology* 46:163-168. [PRESS FOR ABSTRACT]

22. The VirB4 family of proposed traffic nucleoside triphosphatases: common motifs in plasmid RP4 TrbE are essential for conjugation and phage adsorption. Rabel, Christian, Grahn, A. Marika, Lurz, Rudi, Lanka, Erich (2003). *Journal of Bacteriology* 185:1045-1058. [PRESS FOR ABSTRACT]

23. Comparison of shiga toxin production by hemolytic-uremic syndrome-associated and bovine-associated shiga toxin-producing *Escherichia coli* isolates. Ritchie, J. M., Wagner, P. L., Acheson, D. W. K., Waldor, M. K. (2003). *Applied and Environmental Microbiology* 69:1059-1066. [PRESS FOR ABSTRACT]

24. Global phage diversity. Rohwer, F. (2003). *Cell* 113:141. [PRESS FOR ABSTRACT]

25. Los bacteriófagos como herramienta para combatir infecciones en acuicultura [abstract is in English, manuscript is in Spanish]. Ronda, C., Vázquez, M., López, R. (2003). *Revista AquaTIC* 18:3-10. [PRESS FOR ABSTRACT]

26. Seasonal change and fate of coliphages infected to *Escherichia coli* O157:H7 in a wastewater treatment plant. Tanji, Y., Mizoguchi, K., Yoichi, M., Morita, M., Kijima, N., Kator, H., Unno, H. (2003). *Water Research* 37:1136-1142. [PRESS FOR ABSTRACT]

27. Escape from Prisoner's Dilemma in RNA phage phi6. Turner, P. E., Chao, L. (2003). *The American naturalist* 161:497-505. [PRESS FOR ABSTRACT]

28. Use of genetically engineered phage to deliver antimicrobial agents to bacteria: an alternative therapy for treatment of bacterial infections. Westwater, C., Kasman, L. M., Schofield, D. A., Werner, P. A., Dolan, J. W., Schmidt, M. G., Norris, J. S. (2003). *Antimicrobial Agents and Chemotherapy* 47:1301-1307. [PRESS FOR ABSTRACT]

29. Characterization of *Serratia* isolates from soil, ecological implications and transfer of *Serratia proteamaculans* subsp. *quinovora* Grimont et al. 1983 to *Serratia quinivorans* corrig., sp. nov. Ashelford, K. E., Fry, J. C., Bailey, M. J., Day, M. J. (2002). *International journal of systematic and evolutionary microbiology* 52:2281-2289. [PRESS FOR ABSTRACT]

30. Fundamental changes in light scattering associated with infection of marine bacteria by bacteriophage. Balch, W. M., Vaughn, J. M., Novotny, J. F., Drapeau, D. T., Goes, J. I., Booth, E., Lapierre, J. M., Vining, C. L., Ashe, A., Vaughn, JM Jr (2002). *Limnology and Oceanography* 47:1554-1561. [PRESS FOR ABSTRACT]

31. Characterization of the two-component abortive phage infection mechanism AbiT from *Lactococcus lactis*. Bouchard, J. D., Dion, E., Bissonnette, F., Moineau, S. (2002). *Journal of Bacteriology* 184:6325-6332. [PRESS FOR ABSTRACT]

32. Genomic analysis of uncultured marine viral communities. Breitbart, M., Salamon, P., Andresen, B., Mahaffy, J. M., Segall, A. M., Mead, D., Azam, F., Rohwer, F. (2002). *Proceedings of the National Academy of Sciences, USA* 99:14250-14255. [PRESS FOR ABSTRACT]

33. F-specific RNA coliphages: occurrence, types, and survival in natural waters. Brion, G. M., Meschke, J. S., Sobsey, M. D. (2002). *Water Research* 36:2419-2425. [PRESS FOR ABSTRACT]

34. RNA bacteriophage capsid-mediated drug delivery and epitope presentation. Brown, W. L., Mastico, R. A., Wu, M., Heal, K. G., Adams, C. J., Murray, J. B., Simpson, J. C., Lord, J. M., Taylor-Robinson, A. W., Stockley, P. G. (2002). *Intervirology* 45:371-380. [PRESS FOR ABSTRACT]

35. Killing of *Mycobacterium avium* and *Mycobacterium tuberculosis* by a mycobacteriophage delivered by a nonvirulent mycobacterium: A model for phage therapy of intracellular bacterial pathogens. Broxmeyer, L., Sosnowskai, D., Miltner, E., Chacón, O., Wagner, D., McGarvey, J., Barletta, R. G., Bermudez, L. E. (2002). *Journal of Infectious Disease* 186:1155-1160. [PRESS FOR ABSTRACT]

36. Fenogeneticheskaia kharakteristika gruppy gigantskikh Phi KZ-podobnykh bakteriofagov Pseudomonas aeruginosa [Phenogenetic characterization of a group of giant Phi KZ-like bacteriophages of *Pseudomonas aeruginosa*]. Burkal'tseva, M. V., Krylov, V. N., Pleteneva, E. A., Shaburova, O. V., Krylov, S. V., Volkart, G., Sykilinda, N. N., Kurochkina, L. P., Mesianzhinov, V. V. (2002). *Genetika* 38:1470-1479. [PRESS FOR ABSTRACT]

37. Integrity of powdered and powder-free latex examination gloves. Calhoun, A. J., Rodrick, G. E., Brown, F. H. (2002). *Journal of public health dentistry* 62:170-172. [PRESS FOR ABSTRACT]

38. Isolation and characterization of two types of actinophages infecting *Streptomyces scabies* MR13. el Sayed, A. el, el Didamony,

G., Mansour, K. (2002). *Acta Microbiologica et Immunologica Hungarica* 49:469-482. [PRESS FOR ABSTRACT]

39. High control of bacterial production by viruses in a eutrophic oxbow lake. Fischer, U. R., Velimirov, B. (2002). *Aquatic Microbial Ecology* 27:1-12. [PRESS FOR ABSTRACT]

40. The cell surface protein Ag43 facilitates phage infection of *Escherichia coli* in the presence of bile salts and carbohydrates. Gabig, M., Herman-Antosiewicz, A., Kwiatkowska, M., Los, M., Thomas, M. S., Wegrzyn, G. (2002). *Microbiology (Reading England)* 2002 May, 148:1533-1542. [PRESS FOR ABSTRACT]

41. Fluorescent dye labeled bacteriophages--a new tracer for the investigation of viral transport in porous media: 2. Studies of deep-bed filtration. Gitis, V., Adin, A., Nasser, A., Gun, J., Lev, O. (2002). *Water Research* 36:4235-4242. [PRESS FOR ABSTRACT]

42. Fluorescent dye labeled bacteriophages--a new tracer for the investigation of viral transport in porous media: 1. Introduction and characterization. Gitis, V., Adin, A., Nasser, A., Gun, J., Lev, O. (2002). *Water Research* 36:4227-4234. [PRESS FOR ABSTRACT]

43. Observations on cyanobacterial population collapse in eutrophic lake water. Gons, H. J., Ebert, J., Hoogveld, H. L., van den Hove, L., Pel, R., Takkenberg, W., Woldringh, C. J. (2002). *Antonie van Leeuwenhoek* 81:319-326. [PRESS FOR ABSTRACT]

44. Reporter bacteriophage assays as a means to detect foodborne pathogenic bacteria. Goodridge, L., Griffiths, M. (2002). *Food Research International* 35:863-870. [PRESS FOR ABSTRACT]

45. Characterization of phi 12, a bacteriophage related to phi 6: nucleotide sequence of the small and middle double-stranded RNA. Gottlieb, P., Wei, H., Potgieter, C., Toporovsky, I. (2002). *Virology* 293:118-124. [PRESS FOR ABSTRACT]

46. Characterization of phi12, a bacteriophage related to phi6: nucleotide sequence of the large double-stranded RNA. Gottlieb, P., Potgieter, C., Wei, H., Toporovsky, I. (2002). *Virology* 295:266-271. [PRESS FOR ABSTRACT]

47. Viral distribution and activity in Antarctic waters. Guixa-Boixereu, N., Vaque, D., Gasol, J. M., Sanchez-Camara, J., Pedros-Alio, C. (2002). *Deep-Sea Research II* 49:827-845. [PRESS FOR ABSTRACT]

48. Dissemination of the phage-associated novel superantigen gene speL in recent invasive and noninvasive *Streptococcus pyogenes* M3/T3 isolates in Japan. Ikebe, T., Wada, A., Inagaki, Y., Sugama, K., Suzuki, R., Tanaka, D., Tamaru, A., Fujinaga, Y., Abe, Y., Shimizu, Y., Watanabe, H. (2002). *Infection and Immunity* 70:3227-3233. [PRESS FOR ABSTRACT]

49. The effect of storage and lag time on MS2 bacteriophage susceptibility to ultraviolet radiation. Jolis, D. (2002). *Water environment research : a research publication of the Water Environment Federation* 74:516-520. [PRESS FOR ABSTRACT]

50. Degree of ultraviolet radiation damage and repair capabilities are related to G+C content in marine vibriophages. Kellogg, C. A., Paul, J. H. (2002). *Aquatic Microbial Ecology* 27:13-20. [PRESS FOR ABSTRACT]

51. Regeneration of dissolved organic matter by viral lysis in marine microbial communities. Middelboe, M., Lyck, P. G. (2002). *Aquatic Microbial Ecology* 27:187-194. [PRESS FOR ABSTRACT]

52. Regrowth and survival of indicator microorganisms on the surfaces of household containers used for the storage of drinking water in rural communities of South Africa. Momba, M. N. B., Kaleni, P. (2002). *Water Research* 36:3023-3028. [PRESS FOR ABSTRACT]

53. Virus-like particle analysis in a seston-rich coastal pond using transmission electron microscopy. Montanie, H., Hartmann, H. J., Crottereau, C., Trichet, C. (2002). *Aquatic Microbial Ecology* 28:105-115. [PRESS FOR ABSTRACT]

54. The effect of an agglutogen on virus infection: biotinylated filamentous phages and avidin as a model. Nakamura, Michihiro, Tsumoto, Kouhei, Ishimura, Kazunori, Kumagai, Izumi (2002). *FEBS Letters* 520:77-80. [PRESS FOR ABSTRACT]

55. Combined phage typing and amperometric detection of released enzymatic activity for the specific identification and quantification of bacteria . Neufeld, T., Schwartz-Mittelmann, A., Biran, D., Ron, E. Z., Rishpon, J. (2002). *Analytical Chemistry* 75:580-585. [PRESS FOR ABSTRACT]

56. A filterable lytic agent obtained from a red tide bloom that caused lysis of *Karenia brevis* (*Gymnodinum breve*) cultures. Paul, J. H., Houchin, L., Griffin, D., Slifko, T., Guo, M., Richardson, B., Steidinger, K. (2002). *Aquatic Microbial Ecology* 27:21-27. [PRESS FOR ABSTRACT]

57. Marine phage genomics. Paul, J. H., Sullivan, M. B., Segall, A. M., Rohwer, F. (2002). *Comparative Biochemistry and Physiology* 133:463-476. [PRESS FOR ABSTRACT]

58. The *Brucella suis* genome reveals fundamental similarities between animal and plant pathogens and symbionts. Paulsen, I. T., Seshadri, R., Nelson, K. E., Eisen, J. A., Heidelberg, J. F., Read, T. D., Dodson, R. J., Umayam, L., Brinkac, L. M., Beanan, M. J., Daugherty, S. C., Deboy, R. T., Durkin, A. S., Kolonay, J. F., Madupu, R., Nelson, W. C., Ayodeji, B., Kraul, M., Shetty, J., Malek, J., Van Aken, S. E., Riedmuller, S., Tettelin, H., Gill, S. R., White, O., Salzberg, S. L., Hoover, D. L., Lindler, L. E., Halling, S. M., Boyle, S. M., Fraser, C. M. (2002). *Proceedings of the National Academy of Sciences, USA* 99:13148-13153. [PRESS FOR ABSTRACT]

59. Bacteriophage T4 development in *Escherichia coli* is growth rate-dependent. Rabinovitch, A., Fishov, I., Hadas, H., Einav, M., Zaritsky, A. (2002). *Journal of Theoretical Biology* 216:1-4. [PRESS FOR ABSTRACT]

60. Field and laboratory investigations of inactivation of viruses (PRD1 and MS2) attached to iron oxide-coated quartz sand. Ryan, J. N., Harvey, R. W., Metge, D., Elimelech, M., Navigato, T., Pieper, A. P. (2002). *Environmental science & technology* 36:2403-

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62. Site-specific integrative elements of rhizobiophage 16-3 can integrate into proline tRNA (CGG) genes in different bacterial genera. Semsey, S., Blaha, B., Koles, K., Orosz, L., Papp, P. P. (2002). *Journal of Bacteriology* 184:177-182. [PRESS FOR ABSTRACT]

63. Rex-centric mutualism. Slavcev, R. A., Hayes, S. (2002). *Journal of Bacteriology* 184:857-858. [PRESS FOR ABSTRACT]

64. The prevalence and diversity of mobile genetic elements in bacterial communities of different environmental habitats: Insights gained from different methodological approaches. Smalla, K., Sobecky, P. A. (2002). *FEMS Microbiology Ecology* 42:165-175. [PRESS FOR ABSTRACT]

65. Three recently acknowledged *Escherichia* species strikingly differ in the incidence of bacteriocinogenic and lysogenic strains. Smarda, J., Smajs, D., Lhotova, H. (2002). *Journal of Basic Microbiology* 42:429-433. [PRESS FOR ABSTRACT]

66. Otlichiia v sostave genov virulentnosti v shtammakh Vibrio cholerae eltor, vydelennykh iz raznykh istochnikov na territorii Turkmenistana [Differences in virulence genes in *Vibrio cholerae* eltor strains isolated from different sources in Turkmenistan territory]. Smirnova, N. I., Kostromitina, E. A., Cheldyshova, N. B., Kutyrev, V. V. (2002). *Molekuliarnaia genetika* 2002,12-18. [PRESS FOR ABSTRACT]

67. Effectiveness of thermal treatments and biocides in the inactivation of Argentinian *Lactococcus lactis* phages. Suarez, V. B., Reinheimer, J. A. (2002). *Journal of Food Protection* 65:1756-1759. [PRESS FOR ABSTRACT]

68. Mutations in bacteriophage T4 genome. Switala-Jelen, K., Dabrowska, K., Gorski, A., Sliwa, L. (2002). *Acta virologica. English ed* 46:57-62. [PRESS FOR ABSTRACT]

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70. Defektnaia lizogenia Erwinia carotovora [Defective lysogeny in *Erwinia carotovora*]. Tovkach, F. I. (2002). *Mikrobiologiia* 71:359-367. [PRESS FOR ABSTRACT]

71. Ecological and molecular maintenance strategies of mobile genetic elements. Turner, S. L., Bailey, M. J., Lilley, A. K., Thomas, C. M. (2002). *FEMS Microbiology Ecology* 42:177-185. [PRESS FOR ABSTRACT]

72. Removal of indigenous coliphages and fecal coliforms by a novel sewage treatment system consisting of UASB and DHS units. Uemura, S., Takahashi, K., Takaishi, A., Machdar, I., Ohashi, A., Harada, H. (2002). *Water Science and Technology* 46:303-309. [PRESS FOR ABSTRACT]

73. *Pseudoalteromonas* spp. phages, a significant group of marine bacteriophages in the North Sea. Wichels, A., Gerdts, G., Schuett, C. (2002). *Aquatic Microbial Ecology* 27:233-239. [PRESS FOR ABSTRACT]

74. Evolution of lambdoid replication modules. Wrobel, B., Wegrzyn, G. (2002). *Virus Genes* 24:163-171. [PRESS FOR ABSTRACT]

75. River water quality improvement by natural and constructed wetland systems in the tropical semi-arid region of northeastern Brazil. de Ceballos, B. S., Oliveira, H., Meira, C. M., Konig, A., Guimaraes, A. O., de Souza, J. T. (2001). *Water Science and Technology* 44:599-605. [PRESS FOR ABSTRACT]

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87. Bacteriophage lambda and plasmid pUR288 transgenic fish models for detecting in vivo mutations. Winn, R. N., Norris, M., Muller, S., Torres, C., Brayer, K. (2001). *Marine Biotechnology* 3:185-195. [PRESS FOR ABSTRACT]

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89. Increased killing of *Bacillus subtilis* on the hair roots of transgenic T4 lysozyme-producing potatoes. Ahrenholtz, I, Harms, K., De Vries, J., Wackernagel, W. (2000). *Applied and Environmental Microbiology* 66:1862-1865. [PRESS FOR ABSTRACT]

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98. Fate of *Cryptosporidium* oocysts, *Giardia* cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion. Chauret, C., Springthorpe, S., Sattar, S. (1999). *Canadian Journal of Microbiology* 45:257-262. [PRESS FOR ABSTRACT]

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102. Isolation of chloroform-resistant mutants of filamentous phage: localization in models of phage structure. Oh, J. S., Davies, D. R., Lawson, J. D., Arnold, G. E., Dunker, A. K. (1999). *Journal of Molecular Biology* 287:449-457. [PRESS FOR ABSTRACT]

103. Vascular targeting with phage peptide libraries. Pasqualini, R. (1999). *The Quarterly Journal of Nuclear Medicine* 43:159-162. [PRESS FOR ABSTRACT]

104. Genomic structure of phage B40-8 of *Bacteroides fragilis*. Puig, M., Girones, R. (1999). *Microbiology (Reading* 1999 Jul, 145:1661-1670. [PRESS FOR ABSTRACT]

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A. I. (1999). pp. 649-653 in Mornol, M. T., Saygili, H. (eds.) *Proceedings of the 8th International Workshop on Fire Blight*. ISHS, Leuven, Belgium. [no abstract]

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108. Studies on the pustule disease of abalone (*Haliotis discus hannai Ino*) on the Dalian coast. Li, Taiwu, Ding, Mingjin, Zhang, Jian, Xiang, Jianhai, Liu, Ruiyu (1998). *Journal of Shellfish Research* 17:707-711. [PRESS FOR ABSTRACT]

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110. Generation of reactive oxygen species from the photolysis of histidine by near-ultraviolet light: effects on T7 as a model biological system. Paretzoglou, A., Stockenhuber, C., Kirk, S. H., Ahmad, S. I. (1998). *Journal of photochemistry and photobiology. B* 1998 May 15, 43:101-105. [PRESS FOR ABSTRACT]

111. Higher-order predators and the regulation of insect herbivore populations. Rosenheim, J. A. (1998). *Annual Review of Entomology* 43:421-447. [PRESS FOR ABSTRACT]

112. Somatostatin displayed on filamentous phage as a receptor-specific agonist. Rousch, M., Lutgerink, J. T., Coote, J., de Bruine, A., Arends, J. W., Hoogenboom, H. R. (1998). *British Journal of Pharmacology* 125:5-16. [PRESS FOR ABSTRACT]

113. On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. Schubbert, R., Hohlweg, U., Renz, D., Doerfler, W. (1998). *Molecular and General Genetics* 259:569-576. [PRESS FOR ABSTRACT]

114. The influence of environmental factors on microbiological indicators of coastal water pollution. Serrano, E., Moreno, B., Solaun, M., Aurrekoetxea, J. J., Ibarluzea, J. (1998). *Water Science and Technology* 38:195-199. [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. **Association of a bacteriophage with virulence in *Vibrio harveyi*. Austin, B., Pride, A. C., Rhodie, G. A. (2003). *Journal of Fish Diseases* 26:55-58.** The virulence of *Vibrio harveyi*, which is a serious pathogen of penaeids (Karunasagar, Pai, Malathi & Karunasagar 1994; Pizzuto & Hirst 1995; Alvarez, Austin, Alvarez & Reyes 1998) and finfish (Kraxberger-Beatty, McGarey, Grier & Lim 1990; Ishimaru & Muroga 1997), has been associated with possession of double haemolysin genes (Zhang, Meaden & Austin 2001). The study seeks to investigate a possible relationship between virulence and the previously described bacteriophage of *V. harveyi* (Oakey & Owens 2000). The bacteriophage, which has been determined to have an icosahedral head and rigid tail and to contain double stranded linear DNA, has been presumptively assigned to the genus Myovirus (Oakey & Owens 2000).

2. **Detection and drug-susceptibility testing of *M. tuberculosis* from sputum samples using luciferase reporter phage: comparison with the Mycobacteria Growth Indicator Tube (MGIT) system. Bardarov, S. Jr., Dou, H., Eisenach, K., Banaiee, N., Ya, S. u., Chan, J., Jacobs, W. R., Jr., Riska, P. F. (2003). *Diagnostic Microbiology and Infectious Disease* 45:53-61.** Rapid diagnosis of drug-resistant *M.tuberculosis* (Mtb) is desirable worldwide. We (i) describe a new luciferase reporter phage (LRP), phAE142 for this purpose; (ii) compare it to the automated MGIT 960 for time-to-detection of Mtb in clinical specimens; and (iii) evaluate its use for species confirmation and antibiotic susceptibility testing(AST) of Mtb. Twenty sputum samples were inoculated for testing by LRP, or by MGIT 960. After "positives" were identified by either method, the LRP was used for confirmation of Mtb complex (TBC) and for AST. The LRP method proved comparably efficient to MGIT 960 at detecting Mtb. Using an antibiotic uniquely inhibiting TBC with LRP provided species assignment, concurrently with AST, in a median of 3 days, with a sensitivity of 97%. Overall agreement in susceptibility results was 96%. Reliable susceptibility results and identification of TBC can be completed in a median of 12 days (range 8 to 16d) with LRP applied to sputum samples.

3. **On the insertion of foreign DNA into mammalian genomes: mechanism and consequences. Doerfler, W., Grtraud, O., Rainer, S., Katja, F., Hilde, H., Petra, W., Jörg, S. (2003). *Gene* 157:241-245.** We have studied the integration of adenovirus type 12 (Ad12) DNA in transformed and hamster tumor cells over many years. Upon infection of hamster cells with Ad12, viral DNA has been found in association with hamster chromosomes, possibly in part integrated into the host genome. Ad12 DNA integration is not sequence specific. Transcriptionally active sites of the host genome show a preponderance for foreign DNA insertion. We are pursuing the mechanism of Ad12 DNA integrative recombination in a cell-free system prepared from hamster cell nuclear extracts. In a number of Ad12-transformed hamster cell lines or in cell lines carrying foreign DNA, we have located the inserted Ad12 DNA copies on hamster chromosomes by fluorescent in situ hybridization (FISH). Among the consequences of Ad12 DNA integration, we have studied the de novo methylation of the integrated foreign (Ad12) DNA and increases in DNA methylation in several cellular genes and DNA segments in Ad12-transformed and hamster tumor cells. Several lines of evidence argue for the notion that parameters in addition to nucleotide sequence, in particular site of integration and/or the chromatin configuration of the integrated DNA, are important in generating de novo methylation patterns. The de novo methylation of integrated foreign DNA can be interpreted as an old cellular defense mechanism against the activity of foreign genes in an established genome. Pursuing this concept, we have asked for the most likely portal of entry of foreign DNA, supposedly the gastroinstetinal tract in most animals. This hypothesis has been tested by feeding mice linearized or circular, double-stranded

bacteriophage M13mp18 DNA. A small amount of this DNA transiently survives the digestive regime of the animals' GI tract, although in a heavily fragmented form. A minute proportion of the fed M13mp18 DNA can be retrieved from the bloodstream of mice between 2 and 8 h after feeding, mainly associated with the leukocyte population.

4. **CTXphi-independent production of the RS1 satellite phage by *Vibrio cholerae*. Faruque, S. M., Kamruzzaman, M., Sack, D. A., Mekalanos, J. J., Nair, G. B. (2003). *Proceedings of the National Academy of Sciences, USA* 100:1280-1285.** The cholera toxin genes of *Vibrio cholerae* are encoded by the filamentous phage, CTXphi. Chromosomal CTXphi prophage DNA is often found flanked by copies of a related genetic element designated RS1, and RS1 DNA can be packaged into filamentous phage particles (designated RS1phi) by using the CTXphi morphogenesis genes. RS1phi is a satellite phage that further controls expression and dissemination of CTXphi. Here we describe a CTXphi-independent mechanism for production of RS1phi. A nontoxigenic environmental V. cholerae strain (55V71) was identified that supports production of RS1phi. However, newly infected CTX-negative strains did not produce RS1phi, indicating that additional 55V71 genes were involved in production of RS1phi. Analysis of nucleic acids from phage preparations of 55V71 revealed a 7.5-kb single-stranded DNA, whose corresponding replicative form was found in plasmid preparations. This DNA likely corresponds to the genome of a new filamentous phage, which we have designated KSF-1phi. The replicative form DNA of KSF-1phi was cloned into pUC18, and the resulting construct pKSF-1.1 supported the production of RS1phi particles by CTX-negative *V. cholerae* strains. RS1phi particles produced in this way infect recipient *V. cholerae* strains by a mechanism that is independent of the CTXphi receptor, the toxin-coregulated pilus. Thus, KSF-1phi is capable of facilitating the transfer of the RS1 element to strains that do not express toxin coregulated pilus. Given that RS1phi can enhance coproduction of CTXphi particles, KSF-1phi-mediated dissemination of RS1 may indirectly promote the spread of toxin genes among V. cholerae strains. This study also shows that filamentous phages can package diverse DNA elements and thus may play a role in horizontal transfer of more genes than previously appreciated

5. **Reduction of ß-amyloid plaques in brain of transgenic mouse model of Alzheimer's disease by EFRH-phage immunization. Frenkel, D., Dewachter, I., Van Leuven, F., Solomon, B. (2003). *Vaccine* 21:1060-1065.** Antibodies to the epitope EFRH, representing residues 3–6 within the ß-amyloid (Aß) sequence, were previously shown to affect the solubility and disaggregation of Aß fibrils in vitro. Here, we describe a novel method of immunization, using as antigen the EFRH peptide displayed on the surface of the filamentous phage. The EFRH phage evoked effective auto-immune antibodies in amyloid precursor protein [V717I] (APP[V717I]) transgenic mice that recapitulate the amyloid plaques and vascular pathology of Alzheimer's disease (AD). The immunization provoked a considerable reduction in the number of Aß amyloid plaques in the brain of the transgenic mice and may serve as the basis for anti-Aß vaccine.

6. **New insights into the possible role of bacteriophages in host defense and disease. Gorski, A., Dabrowska, K., Switala-Jele, K., Nowaczyk, M., Weber-Dabrowska, B., Boratynski, J., Wietrzyk, J., Opolski, A. (2003). *Medical Immunology* 2:2.** Background: While the ability of bacteriophages to kill bacteria is well known and has been used in some centers to combat antibiotics - resistant infections, our knowledge about phage interactions with mammalian cells is very limited and phages have been believed to have no intrinsic tropism for those cells. ¶ Presentation of the hypothesis: At least some phages (e.g., T4 coliphage) express Lys-Arg-Gly (KGD) sequence which binds b3 integrins (primarily aIIbb3). Therefore, phages could bind b3+ cells (platelets, monocytes, some lymphocytes and some neoplastic cells) and downregulate activities of those cells by inhibiting integrin functions. ¶ Testing the hypothesis: Binding of KGD+ phages to b3 integrin+ cells may be detected using standard techniques involving phage - mediated bacterial lysis and plaque formation. Furthermore, the binding may be visualized by electron microscopy and fluorescence using labelled phages. Binding specificity can be confirmed with the aid of specific blocking peptides and monoclonal antibodies. In vivo effects of phage - cell interactions may be assessed by examining the possible biological effects of b3 blockade (e.g., anti-metastatic activity). ¶ Implication of the hypothesis: If, indeed, phages can modify functions of b3+ cells (platelets, monocytes, lymphocytes, cancer cells) they could be important biological response modifiers regulating migration and activities of those cells. Such novel understanding of their role could open novel perspectives in their potential use in treatment of cardiovascular and autoimmune disease, graft rejection and cancer.

7. **Evaluation of *Bacillus subtilis* and coliphage MS2 as indicators of advanced water treatment efficiency. Huertas, A., Barbeau, B., Desjardins, C., Galarza, A., Figueroa, M. A., Toranzos, G. A. (2003). *Water Science and Technology* 47:255-259.** The assessment of water treatment facilities for their efficiency using alternate indicators is of paramount importance. Current methods for assessing efficiency are limited by the specific characteristics of the microorganisms, such as their different sensitivities to disinfectants. A pilot study was carried out to compare different treatment scenarios for the future upgrade of the Sergio Cuevas Water Treatment plant (the largest in the Caribbean) in San Juan, Puerto Rico. The treatment units under investigation included a coagulation-flocculation-sedimentation unit, dual-media filters, micro-filtration units, intermediate ozone injection and contact columns as well as a biological filtration unit. The plant was challenged at different stages of treatment with *Bacillus subtilis* spores and MS2 coliphages in an attempt to test them as possible alternate indicators of treatment plant performance. These organisms were chosen because of their resistance to disinfection and desiccation, their low analysis costs and ease of detection. The removal of spores and coliphages by each treatment unit tested was calculated by seeding a known concentration (5-7 log10) of spores and coliphages and following the removal or disinfection rates. The seeded indicators were detected using traditional culture techniques. Ballasted clarification was shown to be highly efficient at removing 99.1% (approximately 3 log10) of the spores and 85.1% (approximately 0.86 log10) of MS2. Ozone treatment inactivated 80.37% (approximately 1.4 log10) spores and 99.95% (approximately 3.07 log10) coliphages. The coliphage inactivation rate obtained confirmed data obtained by previous studies indicating that MS2 was less resistant to ozonation than *B subtilis* spores. The membrane technology had the best efficiency in terms of physical removal of spores achieving over 99.9% (> 3 log10) removal. Coliphage removal mechanisms remain to be determined and will be a future focus of the study. Preliminary results indicate that aerobic spores and coliphages may be useful as indicators to determine the efficiency of different drinking water treatment technologies

8. **Effects of lysogeny of Shiga toxin 2-encoding bacteriophages on pulsed-field gel electrophoresis fragment pattern of *Escherichia coli* K-12. Iguchi, A., Osawa, R., KAWANO, J., Shimizu, A., Terajima, J., Watanabe, H. (2003). *Current Microbiology* 46:224-227.** *Escherichia coli* K-12 lysogens of three different Shiga toxin 2 (Stx2)-encoding bacteriophages were examined for variability in their pulsed-field gel electrophoresis (PFGE) fragment patterns. The PFGE fragment patterns could be classified into three types (i.e., PFGE types B, C, and D). For the PFGE type D, a 255-kbp fragment present in the original K-12 strain was apparently shifted by the size of Stx 2-encoding phage genomic DNA (ca. 65 kbp) to the position at 320 kbp. In contrast, the types B and C showed the above fragment shift plus further 6- and 10-fragment differences, respectively, from the original K-12 strain. The evidence suggests that even a single genetic event like lysogeny can cause marked genotypic modification of the host strain

9. **Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Joerger, R. D. (2003). *Poultry*

***science* 82:647640.** Bacteriocins, antimicrobial peptides, and bacteriophage have attracted attention as potential substitutes for, or as additions to, currently used antimicrobial compounds. This publication will review research on the potential application of these alternative antimicrobial agents to poultry production and processing. Bacteriocins are proteinaceous compounds of bacterial origin that are lethal to bacteria other than the producing strain. It is assumed that some of the bacteria in the intestinal tract produce bacteriocins as a means to achieve a competitive advantage, and bacteriocin-producing bacteria might be a desirable part of competitive exclusion preparations. Purified or partially purified bacteriocins could be used as preservatives or for the reduction or elimination of certain pathogens. Currently only nisin, produced by certain strains of *Lactococcus lactis* subsp. *lactis*, has regulatory approval for use in certain foods, and its use for poultry products has been studied extensively. Exploration of the application of antimicrobial peptides from sources other than bacteria to poultry has not yet commenced to a significant extent. Evidence for the ability of chickens to produce such antimicrobial peptides has been provided, and it is likely that these peptides play an important role in the defense against various pathogens. Bacteriophages have received renewed attention as possible agents against infecting bacteria. Evidence from several trials indicates that phage therapy can be effective under certain circumstances. Numerous obstacles for the use of phage as antimicrobials for poultry or poultry products remain. Chiefly among them are the narrow host range of many phages, the issue of phage resistance, and the possibility of phage-mediated transfer of genetic material to bacterial hosts. Regulatory issues and the high cost of producing such alternative antimicrobial agents are also factors that might prevent application of these agents in the near future.

10. **Evidence for horizontal transfer of the EcoT38I restriction-modification gene to chromosomal DNA by the P2 phage and diversity of defective P2 prophages in *Escherichia coli* TH38 strains. Kita, K., Kawakami, H., Tanaka, H. (2003). *Journal of Bacteriology* 185:2296-2305.** A DNA fragment carrying the genes coding for a novel EcoT38I restriction endonuclease (R.EcoT38I) and EcoT38I methyltransferase (M.EcoT38I), which recognize G(A/G)GC(C/T)C, was cloned from the chromosomal DNA of *Escherichia coli* TH38. The endonuclease and methyltransferase genes were in a head-to-head orientation and were separated by a 330-nucleotide intergenic region. A third gene, the C.EcoT38I gene, was found in the intergenic region, partially overlapping the R.EcoT38I gene. The gene product, C.EcoT38I, acted as both a positive regulator of R.EcoT38I gene expression and a negative regulator of M.EcoT38I gene expression. M.EcoT38I purified from recombinant *E. coli* cells was shown to be a monomeric protein and to methylate the inner cytosines in the recognition sequence. R.EcoT38I was purified from *E. coli* HB101 expressing M.EcoT38I and formed a homodimer. The EcoT38I restriction (R)-modification (M) system (R-M system) was found to be inserted between the A and Q genes of defective bacteriophage P2, which was lysogenized in the chromosome at locI, one of the P2 phage attachment sites observed in both *E. coli* K-12 MG1655 and TH38 chromosomal DNAs. Ten strains of *E. coli* TH38 were examined for the presence of the EcoT38I R-M gene on the P2 prophage. Conventional PCR analysis and assaying of R activity demonstrated that all strains carried a single copy of the EcoT38I R-M gene and expressed R activity but that diversity of excision in the ogr, D, H, I, and J genes in the defective P2 prophage had arisen

11. **Comparative genomic analyses of the vibrio pathogenicity island and cholera toxin prophage regions in nonepidemic serogroup strains of *Vibrio cholerae*. Li, M., Kotetishvili, M., Chen, Y., Sozhamannan, S. (2003). *Applied and Environmental Microbiology* 69:1728-1738.** Two major virulence factors are associated with epidemic strains (O1 and O139 serogroups) of *Vibrio cholerae*: cholera toxin encoded by the ctxAB genes and toxin-coregulated pilus encoded by the tcpA gene. The ctx genes reside in the genome of a filamentous phage (CTXphi), and the tcpA gene resides in a vibrio pathogenicity island (VPI) which has also been proposed to be a filamentous phage designated VPIphi. In order to determine the prevalence of horizontal transfer of VPI and CTXphi among nonepidemic (non-O1 and non-O139 serogroups) *V. cholerae*, 300 strains of both clinical and environmental origin were screened for the presence of tcpA and ctxAB. In this paper, we present the comparative genetic analyses of 11 nonepidemic serogroup strains which carry the VPI cluster. Seven of the 11 VPI(+) strains have also acquired the CTXphi. Multilocus sequence typing and restriction fragment length polymorphism analyses of the VPI and CTXphi prophage regions revealed that the non-O1 and non-O139 strains were genetically diverse and clustered in lineages distinct from that of the epidemic strains. The left end of the VPI in the non-O1 and non-O139 strains exhibited extensive DNA rearrangements. In addition, several CTXphi prophage types characterized by novel repressor (rstR) and ctxAB genes and VPIs with novel tcpA genes were found in these strains. These data suggest that the potentially pathogenic, nonepidemic, non-O1 and non-O139 strains identified in our study most likely evolved by sequential horizontal acquisition of the VPI and CTXphi independently rather than by exchange of O-antigen biosynthesis regions in an existing epidemic strain

12. **Reduction of poliovirus 1, bacteriophages, *Salmonella montevideo*, and *Escherichia coli* O157:H7 on strawberries by physical and disinfectant washes. Lukasik, J., Bradley, M. L., Scott, T. M., Dea, M., Koo, A., Hsu, W. Y., Bartz, J. A., Farrah, S. R. (2003). *Journal of Food Protection* 66:188-193.** The efficacy levels of different physical and chemical washing treatments in the reduction of viral and bacterial pathogens from inoculated strawberries were evaluated. *Escherichia coli* O157:H7, *Salmonella Montevideo*, poliovirus 1, and the bacteriophages PRD1, phiX174, and MS2 were used as model and surrogate organisms. Chemicals readily available to producers and/or consumers were evaluated as antimicrobial additives for the production of washes. The gentle agitation of contaminated strawberries in water for 2 min led to reductions in microbial populations ranging from 41 to 79% and from 62 to 90% at water temperatures of 22 and 43 degrees C, respectively. Significant reductions (> 98%) in numbers of bacteria and viruses were obtained with sodium hypochlorite (50 to 300 ppm of free chlorine), Oxine or Carnebon (200 ppm of product generating "stabilized chlorine dioxide"), Tsunami (100 ppm of peroxyacetic acid), and Alcide (100 or 200 ppm of acidified sodium chlorite) washes. Overall, 200 ppm of acidified sodium chlorite produced the greatest reductions of microorganisms. Hydrogen peroxide (0.5%) was slightly less effective than free chlorine in a strawberry wash and caused slight fruit discoloration. Cetylpyridinium chloride (0.1%) was effective in the reduction of bacterial species, while trisodium phosphate (1%) was effective against viruses. The consumer-oriented produce wash Fit was very effective (> 99%) in reducing the numbers of bacteria but not in reducing the numbers of viruses. Another wash, Healthy Harvest, was significantly less effective than Fit in reducing bacterial pathogens but more effective for viruses. The performance of automatic dishwashing detergent was similar to that of Healthy Harvest and significantly better than that of liquid dishwashing detergent. Solutions containing table salt (2% NaCl) or vinegar (10%) reduced the numbers of bacteria by about 90%, whereas only the vinegar wash reduced the numbers of viruses significantly (ca. 95%)

13. **Use of real-time quantitative PCR for the analysis of phiLC3 prophage stability in lactococci. Lunde, M., Blatny, J. M., Lillehaug, D., Aastveit, A. H., Nes, I. F. (2003). *Applied and Environmental Microbiology* 69:41-48.** Bacteriophages are a common and constant threat to proper milk fermentation. It has become evident that lysogeny is widespread in lactic acid bacteria, and in this work the temperate lactococcal bacteriophage phi LC3 was used as a model to study prophage stability in lactococci. The stability was analyzed in six phi LC3 lysogenic *Lactococcus lactis* subsp. *cremoris* host strains when they were growing at 15 and 30 degrees C. In order to perform these analyses, a real-time PCR assay was developed. The stability of the phi LC3 prophage was found to vary with the growth phase of its host *L. lactis* IMN-C1814, in which the induction rate increased

during the exponential growth phase and reached a maximum level when the strain was entering the stationary phase. The maximum spontaneous induction frequency of the phi LC3 prophage varied between 0.32 and 9.1% (28-fold) in the six lysogenic strains. No correlation was observed between growth rates of the host cells and the spontaneous prophage induction frequencies. Furthermore, the level of extrachromosomal phage DNA after induction of the prophage varied between the strains (1.9 to 390%), and the estimated burst sizes varied up to eightfold. These results show that the host cells have a significant impact on the lytic and lysogenic life styles of temperate bacteriophages. The present study shows the power of the real-time PCR technique in the analysis of temperate phage biology and will be useful in work to reveal the impact of temperate phages and lysogenic bacteria in various ecological fields

14. **The prospect for bacteriophage therapy in Western Medicine. Merril, C. R., Scholl, D., Adhya, S. L. (2003). *Nature Reviews* 2:489-497.** Bacteriophage (phage) have been used for clinical applications since their initial discovery at the beginning of the twentieth century. However, they have never been subjted to the scrutiny -- in terms of the determination of efficacy and pharmaco-kinites of therapeutic agents -- that is required in countries that enforce certification of marketed pharmaceuticals. There are a number of historical reasons for this deficiency, including the overshadowing discovery of antibiotics. Neverhteless, present efforts to develop phage into reliable antibacterial agents have been substantially enhanced by knowledge gained concerning the genetics and physiology of phage in molecular detail during the past 50 years. Such efforts will be of importance given the emergence of antibiotic-resistant bacteria.

15. **Comparative reduction of Norwalk virus, poliovirus type 1, F+ RNA coliphage MS2 and *Escherichia coli* in miniature soil columns. Meschke, J. S., Sobsey, M. D. (2003). *Water Science and Technology* 47:85-90.** Norwalk-like viruses (NLVs) are important agents of waterborne illness and have been linked to several groundwater-related outbreaks. The presence of human enteric viruses, in particular the presence of NLVs, is difficult to detect in the environment. Consequently, surrogate organisms are typically used as indicators of viruses from faecal contamination. Whether traditional bacterial indicators are reliable indicators for viral pathogens remains uncertain. Few studies have directly compared mobility and reduction of bacterial indicators (e.g. coliforms, *Escherichia coli*) and other surrogate indicators (coliphages) with pathogenic human viruses in soil systems. In this study the mobility and comparative reduction of the prototype NLV, Norwalk Virus (NV), was compared to poliovirus 1 (PV1), a bacterial indicator (*E coli*, EC) and a viral indicator (coliphage MS2) through miniature soil columns. Replicate, 10 cm deep, miniature columns were prepared using three soils representing a range of soil textures (sand, organic muck, and clay). Columns were initially conditioned, then incubated at 10-14 degrees C, dosed twice weekly for 8 weeks with one column pore volume of virus-seeded groundwater per dose, followed by 8 weeks of dosing with one column pore volume per dose of unseeded, simulated rainwater. Columns were allowed to drain after each dosing until an effluent volume equivalent to an applied dose was collected. Column effluents and doses were assayed for all viruses and EC. Rapid mobility with minimal reduction was observed for all organisms in the sand. Similar reductions were observed in organic muck for most organisms but NV showed a greater reduction. No organisms were shown to pass through the clay columns. Elution of viruses, in particular PV1, from the columns was gradual. After cessation of microbe dosing, E. coli was less detectable than viruses in column effluents and, therefore, unreliable as a virus indicator

16. **Survival of bacterial indicator species and bacteriophages after thermal treatment of sludge and sewage. Moce-Llivina, L., Muniesa, M., Pimenta-Vale, H., Lucena, F., Jofre, J. (2003). *Applied and Environmental Microbiology* 69:1452-1456.** The inactivation of naturally occurring bacterial indicators and bacteriophages by thermal treatment of a dewatered sludge and raw sewage was studied. The sludge was heated at 80 degrees C, and the sewage was heated at 60 degrees C. In both cases phages were significantly more resistant to thermal inactivation than bacterial indicators, with the exception of spores of sulfite-reducing clostridia. Somatic coliphages and phages infecting *Bacteroides fragilis* were significantly more resistant than F-specific RNA phages. Similar trends were observed in sludge and sewage. The effects of thermal treatment on various phages belonging to the three groups mentioned above and on various enteroviruses added to sewage were also studied. The results revealed that the variability in the resistance of phages agreed with the data obtained with the naturally occurring populations and that the phages that were studied were more resistant to heat treatment than the enteroviruses that were studied. The phages survived significantly better than *Salmonella choleraesuis*, and the extents of inactivation indicated that naturally occurring bacteriophages can be used to monitor the inactivation of *Escherichia coli* and *Salmonella*

17. **Genome organization and molecular analysis of the temperate bacteriophage MM1 of *Streptococcus pneumoniae*. Obregon, V., Garcia, J. L., Garcia, E., Lopez, R., Garcia, P. (2003). *Journal of Bacteriology* 185:2362-2368.** The genome of MM1 (40,248 bp), a temperate bacteriophage from the Spain(23F)-1 multiresistant epidemic clone of *Streptococcus pneumoniae*, is organized in 53 open reading frames (ORFs) and in at least five functional clusters. Bioinformatic and N-terminal amino acid sequence analyses enabled the assignment of possible functions to 26 ORFs. Analyses comparing the MM1 genome with those of other bacteriophages revealed similarities, mainly with genomes of phages infecting gram-positive bacteria, which suggest recent exchange of genes between species colonizing the same habitat

18. **Growth and reduction of microorganisms in sediments collected from a greywater treatment system. Ottosson, J., Stenstrom, T. A. (2003). *Letters in Applied Microbiology* 36:168-172.** AIMS: To study the effects of competitive microbiota, temperature and nutrient availability on *Salmonella*, *Enterococcus*, *Campylobacter*[,] spores of sulphite reducing anaerobes and bacteriophages MS2 and phiX174 in sediments from a greywater treatment system. METHODS AND RESULTS: Standard culture methods were used. Bacteria died off rapidly under normal conditions (20 degrees C, competitive microbiota) but remained stable or grew in the other conditions studied. When the sediments became nutrient depleted after 2 weeks, a log-linear die-off was observed for *Salmonella*, which was higher at 20 degrees C than at 4 degrees C. Bacteriophage decay was shown to be log-linear from day 0, with T90 values ranging from 9 (phiX174, 20 degrees C) to 55 days (phiX174, 4 degrees C). The MS2 phage had a significantly higher decay rate in tyndallized sediments (T90 = 17 days) than in original sediments (T90 = 47 days) ($P < 0.001$), with temperature not shown to affect the decay rate. Spores of sulphite-reducing anaerobes were not significantly reduced during the study period (35 days). *Campylobacter* died-off rapidly or entered a viable but non-culturable state and subsequently results were not provided. CONCLUSIONS: Competition was the most important factor to suppress pathogenic bacterial growth in an eutrophic environment. When nutrient depleted conditions prevailed, temperature was more important and log-linear decay of microorganisms could be observed. SIGNIFICANCE AND IMPACT OF THE STUDY: These findings suggest that the normally occurring microbiota will suppress pathogenic bacterial growth in nutrient rich sediments. With lower nutrient status, temperature is the more important factor in reducing pathogens

19. **Use of a phage-based assay for phenotypic detection of mycobacteria directly from sputum. Park, D. J., Drobniewski, F. A., Meyer, A., Wilson, S. M. (2003). *Journal of Clinical Microbiology* 41:680-688.** The phage amplified biologically assay

is a new method for rapid and low-cost phenotypic determination of the drug sensitivities of Mycobacterium tuberculosis isolates and the detection of viable organisms in patient specimens. Infection of slowly growing mycobacteria with phage (phage D29) was followed by chemical virucide destruction of extracellular phage. Infected mycobacteria were mixed in culture with rapidly growing sensor cells, which the phage can also infect; i.e., lytic amplification of phage occurs. The aims of the present study were to optimize the speed and sensitivity of the assay and reduce its cost for developing countries by using an *M. tuberculosis*-spiked sputum model with (i). identification of inhibitory components of sputum and optimization of decontamination methods; (ii). simplification of the washing and development steps; (iii). reduction of the use of high-cost components, e.g., oleate-albumin-dextrose-catalase (OADC) supplement; and (iv). optimization of virucide treatment. The following results were obtained. (i). An inhibitory factor in sputum which could be removed by treatment of the sample with sodium dodecyl sulfate or NaOH decontamination was identified. (ii). A microcentrifuge-based approach with thixotropic silica as a bedding and resuspension agent was developed as an alternative to conventional centrifugation medium exchange. The yield was increased 228-fold, with increased speed and reduced cost. (iii). At present, after extracellular inactivation of phage, the ferrous ammonium sulfate (FAS) virucide is sequestered by dilution with an expensive supplement, OADC. Sodium citrate with calcium chloride was found to be a cost-effective after treatment with the FAS protectant and offered greater protection than OADC. Kinetic-lysis experiments indicated that an infection time of 1 to 3 h prior to FAS addition was optimal. (iv). Amplification of the signal (which corresponded to the burst size) was shown by allowing lysis prior to plating in a spiked medium model (up to 20-fold) and a spiked sputum model (up to 10-fold). A liquid culture detection method capable of detecting approximately 60 viable M. tuberculosis organisms in 1 ml of sputum was developed. Taken together, these improvements support the routine application of the assay to sputum specimens

20. **Pharmacokinetic principles of bacteriophage therapy. Payne, R. J. H., Jansen, V. A. A. (2003). *Clinical Pharmacokinetics* 42:315-325.** Use of bacteriophage to control bacterial infections, including antibiotic-resistant infections, shows increasing therapeutic promise. Effective bacteriophage therapy requires awareness of various novel kinetic phenomena not known in conventional drug treatments. Kinetic theory predicts that timing of treatment could be critical, with the strange possibility that inoculations given too early could be less effective or fail completely. Another paradoxical result is that adjuvant use of an antibiotic can sometimes diminish the efficacy of phage therapy. For a simple kinetic model, mathematical formulae predict the values of critical density thresholds and critical time points, given as functions of independently measurable biological parameters. Understanding such formulae is important for interpreting data and guiding experimental design. Tailoring pharmacokinetic models for specific systems needs to become standard practice in future studies.

21. **Homogeneity of the morphological groups of bacteriophages infecting *Bacteroides fragilis* strain HSP40 and strain RYC2056. Queralt, N., Jofre, J., Araujo, R., Muniesa, M. (2003). *Current Microbiology* 46:163-168.** Bacteriophages infecting *Bacteroides fragilis* strains RYC2056 and HSP40 have been proposed as indicators of water quality. To accomplish this function, homogeneity of the group of phages detected by these strains is necessary to ensure that the final results are not due to the different kinetics of inactivation of the phages. To evaluate homogeneity, we observed by electron microscopy bacteriophages isolated from sewage with two Bacteroides fragilis strains (HSP40 and RYC2056). A predominant group of phages was observed, Siphoviridae with slightly curved tails. Detection of other minority groups, which could be present in the sample, was done with neutralization experiments by using antiserum against the majority group and with host mutants resistant to infection with the predominant phage. Although two other minority groups were observed, results showed that bacteriophages infecting *B. fragilis* strain HSP40 and strain RYC2056 form a homogeneous group, Siphoviridae with slightly curved tails being the most predominant in sewage

22. **The VirB4 family of proposed traffic nucleoside triphosphatases: common motifs in plasmid RP4 TrbE are essential for conjugation and phage adsorption. Rabel, Christian, Grahn, A. Marika, Lurz, Rudi, Lanka, Erich (2003). *Journal of Bacteriology* 185:1045-1058.** Proteins of the VirB4 family are encoded by conjugative plasmids and by type IV secretion systems, which specify macromolecule export machineries related to conjugation systems. The central feature of VirB4 proteins is a nucleotide binding site. In this study, we asked whether members of the VirB4 protein family have similarities in their primary structures and whether these proteins hydrolyze nucleotides. A multiple-sequence alignment of 19 members of the VirB4 protein family revealed striking overall similarities. We defined four common motifs and one conserved domain. One member of this protein family, TrbE of plasmid RP4, was genetically characterized by site-directed mutagenesis. Most mutations in trbE resulted in complete loss of its activities, which eliminated pilus production, propagation of plasmid-specific phages, and DNA transfer ability in *Escherichia coli*. Biochemical studies of a soluble derivative of RP4 TrbE and of the full-length homologous protein R388 TrwK revealed that the purified forms of these members of the VirB4 protein family do not hydrolyze ATP or GTP and behave as monomers in solution

23. **Comparison of shiga toxin production by hemolytic-uremic syndrome-associated and bovine-associated shiga toxin-producing *Escherichia coli* isolates. Ritchie, J. M., Wagner, P. L., Acheson, D. W. K., Waldor, M. K. (2003). *Applied and Environmental Microbiology* 69:1059-1066.** There is considerable diversity among Shiga toxin (Stx)-producing *Escherichia coli* (STEC) bacteria, and only a subset of these organisms are thought to be human pathogens. The characteristics that distinguish STEC bacteria that give rise to human disease are not well understood. Stxs, the principal virulence determinants of STEC, are thought to account for hemolytic-uremic syndrome (HUS), a severe clinical consequence of STEC infection. Stxs are typically bacteriophage encoded, and their production has been shown to be enhanced by prophage-inducing agents such as mitomycin C in a limited number of clinical STEC isolates. Low iron concentrations also enhance Stx production by some clinical isolates; however, little is known regarding whether and to what extent these stimuli regulate Stx production by STEC associated with cattle, the principal environmental reservoir of STEC. In this study, we investigated whether toxin production differed between HUS- and bovine-associated STEC strains. Basal production of Stx by HUS-associated STEC exceeded that of bovine-associated STEC. In addition, following mitomycin C treatment, Stx2 production by HUS-associated STEC was significantly greater than that by bovine-associated STEC. Unexpectedly, mitomycin C treatment had a minimal effect on Stx1 production by both HUS- and bovine-associated STEC. However, Stx1 production was induced by growth in low-iron medium, and induction was more marked for HUS-associated STEC than for bovine-associated STEC. These observations reveal that disease-associated and bovine-associated STEC bacteria differ in their basal and inducible Stx production characteristics.

24. **Global phage diversity. Rohwer, F. (2003). *Cell* 113:141.** Ten new mycobacteriophage genomes presented by Pedulla et al. (2003) show that most phage diversity remains uncharacterized. Extrapolation suggests that less than 0.0002% of the global phage metagenome has been sampled. The new genomes also contain a number of potential virulence factors that may be important in pathogenesis.

25. **Los bacteriófagos como herramienta para combatir infecciones en acuicultura [abstract is in English, manuscript is in Spanish]. Ronda, C., Vázquez, M., López, R. (2003). *Revista AquaTIC* 18:3-10.** Bacteriophages (phages), the most

abundant entities in nature, have been proposed as therapeutic agents since they were isolated in the early years of the last century. The current antibiotic resistance of most pathogenic microorganisms together with the technical achievements in the study of phages has led to reconsider the work carried out for scientists of the former Soviet Union and to propose the use of bacterial viruses as a real therapeutical alternative. In this minireview we analyze the most relevant contribution on phage therapy in Aquaculture as well as the new possibility that offer the use of phage and phage products, like the lytic enzymes, named enzybiotics, as an alternative tool in therapy.

26. **Seasonal change and fate of coliphages infected to *Escherichia coli* O157:H7 in a wastewater treatment plant. Tanji, Y., Mizoguchi, K., Yoichi, M., Morita, M., Kijima, N., Kator, H., Unno, H. (2003). *Water Research* 37:1136-1142.** Seasonal change of virulent phage infected to two *E. coli* O157:H7 strains (O:157-phage) in the influent of a domestic wastewater treatment plant in the central part of Japan and fate of O:157-phage in the plant were monitored almost monthly from March 2001 to February 2002. Coliphage infected to nonpathogenic *E. coli* O157:H7 ATCC43888 (43888-phage) was detected for 1 year. On the other hand, phage infected to pathogenic *E. coli* O157:H7 EDL933 (EDL-phage) was detected intermittently. Concentration of EDL-phage was almost one-tenth of that of 43888-phage. The progressive decrease in phage concentration with the treatment steps was observed. No phage was detected in the supernatant from the secondary settling tank and effluent. PCR amplification of the Stx 2 gene that encodes Shiga toxin (Stx) was observed when O:157-phage concentration in the influent was high $x10^3$ PFU/ml order. Concentration and percentage of suspended O:157-phage decreased with the progress of the wastewater treatment. 933W phage, which encodes Stx 2 gene, was more fragile and sensitive to chlorination than T4 phage. However, addition of 0.02 mg/l chlorine, in conformance with the required concentration of the plant, did not affect the viability of T4 and 933 W phages. On the other hand, 1mg/l chlorine inactivated the 933 W phage significantly

27. **Escape from Prisoner's Dilemma in RNA phage phi6. Turner, P. E., Chao, L. (2003). *The American naturalist* 161:497-505.** We previously examined competitive interactions among viruses by allowing the RNA phage phi6 to evolve at high and low multiplicities of infection (ratio of infecting viruses to bacterial cells). Derived high-multiplicity phages were competitively advantaged relative to their ancestors during coinfection, but their fixation caused population fitness to decline. These data conform to the evolution of lowered fitness in a population of defectors, as expected from the Prisoner's Dilemma of game theory. However, the generality of this result is unknown; the evolution of viruses at other multiplicities may alter the fitness payoffs associated with conflicting strategies of cooperation and defection. Here we examine the change in matrix variables by propagating the ancestor under strictly clonal conditions, allowing cooperation the chance to evolve. In competitions involving derived cooperators and their selfish counterparts, our data reveal a new outcome where the two strategies are predicted to coexist in a mixed polymorphism. Thus, we demonstrate that the payoff matrix is not a constant in phi6. Rather, clonal selection allows viruses the opportunity to escape the Prisoner's Dilemma. We discuss mechanisms that may afford selfish genotypes an advantage during intrahost competition and the relevance in our system for alternative ecological interactions among viruses

28. **Use of genetically engineered phage to deliver antimicrobial agents to bacteria: an alternative therapy for treatment of bacterial infections. Westwater, C., Kasman, L. M., Schofield, D. A., Werner, P. A., Dolan, J. W., Schmidt, M. G., Norris, J. S. (2003). *Antimicrobial Agents and Chemotherapy* 47:1301-1307.** The emergence and increasing prevalence of multidrug-resistant bacterial pathogens emphasizes the need for new and innovative antimicrobial strategies. Lytic phages, which kill their host following amplification and release of progeny phage into the environment, may offer an alternative strategy for combating bacterial infections. In this study, however, we describe the use of a nonlytic phage to specifically target and deliver DNA encoding bactericidal proteins to bacteria. To test the concept of using phage as a lethal-agent delivery vehicle, we used the M13 phagemid system and the addiction toxins Gef and ChpBK. Phage delivery of lethal-agent phagemids reduced target bacterial numbers by several orders of magnitude in vitro and in a bacteremic mouse model of infection. Given the powerful genetic engineering tools available and the present knowledge in phage biology, this technology may have potential use in antimicrobial therapies and DNA vaccine development.

29. **Characterization of *Serratia* isolates from soil, ecological implications and transfer of *Serratia proteamaculans* subsp. *quinovora* Grimont et al. 1983 to *Serratia quinivorans* corrig., sp. nov. Ashelford, K. E., Fry, J. C., Bailey, M. J., Day, M. J. (2002). *International journal of systematic and evolutionary microbiology* 52:2281-2289.** Eleven strains of *Serratia* were isolated from different soils and the guts of invertebrates and characterized by their sensitivity to eight indigenous bacteriophages. They were also classified according to bacteriocin production and sensitivity, BiOLOG plate and API 20E strip profiles and 16S rRNA sequence information. One strain was thus identified as *Serratia plymuthica*, another as *Serratia fonticola*. The remaining strains were shown to be closely related to Serratia *proteamaculans* subsp. *quinovora* Grimont et al. 1983 after DNA-DNA cross-hybridization demonstrated relatedness greater than 70% with the type strain of this subspecies. From an ecological perspective, our results illustrated the wide variation in sensitivity that closely related *Serratia* strains have towards various indigenous soil phages and that these phages have broad host ranges within the genus. Furthermore, the phage and bacteriocin interactions within the *Serratia* strains examined were intricate and did not reflect phylogenetic relationships. These results together imply that complex interactions will occur in soil within the natural community of *Serratia* strains and their bacteriophages. DNA-DNA cross-hybridization and phenotypic characterization showed that *S. proteamaculans* subsp. *quinovora* strains formed a cohesive group at the species level. It is therefore concluded that these strains should be designated as *Serratia quinivorans* corrig., sp. nov

30. **Fundamental changes in light scattering associated with infection of marine bacteria by bacteriophage. Balch, W. M., Vaughn, J. M., Novotny, J. F., Drapeau, D. T., Goes, J. I., Booth, E., Lapierre, J. M., Vining, C. L., Ashe, A., Vaughn, JM Jr (2002). *Limnology and Oceanography* 47:1554-1561.** Bacteria and phytoplankton are key determinants of the ocean's inherent optical properties. Despite their high abundance, marine viruses have generally been thought to play a minor role in ocean optics because of their small scattering cross-sections. Nevertheless, the role of specific viral infection on the optical properties of bacteria and phytoplankton has remained unknown (i.e., as viruses disrupt micron-sized host cells to produce submicron cell debris). Here, we used laboratory and mesocosm cultures of marine bacteria for virus infection experiments in which growth conditions and host-virus specificity were controlled. We report that the chief optical impact of viruses is associated with infection and lysis of their hosts. We quantitatively describe, for the first time, two optical changes associated with infection and lysis of marine bacteria by bacteriophage: (1) rapid, strong shifts in the magnitude and shape of the optical volume scattering function and (2) rapid production of colored dissolved organic material. Qualitatively, these changes result in nearly complete clearing of turbid host bacterial suspensions. Although some optical differences would be expected between infection of bacteria in laboratory cultures versus field populations (mainly because of differences in cell size), these results are applicable to the field, especially for dense host suspensions such as in blooms. Even in nonbloom situations, as long as the host bacteria contribute a significant amount of the total particle backscattering, we expect that virus-induced backscattering changes would be detectable by use of satellite or aircraft remote-sensing techniques

31. **Characterization of the two-component abortive phage infection mechanism AbiT from *Lactococcus lactis*. Bouchard, J. D., Dion, E., Bissonnette, F., Moineau, S. (2002). *Journal of Bacteriology* 184:6325-6332.** During the production of fermented dairy products, virulent bacteriophages infecting *Lactococcus lactis* can delay or stop the milk acidification process. A solution to this biological problem consists of introducing natural phage barriers into the strains used by the dairy industry. One such hurdle is called abortive infection (Abi) and causes premature cell death with no or little phage progeny. Here, we describe the isolation and characterization of a novel Abi mechanism encoded by plasmid pED1 from *L. lactis*. The system is composed of two constitutively cotranscribed genes encoding putative proteins of 127 and 213 amino acids, named AbiTi and AbiTii, respectively. Site-directed mutagenesis indicated that a hydrophobic region at the C-terminal extremity of AbiTi is essential to the antiphage phenotype. The AbiT system is effective against phages of the 936 and P335 species (efficiency of plaquing between 10(-5) and 10(-7)) and causes a 20-fold reduction in the efficiency to form centers of infection as well as a 10- to 12-fold reduction in the burst size. Its efficacy could be improved by raising the plasmid copy number, but changing the intrinsic ratio of AbiTi and AbiTii did not greatly affect the antiphage activity. The monitoring of the intracellular phage infection process by DNA replication, gene expression, and electron microscopy as well as the study of phage mutants by genome mapping indicated that AbiT is likely to act at a later stage of the phage lytic cycle

32. **Genomic analysis of uncultured marine viral communities. Breitbart, M., Salamon, P., Andresen, B., Mahaffy, J. M., Segall, A. M., Mead, D., Azam, F., Rohwer, F. (2002). *Proceedings of the National Academy of Sciences, USA* 99:14250-14255.** Viruses are the most common biological entities in the oceans by an order of magnitude. However, very little is known about their diversity. Here we report a genomic analysis of two uncultured marine viral communities. Over 65% of the sequences were not significantly similar to previously reported sequences, suggesting that much of the diversity is previously uncharacterized. The most common significant hits among the known sequences were to viruses. The viral hits included sequences from all of the major families of dsDNA tailed phages, as well as some algal viruses. Several independent mathematical models based on the observed number of contigs predicted that the most abundant viral genome comprised 2-3% of the total population in both communities, which was estimated to contain between 374 and 7,114 viral types. Overall, diversity of the viral communities was extremely high. The results also showed that it would be possible to sequence the entire genome of an uncultured marine viral community

33. **F-specific RNA coliphages: occurrence, types, and survival in natural waters. Brion, G. M., Meschke, J. S., Sobsey, M. D. (2002). *Water Research* 36:2419-2425.** A small, well-defined watershed was investigated over a 2-year period to determine the prevalence of F-specific RNA coliphage (F + RNA) serotypes as indicators of animal fecal contamination. Sampling sites collected runoff from areas of urban and agricultural land use patterns. F-specific coliphages were concentrated from 2-L freshwater samples by polyethylene glycol precipitation, isolated using the double agar layer (DAL) method, confirmed as F + RNA by RNAse suppression, and serotyped. A subset of serotyped F + RNA were confirmed by genotyping. To determine relative survival, 10 confirmed F + RNA field isolates and 5 prototypic F + RNA were spiked into surface water and incubated at 25 degrees C for 36 days. F-specific coliphage isolation was strongly associated with rainfall events and was infrequent from primarily animal impacted surface waters. Field isolates were predoffiinantly Type I F + RNA (81%) and raw sewage isolates were predominantly Type III F + RNA (57%). Genotyping from either the watershed or raw sewage samples never positively identified Type IV F + RNA. Results from laboratory studies showed that F + RNA differ in their survival in water and that Type IV strains were the least persistent. Type III F + RNA were found to be reliably related to the release of uncontrolled human fecal material in the watershed, but the results of this study suggest that further study is required before utilizing for fecal source identification in natural waters

34. **RNA bacteriophage capsid-mediated drug delivery and epitope presentation. Brown, W. L., Mastico, R. A., Wu, M., Heal, K. G., Adams, C. J., Murray, J. B., Simpson, J. C., Lord, J. M., Taylor-Robinson, A. W., Stockley, P. G. (2002). *Intervirology* 45:371-380.** OBJECTIVE: To use our knowledge of the three-dimensional structure and self-assembly mechanism of RNA bacteriophage capsids to develop novel virus-like particles (VLPs) for drug delivery and epitope presentation. METHODS: Site-directed mutagenesis of a recombinant MS2 coat protein expression construct has been used to generate translational fusions encompassing short epitope sequences. These chimeric proteins still self-assemble in vivo into T = 3 shells with the foreign epitope in an accessible location. Covalent conjugation has also been used to generate RNA stem-loops attached to the toxin, ricin A chain, or to nucleotide-based drugs, that are still capable of stimulating self-assembly of the capsid in vitro. These packaged drugs can then be directed to specific cells in culture by further covalent decoration of the capsids with targeting molecules. RESULTS: Chimeric VLPs are strongly immunogenic when carrying either B or T cell epitopes, the latter generating cytokine profiles consistent with memory responses. Immune responses to the underlying phage epitopes appear to be proportional to the area of the phage surface accessible. Phage shells effectively protect nucleic acid-based drugs and, for the toxin construct, make cell-specific delivery systems with LD50 values in culture sub-nanomolar. CONCLUSION: VLP technology has potential for therapeutic and prophylactic intervention in disease

35. **Killing of *Mycobacterium avium* and *Mycobacterium tuberculosis* by a mycobacteriophage delivered by a nonvirulent mycobacterium: A model for phage therapy of intracellular bacterial pathogens. Broxmeyer, L., Sosnowskai, D., Miltner, E., Chacón, O., Wagner, D., McGarvey, J., Barletta, R. G., Bermudez, L. E. (2002). *Journal of Infectious Disease* 186:1155-1160.** *Mycobacterium avium* causes disseminated infection in patients with acquired immune deficieny syndrome. *Mycobacterium tuberculosis* is a pathogen associated with the deaths of millions of people worldwide annually. Effective therapeutic regimens exist that are limited by the emergence of drug resistance and the inability of antibiotics to kill dormant organisms. The present study describes a system using *Mycobacterium smegmatis,* an avirulent mycobacterium, to deliver the lytic phage TM4 where both *M. avium* and *M. tuberculosis* reside within macrophages. These results showed that treatment of *M. avium*infected, as well as *M. tuberculosis*infected, RAW 264.7 macrophages, with *M. smegmatis* transiently infected with TM4, resulted in a significant time- and titer-dependent reduction in the number of viable intracellular bacilli. In addition, the *M. smegmatis* vacuole harboring TM4 fuses with the *M. avium* vacuole in macrophages. These results suggest a potentially novel concept to kill intracellular pathogenic bacteria and warrant future development.

36. **Fenogeneticheskaia kharakteristika gruppy gigantskikh Phi KZ-podobnykh bakteriofagov Pseudomonas aeruginosa [Phenogenetic characterization of a group of giant Phi KZ-like bacteriophages of *Pseudomonas aeruginosa*]. Burkal'tseva, M. V., Krylov, V. N., Pleteneva, E. A., Shaburova, O. V., Krylov, S. V., Volkart, G., Sykilinda, N. N., Kurochkina, L. P., Mesianzhinov, V. V. (2002). *Genetika* 38:1470-1479.** A comparative study was made of a group of *Pseudomonas aeruginosa* virulent giant DNA bacteriophages similar to phage phi KZ in several genetic and phenotypic properties (particle size, particle morphology, genome size, appearance of negative colonies, high productivity, broad spectrum of lytic activity, ability to overcome the suppressing effect of plasmids, absence of several DNA restriction sites, capability of general transduction, pseudolysogeny). We have recently sequenced the phage phi KZ genome (288,334 bp) [J. Mol. Biol.,

2002, vol. 317, pp. 1-19]. By DNA homology, the phages were assigned to three species (represented by phage phi KZ, Lin68, and EL, respectively) and two new genera (phi KZ and EL). Restriction enzyme analysis revealed the mosaic genome structure in four phages of the phi KZ species (phi KZ, Lin21, NN, and PTB80) and two phages of the EL species (EL and RU). Comparisons with respect to phage particle size, number of structural proteins, and the N-terminal sequences of the major capsid protein confirmed the phylogenetic relatedness of the phages belonging to the phi KZ genus. The origin and evolution of the phi KZ-like phages are discussed. Analysis of protein sequences encoded by the phage phi KZ genome made it possible to assume wide migration of the phi KZ-like phages (wandering phages) among various prokaryotes and possibly eukaryotes. Since the phage phi KZ genome codes for potentially toxic proteins, caution must be exercised in the employment of large bacteriophages in phage therapy

37. **Integrity of powdered and powder-free latex examination gloves. Calhoun, A. J., Rodrick, G. E., Brown, F. H. (2002). *Journal of public health dentistry* 62:170-172.** OBJECTIVES: The difference in permeability between one brand of powdered and another of powder-free latex examination gloves was evaluated to determine leak rates. METHODS: Thirty-one of each type of glove were tested for each of three different conditions: usage by dental personnel (1) for 15 minutes or longer, (2) for less than 15 minutes, and (3) directly from the manufacturer's packaging (zero usage time). Each glove was evaluated in the fingers and the palm. The phiX-174 viral solution in the glove was allowed to penetrate for 15 minutes. Powder (cornstarch) was subsequently added to 20 powder-free gloves, and 15 of these were pierced with a 30-gauge needle. RESULTS: Powdered gloves showed no leakage rates. Because of this, 30-, 27-, and 25-gauge needles were used to pierce five gloves each. One glove with 27- and 25-gauge needle holes showed leakage. Leakage rates for powder-free gloves: 45.1 percent for more than 15 minutes of use, 25.8 percent for less than 15 minutes of use, and 16.1 percent for zero minutes of use. Two of the 20 pierced and one of the five unpierced powder-free gloves with added cornstarch leaked. CONCLUSION: Significant differences in leak results between powdered and powder-free gloves suggest further study is needed

38. **Isolation and characterization of two types of actinophages infecting *Streptomyces scabies* MR13. el Sayed, A. el, el Didamony, G., Mansour, K. (2002). *Acta Microbiologica et Immunologica Hungarica* 49:469-482.** Two types of actinophages, phi S and phi L, were isolated from soil samples by using *Streptomyces scabies* MR13, a potato scab pathogen, as an indicator strain. The phages were partially characterized according to their physicochemical properties, plaques and particles morphology and their host-range. The host-range of these phages was narrow for phi S and wide for phi L. The adsorption rate constants of the phi S and phi L were 3.44 x 10^{-9} and 3.18 x 10^{-9} ml/min, and their burst sizes were 1.61 and 3.75 virions, respectively. One-step growth indicated that phi S and phi L have a latent period of 30 min followed by a rise period of 30 min. The temperate character of these phages was tested in other isolates of Streptomyces. Four of the phages (phi SS3, phi SS12, phi SS13 and phi SS17) were identified as temperate phages, since they were able to lysogenize SS3, SS12, SS13 and SS17. phi SS3, phi SS12 and phi SS13 were homoimmune, and they were heteroimmune with respect to phi SS17. The restriction barriers of lysogenic isolates (SS12, SS13 and SS17) interfered with the blockage of plaques formation by phages (phi SS12, phi SS13 or phi SS17) propagated on them, about 75% of lysogenic isolates had restriction systems. The exposure of the lysogenic isolates (SS12, SS13 and SS17) to UV-irradiation prevented the possible restriction barriers of these isolates, and these barriers could be overcome

39. **High control of bacterial production by viruses in a eutrophic oxbow lake. Fischer, U. R., Velimirov, B. (2002). *Aquatic Microbial Ecology* 27:1-12.** The aim of the study was to test the hypothesis that the magnitude of viral control on bacterial production in a eutrophic oxbow lake of the River Danube would be higher than all average values reported so far in the literature. This assumption was based on the findings of low grazing of heterotrophic nanoflagellates (HNF) in this system, accounting on average for 5% of the bacterial mortality. Several approaches (viral decay method, estimation of the frequency of infected bacterial cells) to determine viral control of bacterial production were applied on a comparative basis. All system-specific parameters necessary to describe virus-bacteria interactions (burst size, bacterial production, contact rates) were monitored simultaneously. The average viral control of bacterial production determined by the different approaches was similar, ranging from 55.7 to 62.7 %, and prevailing over HNF grazing by a factor of more than 11. For individual events, however, we observed large variations between the methods, indicating that the use of one single method is not reliable to decide whether a detected trend is representative of a specific system. We discuss error sources of the applied methods and mathematical models, and accounted for them when calculating the contribution of viruses to bacterial mortality. We demonstrated that viruses could control more than 100 % of the bacterial production in the Alte Donau, which implies that occasionally up to 1.6 % h^{-1} of the bacterial standing stock was removed from the water column. High bacterial mortality due to viruses indicated that a large amount of dissolved organic carbon might be recycled from bacteria by phage-induced cell lysis. On average 15.2 mu g C l^{-1} d^{-1}, corresponding to some 46 % of the bacterial secondary production (BSP), was released into the water column due to viral lysis of bacterial cells and again became available for microheterotrophic consumption

40. **The cell surface protein Ag43 facilitates phage infection of *Escherichia coli* in the presence of bile salts and carbohydrates. Gabig, M., Herman-Antosiewicz, A., Kwiatkowska, M., Los, M., Thomas, M. S., Wegrzyn, G. (2002). *Microbiology (Reading England)* 2002 May, 148:1533-1542.** It was found that infection of *Escherichia coli* by bacteriophage lambda is inhibited in the presence of certain bile salts and carbohydrates when cells are in the "OFF" state for production of the phase-variable cell surface protein antigen 43 (Ag43). The inhibition of phage growth was found to be due to a significant impairment in the process of phage adsorption. Expression of the gene encoding Ag43 (agn43) from a plasmid or inactivation of the oxyR gene (encoding an activator of genes important for defence against oxidative stress) suppressed this inhibition. A mutation, rpoA341, in the gene encoding the alpha subunit of RNA polymerase also facilitated phage adsorption in the presence of bile salts and carbohydrates. The rpoA341 mutation promoted efficient production of Ag43 in a genetic background that would otherwise be in the "OFF" phase for expression of the agn43 gene. Analysis of a reporter gene fusion demonstrated that the promoter for the agn43 gene was more active in the rpoA341 mutant than in the otherwise isogenic rpoA(+) strain. The combined inhibitory action of bile salts and carbohydrates on phage adsorption and the abolition of this inhibition by production of Ag43 was not restricted to lambda, as a similar phenomenon was observed for the coliphages P1 and T4

41. **Fluorescent dye labeled bacteriophages--a new tracer for the investigation of viral transport in porous media: 2. Studies of deep-bed filtration. Gitis, V., Adin, A., Nasser, A., Gun, J., Lev, O. (2002). *Water Research* 36:4235-4242.** Viral transport in deep-bed sand filters was studied by a new method that enables rapid and simple quantitation of labeled viruses. The residence time distribution (RTD) of viruses in the bed was compared to the RTD of a fluorescein dye under conditions that simulate a filter run. The characteristics of the RTD curves for the free dye and the labeled bacteriophages followed very different trends during the filter run. While the retention time of free dye was practically independent of the filtration stage, the average retention time of the labeled bacteriophage depended in a non-linear way on filtration time. Average virus retention time as well as virus-removal efficiency were minimal at the ripening stage, increased during the operational stage and then decreased again

towards the turbidity breakthrough stage. This complex trend reflects two opposing mechanisms that dominate the behavior of the filter. During the ripening stage the accumulation of the kaolin-alum material in the filter increases the adsorption surface area and retards virus mobility. After sufficient kaolin-alum deposit is accumulated in the filter, aging and densification of the alum deposit induces size exclusion phenomenon giving faster apparent mobility of viruses in the filter bed

42. **Fluorescent dye labeled bacteriophages--a new tracer for the investigation of viral transport in porous media: 1. Introduction and characterization. Gitis, V., Adin, A., Nasser, A., Gun, J., Lev, O. (2002). *Water Research* 36:4227-4234.** A new method for the study of pathogen transport in porous media is presented. The method is based on conjugation of fluorescent dyes to target bacteriophages and application of the modified bacteriophages for tracer studies. We demonstrate that the relevant transport determining properties of Rhodamine and several fluorescein-labeled phages are practically identical to those of the native bacteriophages. The advantages of the proposed method relative to direct enumeration of bacteriophages by plaque forming unit method, turbidity, fluorescent microspheres, and other alternative tracers are discussed. Notable advantages include simple quantitation by optical methods, unbiased signals even when virus aggregates are formed, and the ability to decouple inactivation kinetics from transport phenomena. Additionally, the signal reflects the removal and transport of the studied microorganism and not a surrogate

43. **Observations on cyanobacterial population collapse in eutrophic lake water. Gons, H. J., Ebert, J., Hoogveld, H. L., van den Hove, L., Pel, R., Takkenberg, W., Woldringh, C. J. (2002). *Antonie van Leeuwenhoek* 81:319-326.** In two laboratory-scale enclosures of water from the shallow, eutrophic Lake Loosdrecht (the Netherlands), the predominating filamentous cyanobacteria grew vigorously for 2 weeks, but then their populations simultaneously collapsed, whereas coccoid cyanobacteria and algae persisted. The collapse coincided with a short peak in the counts of virus-like particles. Transmission electron microscopy showed the morphotype Myoviridae phages, with isometric heads of about 90 nm outer diameter and > 100-nm long tails, that occurred free, attached to and emerging from cyanobacterial cells. Also observed were other virus-like particles of various morphology. Similar mass mortality of the filamentous cyanobacteria occurred in later experiments, but not in Lake Loosdrecht. As applies to lakes in general, this lake exhibits high abundance of virus-like particles. The share and dynamics of infectious cyanophages remain to be established, and it is as yet unknown which factors primarily stabilize the host-cyanophage relationship. Observations on shallow, eutrophic lakes elsewhere indicate that the cyanophage control may also fail in natural water bodies exhibiting predominance of filamentous cyanobacteria. Rapid supply of nutrients appeared to be a common history of mass mortality of cyanobacteria and algae in laboratory and outdoor enclosures as well as in highly eutrophic lakes

44. **Reporter bacteriophage assays as a means to detect foodborne pathogenic bacteria. Goodridge, L., Griffiths, M. (2002). *Food Research International* 35:863-870.** Bacterial disease due to the consumption of contaminated food is a global problem that has necessitated the need for modern rapid bacterial detection techniques. There has been much recent interest in the use of reporter bacteriophages as a tool to aid in the detection of foodborne, and clinical bacterial pathogens. The reporter bacteriophage concept provides a sensitive method for bacterial detection and sensitivity to antimicrobial agents. This review presents the current status of reporter bacteriophage technology. The bacterial and eucaryotic luciferases, the ice nucleation protein, and the *E. coli* b-galactosidase reporter genes are discussed, along with many examples that demonstrate the usefulness of reporter bacteriophage as tools to detect foodborne bacterial contamination.

45. **Characterization of phi 12, a bacteriophage related to phi 6: nucleotide sequence of the small and middle double-stranded RNA. Gottlieb, P., Wei, H., Potgieter, C., Toporovsky, I. (2002). *Virology* 293:118-124.** The isolation of additional bacteriophages containing segmented double-stranded RNA genomes has expanded the Cystoviridae family to nine members. Comparing the genomic sequences of these viruses has allowed evaluation of important genetic as well as structural motifs. These comparative studies are resulting in greater understanding of viral evolution and the role played by genetic and structural variation in the assembly mechanisms of the cystoviruses. In this regard, the small and middle double-stranded RNA genomic segments of bacteriophage phi 12 were copied as cDNA and their nucleotide sequences determined. This genome's organization is similar to that of the small and middle segments of bacteriophages phi 6, phi 8, and phi 13. Although there is little similarity in the nucleotide sequences, similarity exists in the amino acid sequence of the lysis cassette proteins to those of phi 6. The host cell attachment proteins are found to have marked similarity to the phi 13 attachment proteins

46. **Characterization of phi12, a bacteriophage related to phi6: nucleotide sequence of the large double-stranded RNA. Gottlieb, P., Potgieter, C., Wei, H., Toporovsky, I. (2002). *Virology* 295:266-271.** The isolation of additional bacteriophages besides phi6 containing segmented double-stranded RNA genomes (dsRNA) has expanded the Cystoviridae family to nine members. Comparing the genomic sequences of these viruses has allowed evaluation of important genetic as well as structural motifs. These comparative studies are resulting in greater understanding of viral evolution and the role played by genetic and structural variation in the assembly mechanisms of the cystoviruses. In this regard, the large double-stranded RNA genomic segment of bacteriophage phi12 was copied as cDNA and its nucleotide sequence determined. This genome's organization is similar to that of the large segment of bacteriophages phi6, phi8, and phi13. In the amino acid sequence of the viral RNA-dependent RNA polymerase (P2), similarity was found to the comparable proteins of phi6, phi8, and phi13. Amino acid sequence similarity was also noted in the nucleotide triphosphate phosphorylase (P4) to the comparable proteins of phi8 and phi13

47. **Viral distribution and activity in Antarctic waters. Guixa-Boixereu, N., Vaque, D., Gasol, J. M., Sanchez-Camara, J., Pedros-Alio, C. (2002). *Deep-Sea Research II* 49:827-845.** Variability in abundance of virus-like particles (VLP), VLP decay rates and prokaryotic mortality due to viral infection were determined in three Antarctic areas: Bellingshausen Sea, Bransfield Strait and Gerlache Strait, during December 1995 and February 1996. VLP abundance showed very small spatial variability in the three areas (7×10^6-2×10^7 VLP ml^{-1}). VLP abundance, on the other hand, decreased one order of magnitude from the surface to the bottom, in two stations where deep vertical profiles were sampled. Low seasonal variability in VLP abundance was found when comparing each area separately. Diel VLP variability was also very low. VLP abundance showed the lowest values when solar irradiation was maximal, in two of the three stations where diel cycles were examined. Viral decay rates (VDR) were determined using KCN in two kinds of experiments. Type 1 experiments were performed in 6 stations to determine viral decay. Type 2 experiments were carried out in 2 stations to examine the influence of temperature and organic matter concentration on viral decay. VDR was not influenced by these parameters. Prokaryotic mortality due to viral infection was always higher than that due to bacterivores in the stations where both factors of prokaryotic mortality were measured. Viral infection accounted for all the prokaryotic heterotrophic production in Bellingshausen Sea and Gerlache Strait and for half of the prokaryotic heterotrophic production in Bransfield Strait. These high values of prokaryotic mortality due to viral infection are difficult to reconcile in nature, and more work is necessary to determine the mechanisms involved in the disappearance of viruses

48. **Dissemination of the phage-associated novel superantigen gene speL in recent invasive and noninvasive *Streptococcus pyogenes* M3/T3 isolates in Japan. Ikebe, T., Wada, A., Inagaki, Y., Sugama, K., Suzuki, R., Tanaka, D., Tamaru, A., Fujinaga, Y., Abe, Y., Shimizu, Y., Watanabe, H. (2002). *Infection and Immunity* 70:3227-3233.** In Japan, more than 10% of streptococcal toxic shock-like syndrome (TSLS) cases have been caused by *Streptococcus pyogenes* M3/T3 isolates since the first reported TSLS case in 1992. Most M3/T3 isolates from TSLS or severe invasive infection cases during 1992 to 2001 and those from noninvasive cases during this period are indistinguishable in pulsed-field gel electropherograms. The longest fragments of these recent isolates were 300 kb in size, whereas those of isolates recovered during or before 1973 were 260 kb in size. These 260- and 300-kb fragments hybridized to each other, suggesting the acquisition of an about 40-kb fragment by the recent isolates. The whole part of the acquired fragment was cloned from the first Japanese TSLS isolate, NIH1, and its nucleotide sequence was determined. The 41,796-bp fragment is temperate phage phiNIH1.1, containing a new superantigen gene speL near its right attachment site. The C-terminal part of the deduced amino acid sequence of speL has 48 and 46% similarity with well-characterized erythrogenic toxin SpeC and the most potent superantigen, SmeZ-2, respectively. None of 10 T3 isolates recovered during or before 1973 has speL, whereas all of 18 M3/T3 isolates recovered during or after 1992 and, surprisingly, *Streptococcus equi* subsp. *equi* ATCC 9527 do have this gene. Though plaques could not be obtained from phiNIH1.1, its DNA became detectable from the phage particle fraction upon mitomycin C induction, showing that this phage is not defective. A horizontal transfer of the phage carrying speL may explain the observed change in M3/T3 S. pyogenes isolates in Japan

49. **The effect of storage and lag time on MS2 bacteriophage susceptibility to ultraviolet radiation. Jolis, D. (2002). *Water environment research : a research publication of the Water Environment Federation* 74:516-520.** The susceptibility of MS2 bacteriophage suspensions to UV radiation was assessed using a collimated beam technique. Storage of MS2 bacteriophage cultures at 4 degrees C resulted in a decrease in phage susceptibility to UV radiation over time. After 18 days, the level of MS2 bacteriophage inactivation achieved for the range of UV doses tested decreased by 0.7 to 1.1 logs, but remained constant after that point. Changes in the protein coat of the bacteriophage, a decrease in viability over time, and an increase in coagulation may have played a role in the observed susceptibility decrease. A 2-hour lag time between the preparation of the MS2 suspension and the irradiation test also resulted in a decrease in phage susceptibility

50. **Degree of ultraviolet radiation damage and repair capabilities are related to G+C content in marine vibriophages. Kellogg, C. A., Paul, J. H. (2002). *Aquatic Microbial Ecology* 27:13-20.** A key issue in the ecology of viruses in the marine environment is the rate of viral production and decay. The ultraviolet (UV) radiation in sunlight has been found to cause loss of infectivity in marine bacteriophages at rates nearly equal to all other decay mechanisms combined. There are 2 main host-mediated mechanisms that can repair UV-damaged phage DNA: photoreactivation and excision repair. Both these mechanisms were investigated in 2 marine Vibrio parahaemolyticus hosts as they catalyzed the reactivation of 7 phages. Photoreactivation was the dominant repair mode in all but one case. A significant correlation was found between G+C content of the phage DNAs (16 to 70 %) and degree of DNA damage (r = 0.955), indicating a strong relationship between the number of thymine dimer targets and the capability to photoreactive DNA damage. Evolution of high G+C content may be a strategy for protection from UV damage in marine phages

51. **Regeneration of dissolved organic matter by viral lysis in marine microbial communities. Middelboe, M., Lyck, P. G. (2002). *Aquatic Microbial Ecology* 27:187-194.** The influence of viruses on bacterial net growth and respiration was investigated in batch cultures with natural assemblages of marine bacterioplankton, which were manipulated with respect to abundance of natural virioplankton. In 1 set of cultures (-virus), a virus-free water sample (0.02 mu m filtered) was inoculated with a bacterioplankton concentrate, and in a parallel set of cultures (control) a virus-containing water sample (0.2 mu m filtered) was inoculated with the bacterioplankton concentrate. The 0.02 mu m filtration procedure reduced viral abundance by 62 to 92% in the -virus cultures relative to the parallel control cultures with the natural density of viruses (i.e. the fraction of natural viruses < 0.2 mu m). This approach allowed us to examine the effects of reduced viral densities on the production of natural assemblages of bacteria and viruses and on the distribution of added ^{3}H-thymidine into size fractions (the bacterial size fraction, viral size fraction, dissolved size fraction and respired fraction). The results showed significantly higher bacterial net growth and growth efficiency in cultures with a reduced abundance of viruses relative to control cultures with natural viral abundance, and indicated viral regulation of bacterial abundance in the control cultures. We suggest that viral lysis significantly affected the bacterial carbon cycling in the cultures by liberating a fraction of the organic matter already taken up by the bacteria, thus stimulating recycling of bacterial carbon and reducing the net bacterial production. The implications of such regeneration of dissolved organic matter by viral lysis for pelagic carbon cycling and for measurements of bacterial production are discussed

52. **Regrowth and survival of indicator microorganisms on the surfaces of household containers used for the storage of drinking water in rural communities of South Africa. Momba, M. N. B., Kaleni, P. (2002). *Water Research* 36:3023-3028.** The present study covered two rural communities of South Africa: Ncera and Ntselamanzi villages. Raw water from Ncera river is used by the community of Ncera village for drinking, while the community of Ntselamanzi receives their drinking water from Alice purification system. Treated water is supplied to the community by a public standpipe system. In rural communities of South Africa, many households use polyethylene (PE) and galvanized steel (GS) containers for the storage of their drinking water. To investigate the regrowth and survival of indicator microorganisms on the surface of household containers during the storage of drinking water, PE and GS slides were suspended in the appropriate household containers for a period of 48 h. This period of 48 h was chosen as the study period because results from the questionnaire indicated that the largest percentage (62%) of households store their water for that length of time. The experiment was performed to test drinking water as it is collected and stored by rural communities. No disinfection of household containers or slides was done during the study period. Attached coliphages (F-RNA (FP) and somatic phage (SP), coliform bacteria (total coliform (TC), presumptive *Escherichia coli* (EC), *Salmonella* (Sal) and *Clostridium perfringens* (CP) were measured during the study period. With the exception of CP, attached indicator microorganisms consisted of TC, presumptive *E. coli* and *Salmonella*, somatic and F-RNA coliphages, although the yield (average count) for the last four groups (EC: < 1-3 cfu cm^{-2}, Sal: < 1-15 cfu cm^{-2}, FP: < 1-7 pfu cm^{-2}, SP: < 1-7pfu cm^{-2}) was lower than that of TC (3-183 cfu cm^{-2}). However, the lowest yield of indicator microorganisms was noted for presumptive *E. coli*. Whereas the occurrence and survival of TC was noted on the surface of household containers during the entire period of the experimental study, other indicator microorganisms occurred from time to time. The regrowth of indicator microorganisms occurred 48 h after the exposure of slides to both types of test waters. This length of time mostly resulted in the regrowth of TC (with an increase in bacterial counts) while the persistence of other indicator organism groups on the surface of the slides was apparent. A comparison between PE and GS containers showed that more TC (average count) regrew on PE than on GS containers (for river water, PE: from 36 to 55 cfu cm^{-2}, GS: from 25 to 26 cfu cm^{-2}; for standpipe water, PE: from 147 to 183 cfu cm^{-2}, GS from 3 to 4 cfu cm^{-2}). This study revealed that both types of household containers supported the growth and survival of indicator microorganisms due to the bad quality of the intake water before storage. The storage of drinking water for 48 h mainly

resulted in the regrowth of TC. Nevertheless, the persistence of other indicator microorganisms was observed on the surface of household containers

53. **Virus-like particle analysis in a seston-rich coastal pond using transmission electron microscopy. Montanie, H., Hartmann, H. J., Crottereau, C., Trichet, C. (2002). *Aquatic Microbial Ecology* 28:105-115.** A method was developed to analyse virus-like particles (VLPs) in seston-rich waters and to quantify their dynamics in a coastal marsh of the Bay of Biscay, French Atlantic coast. The method combined clarification and concentration steps with electron microscopy to obtain information on particle abundance, type and size distribution (e.g. presence of tailed phages, Fuselloviridae, etc.). The mean recovery rates of T2-phages using this method were 71 to 79%, higher than other published rates. The transmission electron microscopy (TEM) counts were validated with T2 plaque lysis assay and epifluorescent (DAPI-stained) particle counting: the TEM method was valid for environmental particle concentrations above 1 to 2 x 10^6 VLP ml^{-1}; TEM counts were lower than T2-plaque counts (TEM/lysis median = 0.293) but higher than DAPI counts (TEM/DAPI median = 2.39). The method was used to evaluate the coupling between viral and bacterial dynamics in a marsh pond during 2 months. The VLP abundance varied from 1 to 30 x 10^6 ml super(-1) and the viral population was dominated by small particles (20 to 64 nm). Tailed phages, identified as bacteriophages, were always less abundant than non-tailed VLPs (4 to 23% of total virus), yet their dynamics were better linked with bacterial development than those of total virus. Our results demonstrate that the best way to characterise bacterial lysis from virus in seston-rich coastal environments would be to study the dynamics of tailed phages and virus size-classes rather than the commonly applied total VLPs

54. **The effect of an agglutogen on virus infection: biotinylated filamentous phages and avidin as a model. Nakamura, Michihiro, Tsumoto, Kouhei, Ishimura, Kazunori, Kumagai, Izumi (2002). *FEBS Letters* 520:77-80.** To address the effect of an agglutogen on virus infection, we studied the avidin-associated inhibition of infection by biotinylated M13 phages (BIO-phages). Microscopic observation of mixtures of BIO-phages and avidin-fluorescein conjugates revealed many aggregates. Even at low phage concentrations, avidin induced inhibition of infection significantly. Anti-M13 phage antibody also made aggregates and inhibited the infection but in a different manner from avidin. The inhibition by avidin was at > or = 2 microg/ml, time dependent and marked until 10 min after the mixing of the BIO-phages and *Escherichia coli*. On the other hand, antibody inhibited the infection at > or = 0.1 microg/ml dose dependently, and the inhibition was time dependent and marked until 45 min after the mixing at moderate and low phage concentrations. These results indicate that avidin against BIO-phages and antibodies are agglutogens, and the inhibition of the BIO-phages by avidin is closely related to the tetramerization of avidin. Agglutogens may be novel alternative antiviral drugs

55. **Combined phage typing and amperometric detection of released enzymatic activity for the specific identification and quantification of bacteria . Neufeld, T., Schwartz-Mittelmann, A., Biran, D., Ron, E. Z., Rishpon, J. (2002). *Analytical Chemistry* 75:580-585.** Here, we describe a novel electrochemical method for the rapid identification and quantification of pathogenic and polluting bacteria. The design incorporates a bacteriophage, a virus that recognizes, infects, and lyses only one bacterial species among mixed populations, thereby releasing intracellular enzymes that can be monitored by the amperometic measurement of enzymatic activity. As a model system, we used virulent phage typing and cell-marker enzyme activity (-D-galactosidase), a combination that is specific for the bacterial strain *Escherichia coli* (K-12, MG1655). Filtration and preincubation before infecting the bacteria with the phage enabled amperometric detection at a wide range of concentrations, reaching as low as 1 colony-forming unit/100 mL within 6-8 h. In principle, this electrochemical method can be applied to any type of bacterium-phage combination by measuring the enzymatic marker released by the lytic cycle of a specific phage.

56. **A filterable lytic agent obtained from a red tide bloom that caused lysis of *Karenia brevis* (*Gymnodinum breve*) cultures. Paul, J. H., Houchin, L., Griffin, D., Slifko, T., Guo, M., Richardson, B., Steidinger, K. (2002). *Aquatic Microbial Ecology* 27:21-27.** A filterable lytic agent (FLA) was obtained from seawater in the southeastern Gulf of Mexico during a red tide bloom that caused lysis of *Karenia brevis* (formerly *Gymnodinium breve*) Piney Island. This agent was obtained from <0.2 mu m filtrates that were concentrated by ultrafiltration using a 100 kDa filter. The FLA was propagated by passage on K. brevis cultures, and the filtered supernatants of such cultures resulted in K. brevis lysis when added to such cultures. The lytic activity was lost upon heating to 65 degree C or by 0.02 mu m filtration. Epifluorescence and transmission electron microscopy (TEM) of supernatants of *K. brevis* cultures treated with the lytic agent indicated a high abundance of viral particles (4 x 10^9 to 7 x 10^9 virus-like particles [VLPs] ml^{-1}) compared to control cultures (similar to 10^7 ml^{-1}). However, viral particles were seldom found in TEM photomicrograph thin sections of lysing *K. brevis* cells. Although a virus specific for *K. brevis* may have been the FLA, other explanations such as filterable bacteria or bacteriophages specific for bacteria associated with the *K. brevis* cultures cannot be discounted

57. **Marine phage genomics. Paul, J. H., Sullivan, M. B., Segall, A. M., Rohwer, F. (2002). *Comparative Biochemistry and Physiology* 133:463-476.** Marine phages are the most abundant biological entities in the oceans. They play important roles in carbon cycling through marine food webs, gene transfer by transduction and conversion of hosts by lysogeny. The handful of marine phage genomes that have been sequenced to date, along with prophages in marine bacterial genomes, and partial sequencing of uncultivated phages are yielding glimpses of the tremendous diversity and physiological potential of the marine phage community. Common gene modules in diverse phages are providing the information necessary to make evolutionary comparisons. Finally, deciphering phage genomes is providing clues about the adaptive response of phages and their hosts to environmental cues

58. **The *Brucella suis* genome reveals fundamental similarities between animal and plant pathogens and symbionts. Paulsen, I. T., Seshadri, R., Nelson, K. E., Eisen, J. A., Heidelberg, J. F., Read, T. D., Dodson, R. J., Umayam, L., Brinkac, L. M., Beanan, M. J., Daugherty, S. C., Deboy, R. T., Durkin, A. S., Kolonay, J. F., Madupu, R., Nelson, W. C., Ayodeji, B., Kraul, M., Shetty, J., Malek, J., Van Aken, S. E., Riedmuller, S., Tettelin, H., Gill, S. R., White, O., Salzberg, S. L., Hoover, D. L., Lindler, L. E., Halling, S. M., Boyle, S. M., Fraser, C. M. (2002). *Proceedings of the National Academy of Sciences, USA* 99:13148-13153.** The 3.31-Mb genome sequence of the intracellular pathogen and potential bioterrorism agent, *Brucella suis*, was determined. Comparison of *B. suis* with *Brucella melitensis* has defined a finite set of differences that could be responsible for the differences in virulence and host preference between these organisms, and indicates that phage have played a significant role in their divergence. Analysis of the *B. suis* genome reveals transport and metabolic capabilities akin to soil/plant-associated bacteria. Extensive gene synteny between *B. suis* chromosome 1 and the genome of the plant symbiont *Mesorhizobium loti* emphasizes the similarity between this animal pathogen and plant pathogens and symbionts. A limited repertoire of genes homologous to known bacterial virulence factors were identified

59. **Bacteriophage T4 development in *Escherichia coli* is growth rate-dependent. Rabinovitch, A., Fishov, I., Hadas, H., Einav, M., Zaritsky, A. (2002). *Journal of Theoretical Biology* 216:1-4.** Three independent parameters (eclipse and latent periods, and rate of ripening during the rise period) are essential and sufficient to describe bacteriophage development in its bacterial host. A general model to describe the classical "one-step growth" experiment [Rabinovitch et al. (1999a) J. Bacteriol.181, 1687-1683] allowed their calculations from experimental results obtained with T4 in Escherichia coli B/r under different growth conditions [Hadas et al. (1997) Microbiology143, 179-185]. It is found that all three parameters could be described by their dependence solely on the culture doubling time tau before infection. Their functional dependence on tau, derived by a best-fit analysis, was used to calculate burst size values. The latter agree well with the experimental results. The dependence of the derived parameters on growth conditions can be used to predict phage development under other experimental manipulations.

60. **Field and laboratory investigations of inactivation of viruses (PRD1 and MS2) attached to iron oxide-coated quartz sand. Ryan, J. N., Harvey, R. W., Metge, D., Elimelech, M., Navigato, T., Pieper, A. P. (2002). *Environmental science & technology* 36:2403-2413.** Field and laboratory experiments were conducted to investigate inactivation of viruses attached to mineral surfaces. In a natural gradient transport field experiment, bacteriophage PRD1, radiolabeled with ^{32}P, was injected into a ferric oxyhydroxide-coated sand aquifer with bromide and linear alkylbenzene sulfonates. In a zone of the aquifer contaminated by secondary sewage infiltration, small fractions of infective and ^{32}P-labeled PRD1 broke through with the bromide tracer, followed by the slow release of 84% of the 32P activity and only 0.011% of the infective PRD1. In the laboratory experiments, the inactivation of PRD1, labeled with ^{35}S (protein capsid), and MS2, dual radiolabeled with ^{35}S (protein capsid) and ^{32}P (nucleic acid), was monitored in the presence of groundwater and sediment from the contaminated zone of the field site. Release of infective viruses decreased at a much faster rate than release of the radiolabels, indicating that attached viruses were undergoing surface inactivation. Disparities between ^{32}P and ^{35}S release suggest that the inactivated viruses were released in a disintegrated state. Comparison of estimated solution and surface inactivation rates indicates solution inactivation is approximately 3 times as fast as surface inactivation. The actual rate of surface inactivation may be substantially underestimated owing to slow release of inactivated viruses

61. **Comparative resistance of phage isolates of four genotypes of f-specific RNA bacteriophages to various inactivation processes. Schaper, M., Duran, A. E., Jofre, J. (2002). *Applied and Environmental Microbiology* 68:3702-3707.** The effect of natural inactivation in freshwater, chlorination, ammonia, extreme pHs, temperature, and salt content on phage inactivation was evaluated on mixtures of F-specific RNA bacteriophage isolates belonging to genotypes I, II, III, and IV. The bacteriophages studied were previously but recently isolated from natural samples, characterized as F-specific RNA bacteriophages and genotyped by plaque hybridization with genotype-specific probes. Natural inactivation in river water was modeled by in situ incubation of bacteriophages inside submerged dialysis tubes. After several days bacteriophages of genotype I showed the highest persistence, which was significantly different from that of bacteriophages of genotype II, IV, or III. The pattern of resistance of phages belonging to the various genotypes to extreme pHs, ammonia, temperature, salt concentration, and chlorination was similar. In all cases, phages of genotype I showed the highest persistence, followed by the phages of genotypes II, III, and IV. The phages of genotypes III and IV were the least resistant to all treatments, and resistance of genotypes III and IV to the treatments was similar. Bacteriophages of genotype II showed intermediate resistance to some of the treatments. The resistance of four phages of genotype I to natural inactivation and chlorination did not differ significantly. These results indicate that genotypes III and IV are much more sensitive to environmental stresses and to treatments than the other genotypes, especially than genotype I. This should be taken into consideration in future studies aimed at using genotypes of F-specific RNA bacteriophages to fingerprint the origin of fecal pollution

62. **Site-specific integrative elements of rhizobiophage 16-3 can integrate into proline tRNA (CGG) genes in different bacterial genera. Semsey, S., Blaha, B., Koles, K., Orosz, L., Papp, P. P. (2002). *Journal of Bacteriology* 184:177-182.** The integrase protein of the *Rhizobium meliloti* 41 phage 16-3 has been classified as a member of the Int family of tyrosine recombinases. The site-specific recombination system of the phage belongs to the group in which the target site of integration (attB) is within a tRNA gene. Since tRNA genes are conserved, we expected that the target sequence of the site-specific recombination system of the 16-3 phage could occur in other species and integration could take place if the required putative host factors were also provided by the targeted cells. Here we report that a plasmid (pSEM167) carrying the attP element and the integrase gene (int) of the phage can integrate into the chromosomes of *R. meliloti* 1021 and eight other species. In all cases integration occurred at so-far-unidentified, putative proline tRNA (CGG) genes, indicating the possibility of their common origin. Multiple alignment of the sequences suggested that the location of the att core was different from that expected previously. The minimal attB was identified as a 23-bp sequence corresponding to the anticodon arm of the tRNA

63. **Rex-centric mutualism. Slavcev, R. A., Hayes, S. (2002). *Journal of Bacteriology* 184:857-858.** We asked whether Rex exclusion encoded by a lambda prophage confers a protective or a cell-killing phenotype. We found that the Rex system can channel lysogenic cells into an arrested growth phase that gives an overall protective ability to the host despite some associated killing

64. **The prevalence and diversity of mobile genetic elements in bacterial communities of different environmental habitats: Insights gained from different methodological approaches. Smalla, K., Sobecky, P. A. (2002). *FEMS Microbiology Ecology* 42:165-175.** The pool of mobile genetic elements (MGE) in microbial communities consists of plasmids, bacteriophages and other elements that are either self-transmissible or use mobile plasmids and phages as vehicles for their dissemination. By facilitating horizontal gene exchange, the horizontal gene pool (HGP) promotes the evolution and adaptation of microbial communities. Efforts to characterise MGE from bacterial populations resident in a variety of ecological habitats have revealed a surprisingly vast and seemingly untapped diversity. MGE, conferring such selectable traits as mercury or antibiotic resistance and degradative functions, have been readily acquired from diverse microbial communities. To circumvent the need to isolate microbial hosts, polymerase chain reaction (PCR)-based detection methods have frequently been used to assess the prevalence of MGE-specific sequences resident in the 'microbial community' HGP. As studies continue to reveal novel and distinct MGE, sequencing of newly isolated MGE from diverse habitats is essential for the continued development of DNA probes, PCR primers as well as for gene array and proteomics-based approaches. This minireview highlights insight gained from different methodological approaches, biased albeit largely toward plasmids in Gram-negative bacteria, used to study the HGP of naturally occurring microbial communities from various aquatic and terrestrial habitats

65. **Three recently acknowledged *Escherichia* species strikingly differ in the incidence of bacteriocinogenic and lysogenic strains. Smarda, J., Smajs, D., Lhotova, H. (2002). *Journal of Basic Microbiology* 42:429-433.** The incidence of bacteriocinogeny and lysogeny was followed in bacteria of 3 recently acknowledged species of the genus *Escherichia*: *E.*

hermanii, *E. vulneris* and *E. fergusonii*. Almost all of the strains examined were of human origin. In 30 strains of *E. hermanii* no one was found bacteriocinogenic while 57% were lysogenic, in 30 strains of *E. vulneris* none was found to be bacteriocinogenic and only 10% were lysogenic, and in 50 strains *E. fergusonii* 12% were bacteriocinogenic and 40% lysogenic. From the 6 bacteriocinogenic strains of *E. fergusonii*, 3 were producers of colicin E1, 2 of colicin Ib and 1 of colicin Ia. In addition, 3 *E. fergusonii* strains produced aerobactin

66. **Otlichiia v sostave genov virulentnosti v shtammakh Vibrio cholerae eltor, vydelennykh iz raznykh istochnikov na territorii Turkmenistana [Differences in virulence genes in *Vibrio cholerae* eltor strains isolated from different sources in Turkmenistan territory]. Smirnova, N. I., Kostromitina, E. A., Cheldyshova, N. B., Kutyrev, V. V. (2002). *Molekuliarnaia genetika* 2002,12-18.** Polymerase chain reaction (PCR) detected the presence of various genes associated with virulence in genome of strains *V. cholerae* eltor isolated in Turkmenistan territory during epidemic and epidemic-free perios. It was found that a complete set of virulence genes (ctxA+, tcpA+ and toxR+) contained strains isolated from patients, carriers and environment only in cholera epidemics. Strains isolated from the environment in the period free of epidemics did not contain ctxA and tcpA in 78.2% of cases, but 5.2% of the strains carried a complete set of virulence genes. There were also nontoxigenic strains containing genes tcpA and toxR. Such strains were isolated from the environment (16.6%) and vibrion carriers (42.9%). Isolated were also strains *V.cholerae* eltor carrying bacteriophage CTX phi with incomplete set of virulence genes and having genotype ctxA-, ace+ and zot+. Almost all the strains ctxA-, tcpA+ carry attRS1-site in genome. This shows that such strains may transform into toxigenic as a result of infection with bacteriophage CTX phi

67. **Effectiveness of thermal treatments and biocides in the inactivation of Argentinian *Lactococcus lactis* phages. Suarez, V. B., Reinheimer, J. A. (2002). *Journal of Food Protection* 65:1756-1759.** The thermal and chemical resistance levels of four autochthonal bacteriophages of *Lactococcus lactis* subsp. *lactis*, isolated from cheese processes, was investigated. The times required to obtain 99% inactivation of phages (T99) at 63 and 72 degrees C in three suspension media (M17 broth, reconstituted commercial nonfat skim milk, and Tris magnesium gelatin buffer) were determined. Thermal resistance was dependent on the phage studied, and the results of this study demonstrate that pasteurization treatments used in dairy industries may leave viable viral particles in milk. It was possible to determine that M17 broth was generally the least protective medium, while phosphate buffer was the most protective one. Peracetic acid (0.15%, vol/vol) was the most effective viricidal agent, with exposures of 5 min being sufficient to inactivate high-titer phage suspensions (>10(6) PFU/ml). To achieve total inactivation (<10 PFU/ml) of viral suspensions, sodium hypochlorite was effective at 100 ppm for only two phages, while the other two phages needed concentrations of 200 and 300 ppm. Ethanol at concentrations of 100 and 75% proved to be very efficient in inactivating phages, but isopropanol was not effective against them

68. **Mutations in bacteriophage T4 genome. Switala-Jelen, K., Dabrowska, K., Gorski, A., Sliwa, L. (2002). *Acta virologica. English ed* 46:57-62.** Bacteriophage (phage) T4 belonging to T-even phages is one of the best known phages with a completely deciphered genome sequence. As a model of living systems, T4 phage has many technical advantages. It can be very easily grown in large quantities, manipulated by classical genetics, and engineered by site-directed mutagenesis. Many substances have been first tested for mutagenicity in T-even phages. The results of these tests were very often applicable to higher organisms due to similar mechanisms of mutagenesis. T4 phage is also important in phage therapy, which represents an alternative treatment of bacterial infections since the bacterial resistance to antibiotics has become a serious medical problem. The site-directed mutagenesis is a method that enables to introduce mutations which can influence phage affinity to bacteria and can be a practical technique for enriching phage collections and for widening specificity of phages for new bacterial strains now insensitive to phage therapy

69. **Fate of coliphage in waste water treatment process and detection of phages carrying the Shiga toxin type 2 gene. Tanji, Y., Mizoguchi, K., Akitsu, T., Morita, M., Hori, K., Unno, H. (2002). *Water Science and Technology* 46:285-289.** Abundances of phages specific to *Escherichia coli* in the wastewater treatment process were analyzed. Relatively abundant coliphages were detected in sewage influent. Phages in the influent were found both suspended in liquid phase and attached on the solid particles. Phage concentration was not reduced in the settling tank without chemical agglutination. Anaerobic followed by aerobic treatment of the sewage reduced concentration of suspended phages. Almost no phage was detected as a suspended form in the aerobic tank. Most of the phages were detected as attaching form and were excluded by aggregation with sludge. Using an experimental approach based on the detection of Shiga toxin 2 (Stx 2) gene by a phage enrichment culture followed by nested PCR, bacteriophages carrying Stx 2 gene were detected in the influent, settling tank, and anaerobic tank. It was revealed that the presence of phages carrying Stx 2 gene is common in sewage and these phages are effectively eliminated through sewage treatment process

70. **Defektnaia lizogenia Erwinia carotovora [Defective lysogeny in *Erwinia carotovora*]. Tovkach, F. I. (2002). *Mikrobiologiia* 71:359-367.** The electron microscopic study of several *Erwinia carotovora* strains showed that the SOS-induced cells of this pectolytic phytopathogenic bacterium produce particular phage parts (tails, heads, and baseplates) but do not assemble them into fully functional phage particles. *E. carotovora* cells produced several times greater amounts of phage tails in response to induction by mitomycin C than in response to induction by nalidixic acid. The tails were 128-192 nm in length and 13-21 nm in diameter. Phage heads were characterized by four discrete ranges of diameters: 18, 55-59, 66-75, and 92-98 nm. The diameters of phage baseplates varied from 39 to 53 nm, depending on the particular strain. It was shown that cells of the same species may contain several different types of phage tails and heads. The structural organization of phage tails and baseplates in the nalidixic acid-induced lysate of *E. carotovora* J2 was studied in more detail. The data obtained suggest that pectolytic phytopathogenic erwinia are characterized by defective polylysogeny

71. **Ecological and molecular maintenance strategies of mobile genetic elements. Turner, S. L., Bailey, M. J., Lilley, A. K., Thomas, C. M. (2002). *FEMS Microbiology Ecology* 42:177-185.** This review considers the influence of selection pressure, fitness and population structures on the evolution of mobile genetic elements (including plasmids, phage, pathogenicity islands, transposons and insertion sequences) that constitute the horizontal gene pool of bacteria. These are considered at different scales using examples from in vitro evolutionary studies of *Escherichia* coli and associated bacteriophage, detailed molecular analyses of the broad host-range IncP-1 plasmids, population surveys of pseudomonad plasmids and genomic comparisons of members of the Rhizobiaceae. All biological systems show genetic redundancy (the existence of allelic variation) at some population level, i.e. within a cell, a clone, population or community. We consider the level(s) at which redundancy is expressed and how this will affect and has influenced the evolution of mobile genetic elements

72. **Removal of indigenous coliphages and fecal coliforms by a novel sewage treatment system consisting of UASB and DHS units. Uemura, S., Takahashi, K., Takaishi, A., Machdar, I., Ohashi, A., Harada, H. (2002). *Water Science and***

***Technology* 46:303-309.** A novel sewage treatment system, which consists of an upflow anaerobic sludge blanket (UASB) pre-treatment unit and the following downflow hanging sponge (DHS) unit for polishing up the UASB effluent, was developed as a cost-effective and easy-maintenance sewage treatment system for developing countries. A long-term experiment with actual sewage was conducted in order to evaluate its treatment efficiency of organic substances, nutrients, and pathogen indicator microorganisms such as total coliphages, F^+-specific RNA coliphages (RNA coliphages), and fecal coliforms. The main objective of this paper is to investigate the removal efficiency of those indicator microorganisms by the UASB-DHS combined system. The results obtained from the continuous flow experiment indicated a fairly promising removal of the indicator microorganisms, i.e., the $\log_{10}$ reductions of total coliphages, RNA coliphages, and fecal coliforms (based on sewage and DHS effluent) achieved were 2.01 log, 2.02 log, and 2.57 log, respectively. The UASB-DHS combined system was superior to the conventional activated sludge process in the reduction of fecal coliforms, but in the reductions of total and RNA coliphages, the system showed somewhat less removal efficiency. The vertical reducing patterns of the indicator microorganisms along the DHS reactor were also discussed

73. ***Pseudoalteromonas* spp. phages, a significant group of marine bacteriophages in the North Sea. Wichels, A., Gerdts, G., Schuett, C. (2002). *Aquatic Microbial Ecology* 27:233-239.** The occurrence and distribution of specific bacteriophages of marine *Pseudoalteromonas* spp. in the North Sea (North Sea phages) and their genetic relationship to several previously isolated marine phage species from waters of the Helgoland Roads (German Bight, Helgoland phages) were investigated. During 3 cruises from the Elbe estuary to western Norwegian waters, phages were concentrated by ultrafiltration. Detection and isolation of North Sea phages were performed by plaque assay, with 70 host bacteria of the genus *Pseudoalteromonas*. The genetic relationship between North Sea phages from different stations and Helgoland phages, formerly described as Pseudoalteromonas spp. phages, was assessed by DNA-DNA hybridization. DNA probes were prepared using whole phage DNA derived from 13 Helgoland phages. This approach provides the first information on the distribution of specific Pseudoalteromonas spp. phage-host systems (PHS) in the North Sea. The occurrence of *Pseudoalteromonas* spp. phages, which are specific for the tested *Pseudoalteromonas* spp. host bacteria, was restricted to a narrow geographical region of the German Bight between 53 degree 30' and 57 degree 00' N latitude. Most of the previously isolated Helgoland phages were highly host specific (54%), whereas this was true for only some of the 39 North Sea phages (16%). The most common *Pseudoalteromonas* spp. phage species found in the North Sea belong to the virus family Siphoviridae (species H103/1). Several phage strains within this phage species displayed different host sensitivity patterns

74. **Evolution of lambdoid replication modules. Wrobel, B., Wegrzyn, G. (2002). *Virus Genes* 24:163-171.** Comparison of the putative iteron-binding proteins of lambdoid phages allows us to propose that in the case of lambdoid replication modules, the units on which natural selection acts do not coincide with the open reading frames. Rather, the first replication gene is split into two segments, and its 3' part (corresponding to the C-terminal domain of the iteron-binding protein) forms one unit with the second gene. We also propose from the phylogenetic analysis of phage-encoded homologs of *E. coli* DnaB and DnaC, that the recombination with the host sequences is not frequent. Accessory ATP-ases for helicase loading (*E. coli* DnaC homologs) may not be universal replication proteins. Our analysis may suggest that the bacterial helicase loaders might be of phage origin. The comparison of DnaC homologs of enterobacteria and enterobacterial phages supports the experimental data on residues important in interaction with DnaB. We propose that construction of plasmids carrying the replication origins of lambdoid prophages could be useful not only in further research on DNA replication but also on the role of these prophages in shuttling genes for bacterial virulence. The phage replication sequences could be also useful for identification of clinical enterobacterial isolates

75. **River water quality improvement by natural and constructed wetland systems in the tropical semi-arid region of northeastern Brazil. de Ceballos, B. S., Oliveira, H., Meira, C. M., Konig, A., Guimaraes, A. O., de Souza, J. T. (2001). *Water Science and Technology* 44:599-605.** The efficiencies of a natural Typha spp wetland (Wn) formed on a river bed and its effluent treatment in a constructed wetland (Wc, subsurface horizontal flow) were investigated in northeastern Brazil (Paraiba State). The Wc system (12 tanks with stone gravel, 4.13 m2, 0.22 m3, 20 Typha spp rhizomes, m^{-2} each, with 38, 29, and 19 mm x d^{-1} hydraulic loadings, and 5, 7, and 10 days HRT) was fed daily with effluent from a Wn. Wn removal presented the highest values after Typha spp were cut during the 5th week. Removal values were (1st and 2nd periods or before and after cutting): 75% and 81% BOD5; 10-53% total phosphorus; 13%-55% ammonia; 89%-91% FC; 90-96% coliphages and bacteriophages. Wc removals increased with time with best results on 10 d HRT. Removals were also higher in the 2nd period: 74%-78% BOD5; 58%-82% ammonia; 90% FC; 94-98% FS; and 92%-96% coliphages and bacteriophages. Despite the high remaining values of FC (1.4×10^4 CFU/100 ml) and FX (4×10^3 CFU/100 ml), the removals were satisfactory and HRT dependent, suggesting a gradual optimization of the system with time. The Wc exhibited good efficiency for improving water quality from polluted river

76. **Filamentous Phage Associated with Recent Pandemic Strains of *Vibrio parahaemolyticus*. Ida, T., Hattori, A., Tagomori, K., Nasu, H., Naim, R., Honda, T. (2001). *Emerging Infectious Diseases* 7:477-478.** A group of pandemic strains of *Vibrio parahaemolyticus* has recently appeared in Asia and North America. We demonstrate that a filamentous phage is specifically associated with the pandemic *V. parahaemolyticus* strains. An open reading frame unique to the phage is a useful genetic marker to identify these strains.

77. **Phage therapy in terms of bacteriophage genetics: Hopes, prospects, safety, limitations. Krylov, V. N. (2001). *Russian Journal of Genetics* 37:715-730.** The appearance and spreading of multidrug-resistant bacterial pathogens is a consequence of the large-scale use of antibiotics in medicine. In view of this, claims for the phage therapy were renewed: in recent studies, the natural phages and their products neutralizing various proteins, as well as the bacterial products often controlled by defective prophages (bacteriocins) were applied for treatment of bacterial infections. Constructs obtained by gene engineering are increasingly used to change bacteriophage properties to expand the spectrum of their lytic activity and to eliminate therapeutic drawbacks of some natural phages. In this review, the problem of phage therapy is discussed in general with respect to bacteriophage properties, their genetics, structure, evolution, taking into account long-term experience of the author in the field of bacteriophage genetics. Note that the general concept of phage therapy should be developed to ensure long-term, efficient and harm-less phage therapy.

78. **The effect of bacteriophage treatment to reduce the rapid dissemination of *Salmonella typhimurium* in pigs. Lee, N., Harris, D. L. (2001). *Proceedings of the American Association of Swine Veterinarians* 32:555-557.** Bacteriophage treatment significantly reduced the rapid dissemination of *Salmonella typhimurium* in tonsil and cecum, where the highest number of *Salmonella* was recovered in pigs experimentally infected with *S. typhimurium*. The rapid dissemination of *Salmonella* in market weight pigs prior to slaughter may pose a potential risk in contaminating pork products. Phage treatment

should be considered as an intervention strategy to reduce the number of *Salmonella* in pigs.

79. **Turning the phage on produce pathogens. McBride, J. (2001). *Agricultural Research* 2001:12.** Even bacteria have their nemesis. Tiny viruses, called phages, infect and kill bacteria naturally, including the foodborne pathogens that sometimes make humans so sick, they wish they were dead. ¶ So why not put these phages to work on fresh-cut fruit, thought ARS plant pathologists Britta Leverentz and William S. Conway at the Produce Quality and Safety Laboratory in Beltsville, Maryland. ¶ Since phages home in on a bacterium's surface proteins, they are very selective about their hosts. Phages specific for *Salmonella,* for instance, would leave beneficial bacteria free to multiply on fresh-cut produce and crowd out potential pathogens, Leverentz explains. ¶ What's more, these tiny viruses are natural, safe, and ubiquitous. A small dropperful of fresh water from a stream or lake, for example, contains an average 250 million phages. Before antibiotics, phages were used to treat human infections in the United States and are still used therapeutically in other parts of the world. ¶ Phages are already under study to control pathogens in poultry, meat, and eggs. Leverentz and Conway are the first to investigate their potential to reduce pathogens on fruits and vegetables— both whole and fresh-cut. They are working under a cooperative research and development agreement with Intralytix in Baltimore, Maryland, which is providing known phages for *Salmonella* Enteritidis. A patent application has been filed on the use of phages with produce.

80. **Validity of Escherichia coli, enterovirus, and F-specific RNA bacteriophages as indicators of viral shellfish contamination. Miossec, L., Le Guyader, F., Pelletier, D., Haugarreau, L., Caprais, M. P., Pommepuy, M. (2001). *Journal of Shellfish Research* 20:1223-1227.** The sanitary classification of harvesting areas for bivalve mollusks in France is based on the level of *Escherichia coli* contamination detected in shellfish meat, as defined in EC Directive 91/492 EEC. However, outbreaks of gastroenteritis or hepatitis after consumption of shellfish meeting current bacteriological standards suggest that *E. coli* is a poor indicator of viral contamination. The purpose of this study was to assess the adequacy of enterovirus and F-specific RNA bacteriophages as new indicators of human enteric viruses. Shellfish were sampled over a 37-mo period to characterize microbial contamination in two coastal areas subjected to different sewage contamination inputs. Contamination by E. coli, F-specific RNA bacteriophages (F+ RNA) and human enteric viruses (enterovirus, EV; hepatitis A virus, HAV; Norwalk-like virus, NLV; astrovirus, AV; and rotavirus, RV) was measured in the same samples. *E. coli* analysis was performed by conductance measurement, enteric viruses were detected by reverse-transcription polymerase chain reaction (RT-PCR) and hybridization, and F+ RNA was evaluated by culture according to the ISO 10705-1 method. Statistical analysis based on bootstrap methods was performed on 95 series of paired observations. The validity of *E. coli*, enterovirus, and F-specific RNA bacteriophages as viral indicators was evaluated by measuring their sensitivity and specificity in the presence of enteric viruses. None of the tested indicators proved adequate to protect the public from viral shellfish contamination. The sensitivity of all indicators was better in the highly contaminated zone, and enteroviruses showed the highest specificity for both sites

81. **The UL6 gene product forms the portal for entry of DNA into the herpes simplex virus capsid. Newcomb, W. W., Juhas, R. M., Thomsen, D. R., Homa, F. L., Burch, A. D., Weller, S. K., Brown, J. C. (2001). *Journal of Virology* 75:10923-10932.** During replication of herpes simplex virus type 1 (HSV-1), viral DNA is synthesized in the infected cell nucleus, where DNA-free capsids are also assembled. Genome-length DNA molecules are then cut out of a larger, multigenome concatemer and packaged into capsids. Here we report the results of experiments carried out to test the idea that the HSV-1 UL6 gene product (pUL6) forms the portal through which viral DNA passes as it enters the capsid. Since DNA must enter at a unique site, immunoelectron microscopy experiments were undertaken to determine the location of pUL6. After specific immunogold staining of HSV-1 B capsids, pUL6 was found, by its attached gold label, at one of the 12 capsid vertices. Label was not observed at multiple vertices, at nonvertex sites, or in capsids lacking pUL6. In immunoblot experiments, the pUL6 copy number in purified B capsids was found to be 14.8 ± 2.6. Biochemical experiments to isolate pUL6 were carried out, beginning with insect cells infected with a recombinant baculovirus expressing the UL6 gene. After purification, pUL6 was found in the form of rings, which were observed in electron micrographs to have outside and inside diameters of 16.4 ± 1.1 and 5.0 ± 0.7 nm, respectively, and a height of 19.5 ± 1.9 nm. The particle weights of individual rings as determined by scanning transmission electron microscopy showed a majority population with a mass corresponding to an oligomeric state of 12. The results are interpreted to support the view that pUL6 forms the DNA entry portal, since it exists at a unique site in the capsid and forms a channel through which DNA can pass. The HSV-1 portal is the first identified in a virus infecting a eukaryote. In its dimensions and oligomeric state, the pUL6 portal resembles the connector or portal complexes employed for DNA encapsidation in double-stranded DNA bacteriophages such as 29, T4, and P22. This similarity supports the proposed evolutionary relationship between herpesviruses and double-stranded DNA phages and suggests the basic mechanism of DNA packaging is conserved.

82. **Estabilidad de fagos de *Lactococcus lactis* frente al hipoclorito de sodio y durante el almacenamiento [Stability of *Lactococcus lactis* phages treated with sodium hypochlorite and during storage]. Parada, J. L., De Fabrizio, S. V. (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

83. **Low-molecular-weight plasmid of *Salmonella enterica* serovar *Enteritidis* codes for retron reverse transcriptase and influences phage resistance. Rychlik, I., Sebkova, A., Gregorova, D., Karpiskova, R. (2001). *Journal of Bacteriology* 183:2852-2858.** Retron reverse transcriptases are unusual procaryotic enzymes capable of synthesis of low-molecular-weight DNA by reverse transcription. All of the so-far-described DNA species synthesized by retron reverse transcriptases have been identified as multicopy single-stranded DNA. We have shown that *Salmonella enterica* serovar *Enteritidis* is also capable of synthesis of the low-molecular-weight DNA by retron reverse transcriptase. Surprisingly, *Salmonella* serovar *Enteritidis*-produced low-molecular-weight DNA was shown to be a double-stranded DNA with single-stranded overhangs (sdsDNA). The sdsDNA was 72 nucleotides (nt) long, of which a 38-nt sequence was formed by double-stranded DNA with 19- and 15-nt single-

stranded overhangs, respectively. Three open reading frames (ORFs), encoded by the 4,053-bp plasmid, were essential for the production of sdsDNA. These included an ORF with an unknown function, the retron reverse transcriptase, and an ORF encoding the cold shock protein homologue. This plasmid was also able to confer phage resistance onto the host cell by a mechanism which was independent of sdsDNA synthesis

84. **Molecular properties of *Streptococcus thermophilus* plasmid pER35 encoding a restriction modification system. Solow, B. T., Somkuti, G. A. (2001). *Current Microbiology* 42:122-128.** Bacteriophage attack on lactic fermentation bacteria (LFB) is costly to the dairy industry because it results in product loss. One mechanism used by LFB to protect themselves from bacteriophage attack is restriction of foreign DNA. Three plasmids, pER16, pER35, and pER36, from three different strains of the thermotolerant dairy fermentation bacterium Streptococcus thermophilus were sequenced. One of these plasmids, pER35, isolated from *S. thermophilus* ST135, encoded a type IC restriction-modification (R-M) system very similar to those encoded on plasmids pIL2614 in *Lactococcus lactis* subsp. lactis and pND861 in *Lactococcus lactis* biovar diacetylactis. The high degree of identity between the R-M systems encoded on pER35, pIL2614, and pND861 indicated the potential for horizontal transfer of these genes between different species of lactic fermentation bacteria. Similar to the functional R-M system encoded on pIL2614 that protects the mesophilic *L. lactis* subsp. lactis against phage attack, the R-M system on pER35 most likely functions in the same role in *S. thermophilus* ST135. The plasmid pER16 was found to encode the specificity subunit of the R-M system, but not the R or M subunits. In addition, all three plasmids encoded proteins that are present on other *S. thermophilus* plasmids, including a protein for rolling-circle replication (RepA) and a low-molecular-weight stress protein (Hsp). The presence of a complete R-M system encoded on a plasmid in *S. thermophilus*, a species that often lacks plasmids, is novel and may be beneficial for protecting *S. thermophilus* from bacteriophage attack under dairy fermentation conditions

85. **Male-killing, nematode infections, bacteriophage infection, and virulence of cytoplasmic bacteria in the genus *Wolbachia*. Stevens, L., Giordano, R., Fialho, R. F. (2001). *Annual Review of Ecology and Systematics* 32:519-545.** *Wolbachia* bacteria are cytoplasmic endosymbionts with a wide range of effects on their hosts and are known to infect two major invertebrate groups, arthropods and nematodes. In arthropods *Wolbachia* alter host reproduction, causing unidirectional and bidirectional cytoplasmic incompatibility, parthenogenesis, feminization, and embryonic male killing. *Wolbachia* variation in reproductive effects is indicative of a high degree of evolutionary plasticity. As many as 75% of arthropods may be infected with *Wolbachia*, which in addition to affecting reproduction, can also directly affect host fitness by either increasing or decreasing survival and fecundity. We review the dynamics of embryonic male-killing, including effects on insect mating behavior, as well as the distribution and implication of *Wolbachia* infections in filarial nematodes. Arthropod host-*Wolbachia* phylogenies are not congruent, which is suggestive of horizontal transmission. The opposite has been shown in nematode-*Wolbachia* phylogenies, indicative of long-term association and vertical transmission. Multiple levels of parasitism within arthropods may promote horizontal transmission. Bacteriophage WO has recently been identified and is found in all *Wolbachia*-infected insect hosts so far examined. Extensive horizontal transmission of the phage occurs between different *Wolbachia* strains within a host as well as between *Wolbachia* in different hosts. The phage genome may carry genes important in determining both the effect of Wolbachia on arthropod host reproduction and host fitness and fecundity. The extensive horizontal transmission of the phage may explain the plasticity of Wolbachia's effect on arthropod hosts

86. **Studies on the anti-mitogenic, anti-phage and hypotensive effects of several ribosome inactivating proteins. Wang, H. X., Ng, T. B. (2001). *Comparative biochemistry and physiology. Toxicology & pharmacology* 128:359-366.** An investigation was conducted to compare the anti-mitogenic, anti-phage and hypotensive activities of several ribosome inactivating proteins (RIPs) in order to ascertain whether the RIPs differed in their potencies in the various bioassays. Agrostin, luffin and saporin elicited a dose-dependent suppression of the mitogenic response of murine splenocytes to concanavalin A. The three RIPs were approximately equipotent in this regard, with near maximal inhibition attained at a dose of 83 nM and approximately 50% inhibition at 830 pM. Trichosanthin was slightly more potent than the three aforementioned RIPs. All of these RIPs were capable of inhibiting the replication of phage M13 in the bacterium *Escherichia coli*, the ranking of potencies being luffin>trichosanthin>agrostin when tested at a concentration of 3.5 microM. The RIPs gelonin and saporin did not exert a conspicuous antiviral effect at the same dose. After intravenous administration into normotensive rats via the external jugular vein, the RIPs saporin, trichosanthin, gelonin and momordin evoked a mild hypotensive response while luffin and agrostin were inactive. The hypotensive response, however, lacked dose dependence. The RIPs trichosanthin, momordin and gelonin did not affect the blood pressure response to angiotensin I. Chemical modification of the arginine residues of the RIPs brought about a reduction in their ability to inhibit cell-free translation. It appears that the ranking of potency of RIPs in one bioassay was different from the rankings in other assays

87. **Bacteriophage lambda and plasmid pUR288 transgenic fish models for detecting in vivo mutations. Winn, R. N., Norris, M., Muller, S., Torres, C., Brayer, K. (2001). *Marine Biotechnology* 3:185-195.** We adapted transgenic rodent mutation assays based on fish carrying bacteriophage lambda and plasmid pUR288 vectors to address the needs for improved methods to assess health risks from exposure to environmental mutagens and also to establish new animal models to study in vivo mutagenesis. The approach entails separating the vectors from fish genomic DNA and then shuttling them into specialized strains of *E. coli* bacteria to analyze spontaneous and induced mutations in either lacI and cII or lacZ mutational targets. Fish exhibited low frequencies of spontaneous mutants comparable to the sensitivity of transgenic rodent models. Mutations detected after treating fish with chemical mutagens showed concentration-dependent, tissue-specific, and time-dependent relationships. Spontaneous and induced mutational spectra also were consistent with the specificity of known mutagens, further supporting the utility of transgenic fish for studies of in vivo mutagenesis

88. **Viral transport in a sand and gravel aquifer under field pumping conditions. Woessner, W. W., Ball, P. N., DeBorde, D. C., Troy, T. L. (2001). *Ground Water* 39:886-894.** Ground water supplies contaminated with microbes cause more than 50% of the water-borne disease outbreaks in the United States. Proposed regulations suggest natural disinfection as a possible mechanism to treat microbe-impacted ground water under favorable conditions. However, the usefulness of current models employed to predict viral transport and natural attenuation rates is limited by the absence of field scale calibration data. At a remote floodplain aquifer in western Montana, the bacteriophages MS2, phiX174, and PRD1; attenuated poliovirus type-1 (CHAT strain); and bromide were seeded as a slug 21.5 m from a well pumping at a steady rate of 408 L/min. Over the 47-hour duration of the test, resulting in the exchange of 12 to 13 pore volumes, 77% of the bromide, 55% of the PRD1, 17% of the MS2, 7% of the phiX174, and 0.12% of the poliovirus masses were recovered at the pumping well. Virus transport behavior was controlled by mechanical dispersion, preferential flow, time-dependent nonreversible and reversible attachment, and apparent mass transfer to immobile domains within the sand and gravel dominated aquifer. The percentage of virus recovery appears correlated with reported viral isoelectric point (pI) values. Successful modeling of viral transport in coarse-grained aquifers will require separation of viral specific properties from reported lumped viral-transport system parameters

89. **Increased killing of *Bacillus subtilis* on the hair roots of transgenic T4 lysozyme-producing potatoes. Ahrenholtz, I, Harms, K., De Vries, J., Wackernagel, W. (2000). *Applied and Environmental Microbiology* 66:1862-1865.** Transgenic potato plants expressing the phage T4 lysozyme gene which are resistant to the plant-pathogenic enterobacterium *Erwinia carotovora* subsp. carotovora have been constructed. The agricultural growth of these potatoes might have harmful effects on soil microbiota as a result of T4 lysozyme release into the rhizosphere. To assess the bactericidal effect of roots, we have developed a novel method to associate the cells of *Bacillus subtilis* with hair roots of plants and to quantify the survival of cells directly on the root surface by appropriate staining and fluorescence microscopy. With this technique, we found that the roots of potato plants (Desiree and transgenic control lines) without T4 lysozyme gene display measurable killing activity on root-adsorbed *B. subtilis* cells. Killing was largely independent of the plant age and growth of plants in greenhouse or field plots. Roots from potato lines expressing the T4 lysozyme gene always showed significantly (1.5- to 3.5-fold) higher killing. It is concluded that T4 lysozyme is released from the root epidermis cells and is active in the fluid film on the root surface. We discuss why strong negative effects of T4 lysozyme-producing potatoes on soil bacteria in field trials may not be observed. We propose that the novel method presented here to study interactions of bacteria with roots can be applied not only to bacterial killing but also to interactions leading to growth-sustaining effects of plants on bacteria

90. **Sequence of the genome of *Salmonella* bacteriophage P22. Byl, C. V., Kropinski, A. M. (2000). *Journal of Bacteriology* 182:6472-6481.** The sequence of the nonredundant region of the *Salmonella enterica* serovar *Typhimurium* temperate, serotype- converting bacteriophage P22 has been completed. The genome is 41,724 bp with an overall moles percent GC content of 47.1%. Numerous examples of potential integration host factor and C1-binding sites were identified in the sequence. In addition, five potential rho-independent terminators were discovered. Sixty-five genes were identified and annotated. While many of these had been described previously, we have added several new ones, including the genes involved in serotype conversion and late control. Two of the serotype conversion gene products show considerable sequence relatedness to GtrA and -B from *Shigella* phages SfII, SfV, and SfX. We have cloned the serotype-converting cassette (*gtrABC*) and demonstrated that it results in *Salmonella* serovar Typhimurium LT2 cells which express antigen O1. Many of the putative proteins show sequence relatedness to proteins from a great variety of other phages, supporting the hypothesis that this phage has evolved through the recombinational exchange of genetic information with other viruses.

91. **A filamentous phage associated with recent pandemic *Vibrio parahaemolyticus* O3:K6 strains. Nasu, H., Iida, T., Sugahara, T., Yamaichi, Y., Park, K.-S., Yokoyama, K., Makino, K., Shinagawa, H., Honda, T. (2000). *Journal of Clinical Microbiology* 38:2156-2161.** A specific serotype, O3:K6, of *Vibrio parahaemolyticus* has recently been causing epidemics of gastroenteritis in Southeast Asia, Japan, and North America. To examine whether the new O3:K6 strains possess characteristics that may exacerbate outbreaks, we compared *V. parahaemolyticus* O3:K6 strains with non-O3:K6 strains using strains isolated from individuals with traveler's diarrhea at Kansai Airport Quarantine Station, Osaka, Japan. All 24 O3:K6 strains possessed a common plasmid, pO3K6 (DNA size, 8,782 bp, with 10 open reading frames [ORFs]). The gene organization of pO3K6 was similar to that of Vf33, a filamentous phage previously described in *V. parahaemolyticus*. We isolated a phage (phage f237) from the culture supernatant of *V. parahaemolyticus* O3:K6 strain KXV237, which formed a turbid plaque on an indicator strain. The genome of f237 was single-stranded DNA, and the double-stranded DNA obtained by treatment of the genome with DNA polymerase was identical to that of pO3K6 when analyzed by agarose gel electrophoresis after *Hin*dIII digestion. Furthermore, the N-terminal amino acid sequence of the f237 major coat protein was found in ORF4 of pO3K6. Our results showed that pO3K6 is a replicative form of f237. Among the ORFs found in the f237 genome, the sequence of ORF8 had no significant homology to those of any proteins in databases. ORF8 was located on a region corresponding to the distinctive region of Vf33, and its G+C content was apparently lower than that of the remaining DNA sequence of f237. By colony hybridization, ORF8 was detected only in O3:K6 strains isolated since 1996 and was not found in O3:K6 strains isolated before 1996 and clinical *V. parahaemolyticus* strains other than those of serotype O3:K6. Thus, this study shows that f237 is exclusively associated with recent *V. parahaemolyticus* O3:K6 strains. The ORF8 gene can be a useful genetic marker for the identification of the recently widespread O3:K6 strains of *V. parahaemolyticus*.

92. **The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Parkhill, J., Wren, B. W., Mungall, K., Ketley, J. M., Churcher, C., Basham, D., Chillingworth, T., Davies, R. M., Feltwell, T., Holroyd, S., Jagels, K., Karlyshev, A. V., Moule, S., Pallen, M. J., Penn, C. W., Quail, M. A., Rajandream, M. A., Rutherford, K. M., van Vliet, A. H., Whitehead, S., Barrell, B. G. (2000). *Nature* 403:665-668.** *Campylobacter jejuni*, from the delta-epsilon group of proteobacteria, is a microaerophilic, Gram-negative, flagellate, spiral bacterium-properties it shares with the related gastric pathogen *Helicobacter pylori*. It is the leading cause of bacterial food-borne diarrhoeal disease throughout the world. In addition, infection with *C. jejuni* is the most frequent antecedent to a form of neuromuscular paralysis known as Guillain-Barre syndrome. Here we report the genome sequence of *C. jejuni* NCTC11168. *C. jejuni* has a circular chromosome of 1,641,481 base pairs (30.6% G+C) which is predicted to encode 1,654 proteins and 54 stable RNA species. The genome is unusual in that there are virtually no insertion sequences or phage-associated sequences and very few repeat sequences. One of the most striking findings in the genome was the presence of hypervariable sequences. These short homopolymeric runs of nucleotides were commonly found in genes encoding the biosynthesis or modification of surface structures, or in closely linked genes of unknown function. The apparently high rate of variation of these homopolymeric tracts may be important in the survival strategy of *C. jejuni*

93. **Description of a DNA amplification procedure for the detection of bacteriophages of *Bacteroides fragilis* HSP40 in environmental samples. Puig, M., Pina, S., Lucena, F., Jofre, J., Girones, R. (2000). *Journal of Virological Methods* 89:159-166.** A molecular test based on DNA amplification by PCR was developed for the detection of bacteriophages of *Bacteroides fragilis* strain HSP40 in the environment. These specific phages are associated with faecal contamination of human origin. A homologous DNA region of 1.5 kb, identified previously by hybridisation, was used to design primers for the detection of B. fragilis HSP40 phages. A nested-PCR procedure for the DNA amplification of those phages was developed. The sensitivity of the nested-PCR was between 10^{-1} and 10^{-2} PFU for purified HSP40 phage solutions, sewage and seawater samples, and between 1 and 10 PFU for river water samples. Specific amplification of HSP40 phages was observed when viral suspensions of 10^3 PFU/ml or lower were used. Common levels of *B. fragilis* phages found in sewage are 10^1-10^2 PFU /ml. A total of 24 water samples (sewage, river water and seawater) were tested both by PCR and by plaque assay, to evaluate the efficiency of the molecular method in field samples. The data obtained by PCR in environmental samples showed good concordance with the PFU counts and a higher sensitivity

94. **Transport and attenuation of bacteria and bacteriophages in an alluvial gravel aquifer. Sinton, L. W., Noonan, M. J., Finlay, R. K., Pang, L., Close, M. E. (2000). *New Zealand Journal of Marine and Freshwater Research* 34:175-186.** The

relative attenuation of rhodamine WT dye, two strains of *Escherichia coli*, *Bacillus subtilis* endospores, and the F-RNA bacteriophage MS2 in an alluvial gravel aquifer was investigated in two tracing experiments at Burnham, near Christchurch, New Zealand. A simulated concentration curve was fitted to the observed breakthrough curves using the contaminant transport model AT123D, by optimising hydraulic conductivity (K), longitudinal dispersivity (alpha sub(x)), and a removal constant (lambda) (which includes die-off and physical removal processes) with the Parameter Estimation (PEST) optimisation routine. When comparing the parameters, the hydraulic conductivity was converted to velocity (V). The V ranking was *E. coli* 2690 > *B. subtilis* endospores > rhodamine WT in Experiment 1 and E. coli J6-2 > phage MS2 > rhodamine WT in Experiment 2. These rankings are consistent with the concept of pore size exclusion, whereby larger particles are preferentially transported in the larger interconnected pores where water velocities are higher. The longitudinal dispersivity (alpha sub(x)) rankings were consistent with pore size exclusion in Experiment 1, and broadly consistent in Experiment 2. Of the two parameters, V is considered to provide the more reliable result, because it is easier to determine peak position in time than peak height. Little useful information could be derived from the lambda values in our study, because of high levels of uncertainty associated with determining peak heights, particularly in Experiment 1. Overall, the curve fits were better in Experiment 2, because of a greater number of bores and observations. Although this complicated between-experiment comparisons, an overall retardation (R) ranking of rhodamine WT > phage MS2; *B. subtilis* endospores > *E. coli* J6-2 > E. coli 2690 is broadly consistent with pore size exclusion. Overall, our study showed that the application of the AT123D model to the observed velocities of the tracer curves demonstrated an effect consistent with pore size exclusion

95. **Phages will out: strategies of host cell lysis. Young, R., Wang, I.-N., Roof, W. D. (2000). *Trends in Microbiology* 8:120-128.** Most phages accomplish host lysis using a muralytic enzyme, or endolysin, and a holin, which permeabilizes the membrane at a programmed time and thus controls the length of the vegetative cycle. By contrast, lytic single-stranded RNA and DNA phages accomplish lysis by producing a single lysis protein without muralytic activity

96. **Stable expression of the *Lactobacillus casei* bacteriophage A2 repressor blocks phage propagation during milk fermentation. Alvarez, M. A., Rodriguez, A., Suarez, J. E. (1999). *Journal of Applied Microbiology* 86:812-816.** A general strategy was applied to implement resistance against temperate bacteriophages that infect food fermentation starters through cloning and expression of the phage repressor. Lactobacillus casei ATCC 393 and phage A2 were used to demonstrate its feasibility as milk fermentation is drastically inhibited when the strain is infected by this phage. The engineered strain *Lact. casei* EM40::cI, which has the A2 repressor gene (cI) integrated into the genome, was completely resistant and able to ferment milk whether phage was present or not. In addition, viable phages were eliminated from the milk, probably through adsorption to the cell wall. Finally, the integration of cI in the genome resulted in a stable resistance phenotype, being unnecessary selective pressure during milk fermentation

97. **Reduction of microorganisms in sewage effluent using hypochlorite and peracetic acid as disinfectants. Bonadonna, L., Della, Libera S., Veschetti, E., Cutilli, D., Ottaviani, M., Divizia, M., Donia, D., Gabrieli, R., Pana, A., Martini, C., Anastasi, P. (1999). *Central European Journal of Public Health* 7:130-132.** A comparative study on peracetic acid and sodium hypochlorite in inactivating bacteria and viruses was carried out. Therefore the disinfection actions of peracetic acid, in comparison with sodium hypochlorite, was evaluated against the usual indicators of faecal contamination, the pathogen *Salmonella*, *Pseudomonas* spp., bacteriophages anti-*Escherichia coli*, F+/phage and the phage of *Bactericides fragilis* B40-8 and enteroviruses. Under the experimental conditions, no representative results were obtained for enteroviruses and phages because of their low concentration in the sewage effluent. On the other hand, the indicator organisms were reduced substantially by the sodium hypochlorite and peracetic acid concentrations, while more variable results were obtained against *Pseudomonas* and bacteriophages anti-*Escherichia coli*

98. **Fate of *Cryptosporidium* oocysts, *Giardia* cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion. Chauret, C., Springthorpe, S., Sattar, S. (1999). *Canadian Journal of Microbiology* 45:257-262.** The extent of reduction in selected microorganisms was tested during both aerobic wastewater treatment and anaerobic digestion of sludge at the wastewater treatment plant in Ottawa to compare the removal of two encysted pathogenic protozoa with that of microbial indicators. Samples collected included the raw wastewater, the primary effluent, the treated wastewater, the mixed sludge, the decanted liquor, and the cake. All of the raw sewage samples were positive for *Cryptosporidium* oocysts and *Giardia* cysts, as well as for the other microorganisms tested. During aerobic wastewater treatment (excluding the anaerobic sludge digestion), *Cryptosporidium* and *Giardia* were reduced by 2.96 $\log_{10}$ and 1.40 $\log_{10}$, respectively. *Clostridium perfringens* spores, *Clostridium perfringens* total counts, somatic coliphages, and heterotrophic bacteria were reduced by approximately 0.89 log10, 0.96 log10, 1.58 $\log_{10}$, and 2.02 $\log_{10}$, respectively. All of the other microorganisms were reduced by at least 3.53 $\log_{10}$. Sludge samples from the plant were found to contain variable densities of microorganisms. Variability in microbial concentrations was sometimes great between samples, stressing the importance of collecting a large number of samples over a long period of time. In all cases, the bacterial concentrations in the cake (dewatered biosolids) samples were high even if reductions in numbers were observed with some bacteria. During anaerobic sludge digestion, no statistically significant reduction was observed for *Clostridium perfringens*, *Enterococcus* sp., *Cryptosporidium oocysts*, and *Giardia* cysts. A 1-2 $\log_{10}$ reduction was observed with fecal coliforms and heterotrophic bacteria. However, the method utilized to detect the protozoan parasites does not differentiate between viable and nonviable organisms. On the other hand, total coliforms and somatic coliphages were reduced by 0.35 $\log_{10}$ and 0.09 $\log_{10}$, respectively. These results demonstrate the relative persistence of the protozoa in sewage sludge during wastewater treatment

99. **Little evidence for synergism among deleterious mutations in a nonsegmented RNA virus. Elena, S. F. (1999). *Journal of Molecular Evolution* 49:703-707.** Several models have been proposed to account for the segmentation of RNA viruses. One of the best known models suggests that segmentation, and mixing of segments during coinfections, is a way to eliminate deleterious mutations from the genome. However, for validity, this model requires that deleterious mutations interact in a synergistic way. That is, two mutations together should have a more deleterious effect than the result of adding their individual effects. Here I present evidence that deleterious mutations in foot-and-mouth disease virus produce a decline in fitness but that the relationship between the number of mutations fixed and the magnitude of fitness decline is compatible mainly with a nonsynergistic model. However, the statistical uncertainties associated with the data still give some room for the existence of very weak synergistic epistasis.

100. **Symbiotic properties of transformed *Rhizobium phaseoli* and its susceptibility to phages of the donour and recipient strains. Hammad, A. M. M. (1999). *Egyptian Journal of Microbiology* 34:331-346.** THE PRESENCE of extrachromosomal DNA (viz. plasmid DNA) in *Rhizobium leguminosarum* F21 and R. phaseoli L1 was investigated. On the basis of antibiotic

sensitivity tests both rhizobium strains were found to contain plasmids and carrying streptomycin resistance genes. Plasmid DNA was isolated from both strains and analysed by agarose gel electrophoresis. The plasmid DNA of all strains was detected as one band on the gel, indicating that *R. leguminosarum* F21 and R. phaseoli L1 contains just one plasmid of 21.200 kbp. Transferring of *R. leguminosarum*-plasmid into *R. phaseoli* resulted in transfer of faba bean infectivity to *R. phaseoli*. Moreover, the transformed strain exhibited high efficiency in nodulating roots of both faba bean and beans as well as in fixing nitrogen. Six Bacteriophage isolates specific for either *R. leguminosarum* F21 (donor) or *R. phaseoli*. L1 (recipient) were isolated from soil samples collected from the Experimental farm of Fac. Agric. Minia Univ. The transformed strains were found to be susceptible to the six phages of the recipient strain and to four phages among six of the donor strain. The total proteins of the donor, recipient strains and the transformant were compared by electrophoretical analysis. Although most of the polyeptides were found to be similar in the three strains and migrated with identical mobilities, few differences were detected

101. **Growth characteristics of *Heterosigma akashiwo* virus and its possible use as a microbiological agent for red tide control. Nagasaki, K., Tarutani, K., Yamaguchi, M. (1999). *Applied and Environmental Microbiology* 65:898-902.** The growth characteristics of *Heterosigma akashiwo* virus clone 01 (HaV01) were examined by performing a one-step growth experiment. The virus had a latent period of 30 to 33 h and a burst size of 7.7×10^2 lysis-causing units in an infected cell. Transmission electron microscopy showed that the virus particles formed on the peripheries of viroplasms, as observed in a natural *H. akashiwo* cell. Inoculation of HaV01 into a mixed algal culture containing four phytoplankton species, *H. akashiwo* H93616, *Chattonella antiqua* (a member of the family Raphidophyceae), *Heterocapsa triquetra* (a member of the family Dinophyceae), and *Ditylum brightwellii* (a member of the family Bacillariophyceae), resulted in selective growth inhibition of *H. akashiwo*. Inoculation of HaV01 and *H. akashiwo* H93616 into a natural seawater sample produced similar results. However, a natural *H. akashiwo* red tide sample did not exhibit any conspicuous sensitivity to HaV01, presumably because of the great diversity of the host species with respect to virus infection. The growth characteristics of the lytic virus infecting the noxious harmful algal bloom-causing alga were considered, and the possibility of using this virus as a microbiological agent against *H. akashiwo* red tides is discussed.

102. **Isolation of chloroform-resistant mutants of filamentous phage: localization in models of phage structure. Oh, J. S., Davies, D. R., Lawson, J. D., Arnold, G. E., Dunker, A. K. (1999). *Journal of Molecular Biology* 287:449-457.** Interaction of fd or M13 filamentous phage with a chloroform/water interface induces morphological change, contracting the filaments sequentially into shortened rods (I-forms), and then into spheroidal particles (S-forms). To further investigate this phage contraction, 34 and 26 chloroform-resistant isolates of fd and M13, respectively, were selected after chloroform treatment of wild-type phages at pH 8. 2 and 4 degrees C. DNA sequencing of gene VIII of the 34 fd isolates revealed five different mutants: these were D5H, M28L, V31L, I37T, and S50T. All 26 M13 isolates were I37T. These mutants exhibited variable sensitivity to chloroform, but all contracted much more slowly than wild-type phage during treatment at 4 degrees C. They all contracted like wild-type phage at 37 degrees C. Site-directed mutagenesis showed that the indicated single mutations carried the chloroform resistance. In structural models of the phage, the D5H locus is on the outside and the S50T locus is on the inside. The M28L and I37T loci are buried in a mostly hydrophobic region in the middle. Although these four mutants are spread out radially, they are localized in the axial direction into a thin disk in the model. The last mutant locus, V31L, is out of this disk, but this locus is proximal to the M28L and I37T loci and also in contact with the surface via a deep hydrophobic hole or depression. These five mutants, their locations, and their variable affects on contraction suggest that chloroform-induced contraction involves a specific mechanism rather than a generalized solvent-induced denaturation and that the critical structural changes occur in a localized level in the phage. These results add weight to suggestions that the sequential contraction of filaments-->I-forms-->S-forms mimic corresponding steps in phage penetration, and, in the reverse order, for phage assembly.

103. **Vascular targeting with phage peptide libraries. Pasqualini, R. (1999). *The Quarterly Journal of Nuclear Medicine* 43:159-162.** We have developed an in vivo selection system in which phage capable of selective homing to different tissues are recovered from a phage display peptide library following intravenous administration. Using this strategy, we have isolated several organ and tumor-homing peptides. We have shown that each of those peptides binds to different receptors that are selectively expressed on the vasculature of the target tissue. The tumor-homing peptides bind to receptors that are upregulated in tumor angiogenic vasculature. Targeted delivery of doxorubicin to angiogenic vasculature using these peptides in animal models decreased toxicity and increased the therapeutic efficacy of the drug. Vascular targeting may facilitate the development of other treatment strategies that rely on inhibition of angiogenesis and lead to advances in cancer treatment. Our technology is also likely to extend the potential for targeting of drugs, genes, and radionuclides in the context of many diseases

104. **Genomic structure of phage B40-8 of *Bacteroides fragilis*. Puig, M., Girones, R. (1999). *Microbiology (Reading* 1999 Jul, 145:1661-1670.** Very few data are available on the molecular biology of *Bacteroides fragilis* bacteriophages, which have been considered in several studies as indicators of faecal contamination. Phage B40-8, initially isolated from an urban sewage sample using a strain of *B. fragilis* (HSP40) isolated from a clinical specimen, was chosen in this study as a prototype for morphological and molecular studies. Like most of the phages infective for *B. fragilis*, B40-8 belongs to the Siphoviridae family. Its genome has been found to be a double-stranded DNA molecule, of approximately 51.7 kb, containing a rather low percentage (38.9 mol%) of G + C. The ends of the molecule appeared not to be cohesive but permuted, with a terminal redundancy of 7.3%. A genomic map was constructed. Three major proteins (MP) out of 15 peptides in the SDS-PAGE profile were selected for N-terminal sequencing. From these data, degenerate probes were designed to locate the ORFs in the genomic map. Immunodetection by electron microscopy revealed that MP1 and MP3 were structural proteins of the phage head and that MP2 was a constituent of the tail. A genomic library of the phage was prepared, and a clone including the MP2 ORF was identified and sequenced

105. **Bacteriophage of *Erwinia amylovora* and their potential for biocontrol. Schnabel, E. L., Fernando, W. G. D., Meyer, M. P., Jones, A. I. (1999). pp. 649-653 in Mornol, M. T., Saygili, H. (eds.) *Proceedings of the 8th International Workshop on Fire Blight*. ISHS, Leuven, Belgium.**

106. **Characterization of *Pseudomonas aeruginosa* bacteriophage UNL-1, a bacterial virus with a novel UV-A-inducible DNA damage reactivation phenotype. Shaffer, J. J., Jacobsen, L. M., Schrader, J. O., Lee, K. W., Martin, E. L., Kokjohn, T. A. (1999). *Applied and Environmental Microbiology* 65:2606-2613.** UNL-1, a lytic virus of *Pseudomonas aeruginosa*, was observed to express a novel inducible DNA damage reactivation activity in UV-A-irradiated P. aeruginosa host cells. The expression of bacteriophage reactivation was quantified in hosts exposed to either UV-C or UV-A radiation. While reactivation of UV-C-damaged UNL-1 was not inducible in UV-C-irradiated host cells, an approximately 13-fold induction was observed in UV-A-irradiated host cells. When host cells were exposed to sunlight, reactivation of damaged UNL-1 virus increased eightfold. The UV-A induction of UNL-1 DNA damage reactivation was supported in hosts lacking recA gene function. This report is the first

description of a recA-independent, UV-inducible virus DNA damage repair system. Our findings suggest that a combination of both host and virus DNA repair processes contribute to the persistence and sustained replication of some bacterial viruses in aquatic environments

107. **Algal viruses (*Phycodnaviridae*). van Etten, J. (1999). pp. 44-50 in Webster, R. G., Granoff, A. (eds.) *Encyclopedia of Virology*. Academic Press, London.**

108. **Studies on the pustule disease of abalone (*Haliotis discus hannai Ino*) on the Dalian coast. Li, Taiwu, Ding, Mingjin, Zhang, Jian, Xiang, Jianhai, Liu, Ruiyu (1998). *Journal of Shellfish Research* 17:707-711.** Since first observed in the summer of 1993, a serious pustule disease has spread among several abalone hatcheries in the Dalian area. It affects different growth stages of the abalone. Mortality has been as high as 50-60%. Three strains of bacteria (D, T, and N) were obtained, isolated, and purified from the Aquacultural Company of Dalian (D), the Aquacultural Company of Precious Sea Food of the Pacific Ocean (T), and the Dalian New Harbor Aquacultural Company (N). Observation by electron microscopy showed that the three strains were all short rod bacteria with a single polar flagellum. They all grew in 1% tryptone water in the ranges, 15-42 degree C; salinity, 0-70ppt; and pH, 5.5-11. Physiological and biochemical analyses gave the same result as in an earlier study by the authors. The bacteriophage of the D strain, which was isolated by the authors (reported elsewhere) could propagate and replicate on the N and T strains. Thus, the three strains are concluded to belong to the same species of bacteria, one earlier identified as *Vibrio fluvialis*-II. Results of experimental trials indicated that the pustule disease is transmitted through lesions in the foot. Infection quickly followed intramuscular injection. Infection did not result when bacteria were included in the food or when present in the surrounding seawater

109. **Efficacy and mechanisms of action of sodium hypochlorite on *Pseudomonas aeruginosa* PAO1 phage F116. Maillard, J. Y., Hann, A. C., Baubet, V., Perrin, R. (1998). *Journal of Applied Microbiology* 85:925-932.** The *Pseudomonas aeruginosa* PAO1 phage F116 was used to investigate the viricidal activity and the mechanism of action of sodium hypochlorite. The bacteriophage was inactivated with a low concentration (0.0005% available chlorine) of the biocide prepared in tap water but it was less sensitive to a sodium hypochlorite solution prepared in ultra-pure water (0.0075% available chlorine). For all the effective concentrations of sodium hypochlorite (i.e. producing at least 4 log reduction in phage titre), F116 was readily inactivated within 30 s. Electron microscopical investigations of the phage particles challenged with sodium hypochlorite showed a wide variety of deleterious effects, some of which have not been previously observed with other biocides. The wide range of structural alterations observed suggested that sodium hypochlorite has multiple target sites against F116 bacteriophage. A 30 s exposure to sodium hypochlorite (0.001% available chlorine) produced severe damage, the number and severity of which increased with a higher concentration (0.0075% available chlorine) and with a longer contact time. These observations suggested that sodium hypochlorite inactivated F116 bacteriophage by causing structural alterations to the phage head, tail and overall structure, hence possibly releasing the viral genome from damaged capsids in the surrounding media

110. **Generation of reactive oxygen species from the photolysis of histidine by near-ultraviolet light: effects on T7 as a model biological system. Paretzoglou, A., Stockenhuber, C., Kirk, S. H., Ahmad, S. I. (1998). *Journal of photochemistry and photobiology. B* 1998 May 15, 43:101-105.** Near-ultraviolet (NUV) light (280-400 nm) has a variety of effects on biological systems; these effects are increased, often synergistically, in the presence of sensitizers. A variety of both man-made and naturally occurring sensitizers have been identified, but their precise roles and relative contributions to cellular damage are not yet fully established. DNA seems to be a major target and a variety of types of damage have been observed. In this report we present evidence that histidine can also act as a sensitizer of NUV. Upon NUV photolysis a variety of reactive oxygen species, including superoxide anions, hydroxyl radicals and hydrogen peroxide, are produced as determined by the effects of various scavengers. pH influences the reaction, alkaline media being most effective, as has previously been reported for the photolysis of H2O2, tyrosine, phenylalanine and tryptophan. Exposure of phage T7 to a combination of histidine and NUV leads to synergistic inactivation and scavengers of $O^{2.-}$, .OH and H_2O_2 reduce this effect. These results point to a possible involvement of sunlight-induced histidine photolysis in cellular damage. The fact that photolysis is maximal at high pH indicates that biological effects are likely to be highly localized, e.g., at enzyme active sites

111. **Higher-order predators and the regulation of insect herbivore populations. Rosenheim, J. A. (1998). *Annual Review of Entomology* 43:421-447.** Empirical research has not supported the prediction that populations of terrestrial herbivorous arthropods are regulated solely by their natural enemies. Instead, both natural enemies (top-down effects) and resources (bottom-up effects) may play important regulatory roles. This review evaluates the hypothesis that higher-order predators may constrain the top-down control of herbivore populations. Natural enemies of herbivorous arthropods generally are not top predators within terrestrial food webs. Insect pathogens and entomopathogenic nematodes inhabiting the soil may be attacked by diverse micro- and mesofauna. Predatory and parasitic insects are attacked by their own suite of predators, parasitoids, and pathogens. The view of natural enemy ecology that has emerged from laboratory studies, where natural enemies are often isolated from all elements of the biotic community except for their hosts or prey, may be an unreliable guide to field dynamics. Experimental work suggests that interactions of biological control agents with their own natural enemies can disrupt the effective control of herbivore populations. Disruption has been observed experimentally in interactions of bacteria with bacteriophages, nematodes with nematophagous fungi, parasitoids with predators, parasitoids with hyperparasitoids, and predators with other predators. Higher-order predators have been little studied; manipulative field experiments will be especially valuable in furthering our understanding of their roles in arthropod communities

112. **Somatostatin displayed on filamentous phage as a receptor-specific agonist. Rousch, M., Lutgerink, J. T., Coote, J., de Bruine, A., Arends, J. W., Hoogenboom, H. R. (1998). *British Journal of Pharmacology* 125:5-16.** 1. In search of methods to identify bio-active ligands specific for G protein-coupled receptors with seven transmembrane spanning regions, we have developed a filamentous phage-based selection and functional screening method. 2. First, methods for panning peptide phage on cells were established, using the hormone somatostatin as a model. Somatostatin was displayed on the surface of filamentous phage by cloning into phage(mid) vectors and fusion to either pIII or pVIII viral coat proteins. Peptide displaying phage bound to a polyclonal anti-somatostatin serum, and, more importantly, to several somatostatin receptor subtypes (Sst) expressed on transfected CHO-K1 cells, in a pattern which was dependent on the used display method. Binding was competed with somatostatin, with an IC50 in the nanomolar range. The phage were specifically enriched by panning on cells, establishing conditions for cell selections of phage libraries. 3. Binding of somatostatin displaying phage to sst2 on a reporter cell line, in which binding of natural ligand reduces secretion of alkaline phosphatase (via a cyclic AMP responsive element sensitive promoter), proved that the phage particles act as receptor-specific agonists. Less than 100 phage particles per cell were required for this activity, which is approximately 1000 fold less than soluble somatostatin, suggesting that phage binding interferes with normal receptor desensitization and/or recycling. 4. The combination of biopanning of phage libraries on cells with

functional screening of phage particles for receptor triggering activity, may be used to select novel, bio-active ligands from phage libraries of random peptides, antibody fragments, or libraries based on the natural receptor ligand

113. **On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. Schubbert, R., Hohlweg, U., Renz, D., Doerfler, W. (1998). *Molecular and General Genetics* 259:569-576.** We have previously shown that, when administered orally to mice, bacteriophage M13 DNA, as a paradigm foreign DNA without homology to the mouse genome, can persist in fragmented form in the gastrointestinal tract, penetrate the intestinal wall, and reach the nuclei of leukocytes, spleen and liver cells. Similar results were obtained when a plasmid containing the gene for the green fluorescent protein (pEGFP-C1) was fed to mice. In spleen, the foreign DNA was detected in covalent linkage to DNA with a high degree of homology to mouse genes, perhaps pseudogenes, or to authentic E. coli DNA. We have now extended these studies to the offspring of mice that were fed regularly during pregnancy with a daily dose of 50 microg of M13 or pEGFP-C1 DNA. Using the polymerase chain reaction (PCR) or the fluorescent in situ hybridization (FISH) method, foreign DNA, orally ingested by pregnant mice, can be discovered in various organs of fetuses and of newborn animals. The M13 DNA fragments have a length of about 830 bp. In various organs of the mouse fetus, clusters of cells contain foreign DNA as revealed by FISH. The foreign DNA is invariably located in the nuclei. We have never found all cells of the fetus to be transgenic for the foreign DNA. This distribution pattern argues for a transplacental pathway rather than for germline transmission which might be expected only after long-time feeding regimens. In rare cells of three different fetuses, whose mothers have been fed with M 13 DNA during gestation, the foreign DNA was detected by FISH in association with both chromatids. Is maternally ingested foreign DNA a potential mutagen for the developing fetus?

114. **The influence of environmental factors on microbiological indicators of coastal water pollution. Serrano, E., Moreno, B., Solaun, M., Aurrekoetxea, J. J., Ibarluzea, J. (1998). *Water Science and Technology* 38:195-199.** The relationships between bacteriological and viral indicators of sewage pollution (TC, FC, E coli, FS, *Salmonella*, somatic coliphages and F-RNA phages) and environmental variables in coastal water and weather were studied at three beaches in San Sebastian, the Basque Country. The microbiological indicators in bathing water presented high counts associated with the following conditions: early morning, overcast skies, low and high tides, groundswell, intense turbidity and the presence of flotsam (P = <0.05). Coliphage density was significantly related (P = <0.05) to cloud cover, groundswell and flotsam. Correlations between microbiological indicators proved high (0.62 less than or equal to or less than or equal to 0.90, P = <0.001). The percentage of *Salmonella* presented significant (<0.05), albeit low (r < 0.4), correlations with all microbiological parameters. Somatic coliphages also revealed highly significant (0.32 less than or equal to r less than or equal to 0.66) correlations (P = <0.001). Those obtained for F-RNA phages, in contrast, were low (r less than or equal to 0.33). The equations obtained using a multiple regression analysis with a view to predicting microbiological, viral, and *Salmonella* indicator density demonstrated that environmental variables facilitate the construction of highly significant equations, but that these have low predictive capability (R^2 = <0.50)

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