

Bacteriophage Ecology Group (BEG) News

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

© Stephen T. Abedon (editor)

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April 1, 2001 issue (volume 8)

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Editorial

Editorials should be written on subjects relevant to The Bacteriophage Ecology Group as an organization, to *BEG News* (either the concept or a given issue of *BEG News*), or the science of Bacteriophage Ecology. While my assumption is that I will be writing the bulk of these editorials, <u>I wish to encourage as many people as possible to seek to relieve me of this duty</u>, <u>as often as possible</u>. Additionally, I welcome suggestions of topics that may be addressed. Please address all correspondences to <u>abedon.1@osu.edu</u> 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.

The Best of Times, the Worst of Times

Phage biologists are accustomed to being treated as relics, hoary apostles of a classical discipline pedagogically useful but past its prime. It is thus heartening to see the burgeoning interest in bacteriophage at all levels and from so many different perspectives. Of course, there are legions of protein biochemists who suddenly have to titer M13 to monitor the enrichment cycles of their phage display libraries. Even a purely methodological interest makes them stakeholders, however marginally, in phage biology. More impressive is the wave of other scientists who have come to phage as an active research field in the pursuit of seemingly unrelated goals. Suddenly the virulence of otherwise harmless bacteria turns out to be due to pathogenesis islands, which turn out to be prophages. Indeed, phage don't just carry virulence characteristics but in fact, suddenly, it develops that major diseases like cholera and enterohemorrhagic diarrhea are fundamentally phage-borne diseases, that in a sense the bacteria are victims as much as the human hosts. Suddenly, understanding pathogenesis requires understanding the inheritance, organization and expression of phage genes. *Mirabile dictu*, suddenly phage ecology is discovered to have been ignored; we know more about kangaroo rats than about the "where"'s, "when"'s and "how many"'s of phage populations. We don't know where these disease-factor phages are, how they are transmitted, how they change, what makes them tick. Until study sections wake up to this, clearly our ability to analyze, understand, and predict the

emergence of new infectious disease is limited.

It is not just molecular pathogenesis and epidemiologists who are scurrying for their dusty phage texts; now it's the drug companies and clinicians who have the bug as the "new" concept of phage therapy is making news and attracting investors. Ironically, there is little known about what is available to attack various pathogens, and few people have actually done phage hunts. Thus decades-old phage collections assembled in Stalin's Caucasus by contemporaries of Lysenko are now attracting U.S. government research funding.

All of this serves to bring the word phage back into play in public and general scientific discourse, which is good. Its also the best of times since the Golden Age for phage biology proper. Through the dogged efforts of a few people interested in phage *per se* and also, *pari passu*, as a result of the sequencing of so many bacterial genomes that contain multiple prophages, suddenly we have a phage genomics. Suddenly phage evolution turns out to be stunningly inventive and articulated. The momentum in phage biology extends beyond primary structure to tertiary and quaternary structure: suddenly self-assembly of phage virions has been revealed at the atomic level by focusing modern x-ray crystallography on genetically tractable bacteriophage. Suddenly, through crystallography and high-resolution cryo-electron microscopy, we have a crisp picture of the operation of a phage injection system, geared by both RNA and protein components. The best of times.

But it is also the worst of times. Few students and post-doc's are being trained in the classical traditions of phage biology. Classical phage systems are being depopulated through super-annuation; you can count the combined number of P1, ϕ X174, and T5 labs on one hand and have fingers left over. Almost no grant proposals are being written on phage systems. In the early '90s I served on one of the NIH study sections that traditionally supported phage biology. The panel always had several phage people, as well as individuals who had been trained in the phage biology tradition. Now that same study section has about a third as many R01's to consider, the proportion of phage grants is even less, and the number of phage biologists on the panel is now exactly one.

And now comes the pitch. The professional association of phage biologists is under siege. Division M of ASM has been placed on probationary status because our membership has fallen below the minimum 150, out of 19,000 total members. We can no longer vote in the ASM council. To use an analogy very familiar to Texans, it is like being moved to the isolation chamber shortly before execution. As the 2000-1 chair, I am appealing to the phage community to help redress the situation. We need to recruit for Division M. We need past members who have let their dues lapse to renew their memberships. We need our colleagues who are doing phage biology to join ASM and select Division M as their primary division. We need to enroll graduate students as student members, to look to the future of the Division.

Division M has a lot to offer. Being a small division is not all bad; smallness means we can be cohesive and organized. Despite being less than 1% of the membership, we have influence at the top. For example, this year's General Meeting is chaired by Lucia Rothman-Denes of Division M. All this means is that new members get to be part of a small and influential group and can make an impact immediately; just come to our Division meeting at Orlando this year and see!

If you are interested in joining ASM and Division M, please check out the "HomePhage," the Division's website, at http://www.asmusa.org/division/m/M.html and the ASM membership link at http://www.asmusa.org/mbrsrc/mbr1.htm. Or contact me by phone (979-845-2087) or email (ryland@tamu.edu) and I will get you enrolled. Remember, it's the new Age of Phage. Get with the in-crowd before it becomes cool to do so.

by Ry Young, Chair, Division M (Bacteriophage), ASM

Editor's note: Don't forget to put a link to the "HomePhage" from your wed site: http://www.asmusa.org/division/m/M.html (see "New Links" below).

Editorial Archive

- BEG: What we are, Where we are, Where we're going by Stephen T. Abedon
- When Grown In Vitro, do Parasites of Multicellular Organisms (MOPs) become Unicellular Organism Parasites (UOPs)? by Stephen T. Abedon
- Bacteriophages as Model Systems by Stephen T. Abedon
- 2000 and Sun: A Phage Odyssey by Stephen T. Abedon
- Lytic, Lysogenic, Temperate, Chronic, Virulent, Quoi? by Stephen T. Abedon
- Which Ecology are You? by Stephen T. Abedon
- Science NetWatch October 13, 2000
- The Best of Times, the Worst of Times by Ry Young

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New BEG Members

The **BEG members list** can be found at **www.phage.org/beg_members.htm** as well as on the **BEG home page**. As we add new members, these individuals will be introduced in this section. Note that, in fact, there are two ways of "joining" BEG. One, the "traditional" way, is to have your name listed on the web page and on the list server. The second, the "non-traditional" way, is to have your name only listed on the list server. The latter I refer to as "non-members" on that list. Members, e.g., individuals listed on the **BEG home page**, should be limited to individuals who are actively involved in science and who can serve as a phage ecology resource to interested individuals. If you have an interest in phage ecology

but no real expertise in the area, then you should join as a non-member. To join as a member, please contact BEG using the following link: abedon.1@osu.edu. Include:

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

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

Please welcome our newest members

name (home page links)	status	e-mail	address	
	PI	lssayc @zsu.edu.cn	Director, Lab of Molecular Microbiolgoy and Antimicrobial Drugs, Department of Biochemistry and Microbiology, School of Life Sciences, Zhongshan University, Guangzhou 510275, P R China	
Yun-Can Al	interests:	My lab is focusing on the Phage-bacteria community ecology in the South China Sea, Pacific Ocean Coast. The coevolution of phage and pathogenic bacteria, horizontal gene transfer, effects of stress induced by antibiotics. Bacteriophages isolation and their role in gene transfer within the bacterial community, particularly pathogenicity islands. Bacteriophage genomics and evolution. (contents BEG members top of page)		
	PI	rgoodnow @tranquility.net	Goodnow Microbes Lab/Reg. Aff. Contractors, Inc., 2909 Yukon Dr., Columbia, MO 65202	
Robert A. Goodnow	interests:	Phage inactivation (MS2 0X174 and P22) by chemical/light combination and anti- <i>Pasteurella</i> phage therapy. Am also interested in helpful hints on phage isolation and propagation. (contents BEG members top of page)		
		rgoldman @pop.uh.edu	Department of Biology and Biochemistry, University of Houston, Houston, TX 77204	
Robert Goldman	interests:	Role of phage in natural microbial communities, particularly soil environments; the isolation and characterization of phage from natural communities; the use of phage and bacteria as experimental systems to test evolutionary and ecological hypotheses; phage therapy; diversity and abundance of phage in microbial communities associated with native prairie grasses. (contents BEG members top of page)		
Park Se-Chang		parksc @ipc.hiroshima- u.ac.jp	Laboratory of Fish Pathology, Fac. Appl. Bio. Sci., Hiroshima University, Kagamiyama 1-4-4, Higashihiroshima 739, Japan	
	interests:	Bacteriophage therapeutics. (contents BEG members top of page)		
Grieg E Steward		gsteward @cats.ucsc.edu	Ocean Sciences Department, A451 Earth and Marine Science Bldg., University of California, 1156 High St., Santa Cruz, CA 95064	
Chey I. Oleward	interests:	Molecular diversity and population dynamics in	d ecology of bacteria and viruses. The influence of phages on bacterial n aquatic ecosystems. (contents BEG members top of page)	
Heather Uwing		pdf_hku @hotkey.net.au	Environmental Sciences, Griffith University, Nathan Qld	
	interests:	Development of metho BEG members top of	ods to measure bacteriophage replication using isotope tagging. (contents page)	

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New Links

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

New Bacteriophage Ecology (Etc.) Links

No new links this quarter, sorry. Here, however, are some interesting Google searches and stats (searches done Friday, March 30,

2001):

Search Term	Hits
sex	39,800,000
God	20,300,000
environment	19,100,000
AIDS	5,570,000
virus	5,460,000
ecology	2,310,000
virus -AIDS -HIV	1,350,000
bacteria	1,290,000
"safe sex"	179,000
phage OR phages OR bacteriophage OR bacteriophages	71,600
phage	71,300
bacteriophage	46,100
evolution and phage OR phages OR bacteriophage OR bacteriophage	14,900
phages	12,600
bacteriophages	9,860
ecology and phage OR phages OR bacteriophage OR bacteriophage OR bacteriophages	6,080
library OR libraries and "phage display"	4,570
ASM and phage OR phages	1,360
Bdelovibrio	1,190
"pathogenicity island"	785
"phage therapy"	346
link: www.phage.org	209
"bacteriophage ecology"	145
"Bacteriophage Ecology Group"	97
"phage ecology"	69
link: "HomePhage"	69
"division M" and phage OR phages	34
"phage evolution"	52
HomePhage	32
HomePhage and phage OR bacteriophage	7

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New Features

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

Bacteriophage Ecology Web Ring:

WebRing®
Find rings on any topic:
Go

Is this your site? click here.

The web ring is functioning properly now. To join the ring directly, go to this web page: http://edit.webring.yahoo.com/cgibin/membercgi?ring=bacteriophageeco&addsite. Thanks!

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Upcoming Meetings

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

Evergreen International Phage Meeting

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



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Jobs

Looking for job? Looking to fill a position? Please send advertisement and information to **abedon.1@osu.edu** or to "Jobs", Bacteriophage Ecology Group News, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Please send all information as text (e.g., as an e-mail) or as Microsoft Word documents, if possible (I'll let you know if I have trouble converting any other document formats), and in English. I will update this section as I receive material, regardless of what date this issue of BEG News goes live.

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Submissions

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

Selling Phage Candy

In grade school we were asked to sell candy to neighbors to raise money for who can remember what. The kid with the largest haul always earned some prize and, of course, I never did. Early on I realized that attitude was everything. You had to believe (or, at least, sound as though you believed) that you were actually doing people a favor as their local pusher of sucrose saturated lipid! Pretty good for an eight-year-old, but still naïve. Even the greatest attitude

won't buy you a cup of coffee, bring home the (soy/faux) bacon, get that grant funded, or pad one's bank account with a million bucks before others have even earned tenure. No, what really matters in this world is marketing. Never mind whether you really mean it (or even believe it). In the short term, at least, the pitch is everything. So now that we have a number of companies dedicated to phagology and the competition for clicks (and dollars?) that is the World Wide Web, it is time to get down to the dirty business of reviewing phage company logos and, especially, animated graphics. May the marketing begin...

The Bacteriophage Ecology Group maintains of list of phage-oriented companies at:

• http://www.phage.org/beg_links.htm#phage_companies

Included in this list are descriptions, contact information, and homepage URLs. To date the list includes, in alphabetical order:

- Biophage Inc.
- Exponential Biotherapies, Inc.
- Intralytix
- MicroPeace Biotechnology Consulting
- Phage Biotech, Ltd.
- Phage Therapeutics International Inc. (PhageTx)
- PhageTech Inc.

See http://www.phagetherapy.com/ for a similar list. I'll try to avoid being too critical in this review, but here goes.

MicroPeace Biotechnology Consulting and PhageTech Inc. both lack home pages. Micropeace has a relatively small web presence (3 hits via a Google search). PhageTech does far better Google-wise (49), and they apparently own a URL (www.phagetech.com) but as yet have no homepage. Exponential Biotherapies, Inc. has a homepage, but it is only a single page and there are no graphics! On the other hand, their prose may be worth a thousand gifs:

"Our first product - now in manufacturing - combats a strain of bacteria whose rapid spread is compromising the safety and profitability of some of the nation's major hospital centers. Human clinical trials are projected to begin in the first quarter of 2001."

Winner of our award for the most imformative gif is Phage Therapeutics International Inc. (PhageTx) who provide us with nice logo:



But what really scores points is their interactive timeline (which, unfortunately, has such complex coding that I won't even attempt to duplicate it here).

The logo on the Biophage Inc. site is gorgeous, but what the heck is it? Idealized animal cells? You decide:



The most scientifically informative image is presented by Phage Biotech, Ltd., though this is more graphic than logo. It is an animated Shockwave showing phages (T-even, of course) adsorbing to a red coccobacillus. Adsorption is followed by virion maturation and then host lysis with progeny eagerly pushing their way out of the ghost-like remnants of their cellular incubator. The 2001-like silence of the process is eerie, however, with the viewer perhaps hoping for a Star Wars-ish pseudo-space-wizardry soundtrack.

Perhaps it is indeed Star Wars that Intralytix had in mind as they put together their introductory graphic. It has stars. It has a soundtrack. It also tells a story of sorts with words alternatively flashing on and then fading (lysis-like) away:

What if we could harness the natural predators and use them to our benefit?

What if we could use these creatures to infect and destroy bacteria such as Listeria, Salmonella, or E. coli?

Even bacteria that can not be killed with antibiotics!

We can!





Say hello to phage.

INTRALYTIX

Harnessing the Power

Alternatively, you can skip the graphics. Either way, you will be treated to a logo that is not nearly as sexy as the opening graphic:



MicroDude, a.k.a., Stephen T. Abedon

is the Developer and Editor of The Bacteriophage Ecology Group web site which is dedicated to the ecology and evolutionary biology of the parasites of unicellular organisms (UOPs)

Submissions Archive

 On an Invisible Microbe Antagonistic to the Dysentery Bacillus by Felix d'Herelle Obituary: Hansjürgen Raettig - Collector of Bacteriophage References (October 12, 1911 - December 1, 1997) Some Quotations • Bacteriophages: A Model System for Human Viruses

- - How Big is 10^{30} ?
 - Selling Phage Candy

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Letters & Questions

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

Looking for RNA Coliphages

Marek Kirs, marek@gso.uri.edu

earlier ... my name is marek and i'm a grad student at the uri, currently doing a side project, maybe-maybe PhD, on

coliphages. i'm trying to modify methodology for group I, II III and IV F-RNA coliophage(Leviviridae) detection from Griffin, 2000 using molecular beacons. And i have some ideas for future if we get here a right machine like ABIPrism. Anyway, i have some problems - do you have any idea were i can get positive controls for my experiment. Positive controls for Leviviridae subgroups:

- Subgroup I i'm all set, I have MS2
- Subgroup II -strains that i am aware are BZ13, JP 34, TH1, GA, KU1; none of them in ATCC.
- Subgroup III ATCC has Q-Beta for \$165
- Subgroup IV ATCC has F1 for \$175

What I need is:

- Levivirus subgroup II GA or KUI1
- Allelovirus subgroup III Q-Beta (has anyone a full sequence of this genome? the probe which is usually used for this group is impossible to fold to a beacon due to secondary structures as well I discovered 3b mismatch in one end, so i need to find another target area)
- Allelovirus subgroup IV SP or FI (does SP grow on E.coli? E.coli FAMP is the only host I have in culture)

Do you know anybody who I can contact to ask this material. Prices for SP, FI and QBeta in ATCC are too high to my budget(each \$165-175)).

later... It took me awhile to finish subgroup II and III beacons, also I needed to switch an office and machine...

I'm still looking for sequence(s) of F1. Also TW28 would not hurt, but I doubt that anybody has anything from it. I have only GenBank sequences and there are only couple of very short sequences of F1, definetly not enough for designing a beacon for this group.

Could you please forward this request in suitable form to the list?

T1 Contamination Problems

Ted Schram, tschram@incyte.com

what info do you have on T1 phage? we have it here in our lab, infecting our cells and such.

About Lambda Phage

Devanagoud Patil, dpatil@bioch.ox.ac.uk

I am a post-doctoral fellow working in the Department of Biochemistry, University of Oxford,UK. I am looking for the following information and would grately appreciate your help.

- 1. Antibodies (for ELISA and western) against any of the lambda phage proteins expressed on the outer surface of head or tail.
- 2. A filteration based procedure for preparataion and concentration of lambda phage from 1 to 5 liter culture (final concentrated volume 5-10 ml of Tris-Mg++).

Is there a phage newsgroup or a forum to post this message?

Thank you very much for your help and time.

Looking for Phage O-1

Lydia Dayang, lyds_dayang@yahoo.com

Greetings from the Philippines!

May I inquire how could I possibly get phage O-1 which I will be needed for my screening of Salmonella from feces. I hope you can help me.

Thank you and more power!

Interest in Phage Therapy

Mary Shelton, plytmrs@nottingham.ac.uk

I am a final year microbiologist at Nottingham University. For my Dissertation I am reviewing the scope of Bacteriophage as Therapeutic agents.

I am focusing on the potential of clinical applications and reviewing the practical problems have been overcome.

I would greatly appreciate any help or direction you can provide. I am interested in the following areas:

- rapid clearance of phage from the circulation system by RES
- isolation of long circulating mutants, by cycling through mice
- efficiency of recent trials
- irradication of bacteria, sterilisation or reduction of bacterial load
- comparisons to antibiotic therapy (efficacy and efficiency)
- scope of combination (Antibiotics AND phage) therapy
- realistic risk of resistance, and spread of pathogenisity islands by lysogenic phage
- enhancing pysiological properties of phage to increase therapeutic qualities

This is only a few of the areas i am investigating, of which i have some specific questions to pose.

I would appreciate any contacts, journal citations/references, and suggestions of alternative directions.

This is a critical discussion of the clinical scope of bacteriophage as therapeutic agents and i would value your opinions highly

Thankyou in anticipation

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Phage Images

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



Phage Image Archive

Phage T4 on the pedestal outside of Barker Hall at Berkeley. Does anybody have a better photo(s) of the above as well as an idea as to its history and current status? I would love to feature it. Contact abedon.1@osu.edu with information. Thanks!

- BEG Phage Images Page
- The Face of the Phage
- Bacteriophage T2 by H.-W. Ackermann
 - SSV1-Type Phage
- Saline Lake Bacteriophage David Bird
- Coliphage LG1 Larry Goodridge
 - Bacteriophage HK97 Bob Duda
 - Phage T4 (art) Francis S. Lin
- PhageT4 on the pedestal outside of Barker Hall at Berkeley

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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. The Vibrio cholerae VPI?/CTX?/TCP: Interactions of PHAGE-PHAGE-bacterium. Ai, Y.-C., Meng, F. (2001). Acta Microbiologica Sinica 41. [no abstract]

- 2. Comparative phage genomics and the evolution of Siphoviridae: Insights from dairy phages. Brussow, H., Desiere, F. (2001). Molecular Microbiology 39:213-222. [PRESS FOR ABSTRACT]
- 3. Variable assortment of prophages provides a transferable repertoire of pathogenic determinants in *Salmonella*. Figueroa-Bossi, N., Uzzau, S., Maloriol, D., Bossi, L. (2001). *Molecular Microbiology* 39:260-271. [PRESS FOR ABSTRACT]
- 4. First evidence of lysogeny in *Propionibacterium freudenreichii* subsp. shermanii. Herve, C., Coste, A., Rouault, A., Fraslin, J. M., Gautier, M. (2001). *Applied and Environmental Microbiology* 67:231-238. [PRESS FOR ABSTRACT]
 - Isolation and characterization of five Erwinia amylovora bacteriophages and assessment of phage resistance in strains of Erwinia amylovora. Schnabel, E. L., Jones, A. L. (2001). Applied and Environmental Microbiology 67:59-64. [PRESS FOR ABSTRACT]
- 6. Understanding bacteriophage therapy as a density-dependent kinetic process. Payne, R. J. H., Jansen, V. A. A. (2001). Journal of Theoretical Biology 208:37-48. [PRESS FOR ABSTRACT]
 - 7. Antibody responses to bacteriophage variant phiX-174 in human subjects exposed to the Antarctic winter-over model of spaceflight. Shearer, W. T., Lugg, D. J., Rosenblatt, H. M., Nickolls, P. M., Sharp, R. M., Reuben, J. M., Ochs, H. D. (2001). Journal of Allergy and Clinical Immunology 107:160-164. [PRESS FOR ABSTRACT]
- 8. Rapid coliphage detection assay. Stanek, J. E., Falkinham, J. O., III (2001). *Journal of Virological Methods* 91:93-98. [PRESS FOR ABSTRACT]
- 9. Predation in the presence of decoys: An inhibitory factor on pathogen control by bacteriophages or bdellovibrios in dense and diverse ecosystems. Wilkinson, M. H. F. (2001). *Journal of Theoretical Biology* 208:27-36. [PRESS FOR ABSTRACT]

10. The evolution of pathogen-host interactions mediated by bacteriophages. Ai, Y.-C., Meng, F., Zeng, Y. (2000). Acta Microbiologica Sinica 40:657-661. [no abstract]

- Phage resistance in Lactococcus lactis subsp. lactis strains isolated from traditional fermented milk products in Turkey. Akcelik, M., Sanlibaba, P., Tükel, C. (2000). International Journal of Food Science & Technology 35:473-481. [PRESS FOR ABSTRACT]
 - 12. The presence of viruses and bacteria along the Adriatic Coast. Aulicino, F. A., Ammazzalorso, P., Ercolessi, M., Banini, L., Silverii, G., Orsini, P., Mastrantonio, A., Bellucci, C., Carere, M. (2000). *Igiene Moderna* 113:99-116. [PRESS FOR ABSTRACT]
 - 13. Evolvability of an RNA virus is determined by its mutational neighbourhood. Burch, C. L., Chao, L. (2000). *Nature (London)* 406:625-628. [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. The Vibrio cholerae VPI?/CTX?/TCP: Interactions of PHAGE-PHAGE-bacterium. Ai, Y.-C., Meng, F. (2001). Acta Microbiologica Sinica 41.

2. Comparative phage genomics and the evolution of Siphoviridae: Insights from dairy phages. Brussow, H., Desiere, F. (2001). *Molecular Microbiology* 39:213-222. Comparative phage genomics can retrace part of the evolutionary history of phage modules encoding phage-specific functions such as capsid building or establishment of the lysogenic state. The diagnosis of relatedness is not based exclusively on sequence similarity, but includes topological considerations of genome organization. The gene maps from the lambda-, psiM2-, L5-, Sfi21-, Sfi11-, phiC31-, sk1- and TM4-like phages showed a remarkable synteny of their structural genes defining a lambda supergroup within Siphoviridae (Caudovirales with long non-contractile tails). A hierarchy of relatedness within the lambda supergroup suggested elements of vertical evolution in the capsid module of Siphoviridae. Links to P22-like Podoviridae and P2-like Myoviridae were also detected. Numerous cases of horizontal gene transfer were observed, but recent transfers were limited to interbreeding phage populations. We suggest that tailed phages are the result of both vertical and horizontal evolution and are thus a good model system for web-like phylogenies

3. Variable assortment of prophages provides a transferable repertoire of pathogenic determinants in Salmonella. Figueroa-Bossi, N., Uzzau, S., Maloriol, D., Bossi, L. (2001). Molecular Microbiology 39:260-271. Gene transfer between separate lineages of a bacterial pathogen can promote recombinational divergence and the emergence of new pathogenic variants. Temperate bacteriophages, by virtue of their ability to carry foreign DNA, are potential key players in this process. Our previous work has shown that representative strains of Salmonella typhimurium (LT2, ATCC14028 and SL1344) are lysogenic for two temperate bacteriophages: Gifsy-1 and Gifsy-2. Several lines of evidence suggested that both elements carry genes that contribute to Salmonella virulence. One such gene, on the Gifsy-2 prophage, codes for the (Cu, Zn) superoxide dismutase SodCI. Other putative pathogenicity determinants were uncovered more recently. These include genes for known or presumptive type III-translocated proteins and a locus, duplicated on both prophages, showing sequence similarity to a gene involved in Salmonella enteropathogenesis (pipA). In addition to Gifsy-1 and Gifsy-2, each of the above strains was found to harbour a specific set of prophages also carrying putative pathogenicity determinants. A phage released from strain LT2 and identified as phage Fels-1 carries the nanH gene and a novel sodC gene, which was named sodCIII. Strain ATCC14028 releases a lambdoid phage, named Gifsy-3, which contains the phoP/phoQ-activated pagJ gene and the gene for the secreted leucine-rich repeat protein SspH1. Finally, a phage specifically released from strain SL1344 was identified as SopEPHI. Most phage-associated loci transferred efficiently between Salmonella strains of the same or different serovars. Overall, these results suggest that lysogenic conversion is a major mechanism driving the evolution of Salmonella bacteria

4. First evidence of lysogeny in Propionibacterium freudenreichii subsp. shermanii. Herve, C., Coste, A., Rouault, A., Fraslin, J. M., Gautier, M. (2001). Applied and Environmental Microbiology 67:231-238. Dairy propionic acid bacteria, particularly the species Propionibacterium freudenreichii, play a major role in the ripening of Swiss type cheese. Isometric and filamentous bacteriophages infecting *P. freudenreichii* have previously been isolated from cheese. In order to determine the origin of these bacteriophages, lysogeny of *P. freudenreichii* was determined by isometric bacteriophage type analysis. The genomic DNA of 76 strains were hybridized with the DNA of nine bacteriophages isolated from Swiss type cheeses, and the DNA of 25 strains exhibited strong hybridization. Three of these strains released bacteriophage particules following UV irradiation (254 nm) or treatment with low concentrations of mitomycin C. A prophage-cured derivative of *P. freudenreichii* was readily isolated and subsequently relysogenized. Lysogeny was therefore formally demonstrated in *P. freudenreichii*

5. Isolation and characterization of five *Erwinia amylovora* bacteriophages and assessment of phage resistance in strains of *Erwinia amylovora*. Schnabel, E.L., Jones, A. L. (2001). *Applied and Environmental Microbiology* 67:59-64. Phages able to infect the fire blight pathogen *Erwinia amylovora* were isolated from apple, pear, and raspberry tissues and from soil samples collected at sites displaying fire blight symptoms. Among a collection of 50 phage isolates, 5 distinct phages, including relatives of the previously described phages variant phiEa1 and variant phiEa7 and 3 novel phages named variant phiEa100, variant phiEa125, and variant phiEa116C, were identified based on differences in genome size and restriction fragment pattern. variant phiEa1, the phage distributed most widely, had an approximately 46-kb genome which exhibited some restriction site variability between isolates. Phages variant phiEa100, variant phiEa125 each had genomes of approximately 35 kb and could be distinguished by their EcoRI restriction fragment patterns. variant phiEa116C contained an approximately 75-kb genome. variant phiEa1, variant phiEa7, variant phiEa100, variant phiEa125, and variant phiEa116C were able to infect 39, 36, 16, 20, and 40, respectively, of 40 *E. amylovora* strains isolated from apple orchards in Michigan and 8, 12, 10, 10, and 12, respectively, of 12 *E. amylovora* strains isolated from raspberry fields (*Rubus* spp.) in Michigan. Only 22 of 52

strains were sensitive to all five phages, and 23 strains exhibited resistance to more than one phage. variant phiEa116C was more effective than the other phages at lysing *E. amylovora* strain Ea110 in liquid culture, reducing the final titer of Ea110 by >95% when added at a ratio of 1 PFU per 10 CFU and by 58 to 90% at 1 PFU per 105 CFU

- 6. Understanding bacteriophage therapy as a density-dependent kinetic process. Payne, R. J. H., Jansen, V. A. A. (2001). Journal of Theoretical Biology 208:37-48. Studies of bacteriophage as therapeutic agents have had mixed and unpredictable outcomes. We argue that interpretation of these apparently paradoxical results requires appreciation of various density-dependent threshold effects. We use a mathematical model to delineate different categories of outcome, including therapy by simple inundation, by active biocontrol, and by delayed active biocontrol. Counter-intuitively, there are situations in which earlier inoculation can be less efficacious, and simultaneous inoculation with antibiotics can be detrimental. Predictions of therapeutic responses are made using formulae dependent on biologically meaningful parameters; experimental measurement of the parameters will be a prerequisite of application of the model to particular study systems. Such modelling can point to which aspects of phage biology might most fruitfully be engineered so as to enhance the viability of bacteriophage therapy
- 7. Antibody responses to bacteriophage variant phiX-174 in human subjects exposed to the Antarctic winter-over model of spaceflight. Shearer, W. T., Lugg, D. J., Rosenblatt, H. M., Nickolls, P. M., Sharp, R. M., Reuben, J. M., Ochs, H. D. (2001). Journal of Allergy and Clinical Immunology 107:160-164. Background: It has been proposed that exposure to long-term spaceflight conditions (stress, isolation, sleep disruption, containment, microbial contamination, and solar radiation) or to ground-based models of spaceflight will alter human immune responses, but specific antibody responses have not been fully evaluated. Objective: We sought to determine whether exposure to the 8-month Antarctic winter-over model of spaceflight would alter human antibody responses. Methods: During the 1999 Australian National Antarctic Research Expeditions, 11 adult study subjects at Casey, Antarctica, and 7 control subjects at Macquarie Island, sub-Antarctica, received primary and secondary immunizations with the T cell-dependent neoantigen bacteriophage variant phiX-174. Periodic plasma samples were analyzed for specific antibody function. Results: All of the subjects from Casey, Antarctica, cleared bacteriophage variant phiX-174 normally by 1 week after primary immunization, and all had normal primary and secondary antibody responses, including immunologic memory amplification and switch from IgM to IgG antibody production. One subject showed a high normal pattern, and one subject had a low normal pattern. The control subjects from Macquarie Island also had normal immune responses to bacteriophage variant phiX-174. Conclusions: These data do not support the hypothesis that de novo specific antibody responses of subjects become defective during the conditions of the Antarctic winter-over. Because the Antarctic winter-over model of spaceflight lacks the important factors of microgravity and solar radiation, caution must be used in interpreting these data to anticipate normal antibody responses in long-term spaceflight
- 8. Rapid coliphage detection assay. Stanek, J. E., Falkinham, J. O., III (2001). Journal of Virological Methods 91:93-98. A rapid coliphage detection assay was developed, based on the phage-induced release of beta-galactosidase from cells of *Escherichia coli*. The assay could detect as few as five coliphage per sample without an overnight incubation period. The range of acceptable assay parameters was identified
- 9. Predation in the presence of decoys: An inhibitory factor on pathogen control by bacteriophages or bdellovibrios in dense and diverse ecosystems. Wilkinson, M. H. F. (2001). Journal of Theoretical Biology 208:27-36. Several attempts have been made at the removal of specific pathogens from the intestinal microflora using either bacteriophages or "predatory" bacteria such as *Bdellovibrio* spp. To date these attempts have had mixed success. A mechanism explaining these findings based on competitive hindrance by non-prey, or decoy species is put forward. It is shown that this hindrance tends to damp out predator-prey oscillations, and therefore reduces the probability of prey extinction. Possible experiments to verify this theory are discussed. The decoy effect may play a role in any system with high densities of bacteria or other particulate matter, such as activated sludge or biofilms

10. The evolution of pathogen-host interactions mediated by bacteriophages. Ai, Y.-C., Meng, F., Zeng, Y. (2000). Acta Microbiologica Sinica 40:657-661.

- 11. Phage resistance in Lactococcus lactis subsp. lactis strains isolated from traditional fermented milk products in Turkey. Akcelik, M., Sanlibaba, P., Tükel, C. (2000). International Journal of Food Science & Technology 35:473-481. Lactococcus lactis subsp. lactis strains isolated from traditional fermented milk products in Turkey were used to determine their phage resistance against three different lactic phages. The following modes of action were examined: phage adsorption inhibition in five strains, abortive infection (heat sensitive phage resistance) in three strains, restriction/modification in four strains and blocking of phage DNA injection in one strain. The genetic nature of the phage resistance systems in these strains was determined by comparison of phage proliferation parameters, e.g. adsorption (%), EOP, burst size, latent period and production of major capsid protein, between wild-type strains and their plasmid-cured derivatives
- 12. The presence of viruses and bacteria along the Adriatic Coast. Aulicino, F. A., Ammazzalorso, P., Ercolessi, M., Banini, L., Silverii, G., Orsini, P., Mastrantonio, A., Bellucci, C., Carere, M. (2000). Igiene Moderna 113:99-116. A study was carried out on seawater samples, collected from the Adriatic sea near the coast of Pesaro, to determine the presence of enteric viruses and *Escherichia coli* bacteriophages besides the common indicators of fecal pollution and of trophic conditions of the marine environment (*Pseudomonas, Vibrio*, algae). During 1994-95, seawater samples were tested in 8 stations located in seaside resorts; in 1994 samples of sediment were also analyzed. Generally the results showed a good situation from the microbiological and eutrophic point of view. Only 2 stations showed fecal pollution. Enteroviruses were not detected while Reovirus was isolated from samples of the two most contaminated stations and from a not polluted area
- 13. Evolvability of an RNA virus is determined by its mutational neighbourhood. Burch, C. L., Chao, L. (2000). Nature (London) 406:625-628. The ubiquity of mechanisms that generate genetic variation has spurred arguments that evolvability, the ability to generate adaptive variation, has itself evolved in response to natural selection. The high mutation rate of RNA viruses is postulated to be an adaptation for evolvability, but the paradox is that whereas some RNA viruses evolve at high rates, others are highly stable. Here we show that evolvability in the RNA bacteriophage phi6 is also determined by the accessibility of advantageous genotypes within the mutational neighbourhood (the set of mutants one or a few mutational steps away). We found that two phi6 populations that were derived from a single ancestral phage repeatedly evolved at different rates and toward different fitness maxima. Fitness measurements of individual phages showed that the fitness distribution of mutants differed between the two populations. Whereas population A, which evolved toward a higher maximum, had a distribution that contained only deleterious mutants. We interpret these distributions to measure the fitness effects of genotypes that are mutationally available to

the two populations. Thus, the evolvability of phi6 is constrained by the distribution of its mutational neighbours, despite the fact that this phage has the characteristic high mutation rate of RNA viruses

- 14. Inactivation of indicator microorganisms in estuarine waters. Burkhardt, W., III, Calci, K. R., Watkins, W. D., Rippey, S. R., Chirtel, S. J. (2000). Water Research 34:2207-2214. In the United States, shellfish growing areas are classified, in part, using standards based on the densities of either the total or fecal coliform groups in surface waters. However, the standards currently employed may not reliably index the presence of certain enteric pathogens, particularly enteric viruses responsible for human illnesses, even though both the pathogens and indicators derive from the same fecal contamination. To some extent, this may be due to differences in the survival of these pathogens in the environment relative to that of the bacterial indicators. This investigation was conducted to assess the effects of temperature, salinity, dissolved oxygen, geographic location, season, and solar radiation on the survival of selected indicator microorganisms in estuarine waters. The indicators examined included fecal coliforms, Escherichia coli, Clostridium perfringens, and male-specific bacteriophage (MSB), a potential indicator of enteric viruses. In situ experiments were performed in estuarine waters of Alabama and Rhode Island. Among the parameters examined, sunlight and/or temperature most significantly affected indicator decay rates. In general, the effects from exposure to sunlight accounted for up to 83, 84, and 99% of the density reductions of MSB, C. perfringens and fecal coliforms, respectively. Thus, the effects from sunlight were greatest on fecal coliforms and much less pronounced on MSB and C. perfringens. For fecal coliforms, the effect of sunlight was more pronounced during the winter than the summer. In the absence of sunlight, the rate of MSB decline was strongly negatively correlated with estuarine water temperatures and dissolved oxygen. Overall, fecal coliform decay rates were dissimilar to those found for MSB. From this, it would appear that fecal coliforms may not be reliable indicators of viruses in estuarine waters
 - 15. Large-plaque mutants of Sindbis virus show reduced binding to heparan sulfate, heightened viremia, and slower clearance from the circulation. Byrnes, A. P., Griess, G. A. (2000). Journal of Virology 74:644-651. Laboratory strains of Sindbis virus must bind to the negatively charged glycosaminoglycan heparan sulfate in order to efficiently infect cultured cells. During infection of mice, however, we have frequently observed the development of large-plaque viral mutants with a reduced ability to bind to heparan sulfate. Sequencing of these mutants revealed changes of positively charged amino acids in putative heparin-binding domains of the E2 glycoprotein. Recombinant viruses were constructed with these changes as single amino acid substitutions in a strain Toto 1101 background. All exhibited decreased binding to heparan sulfate and had larger plaques than Toto 1101. When injected subcutaneously into neonatal mice, large-plaque viruses produced higher-titer viremia and often caused higher mortality. Because circulating heparin-binding proteins are known to be rapidly sequestered by tissue heparan sulfate, we measured the kinetics of viral clearance following intravenous injection. Much of the parental small-plaque Toto 1101 strain of Sindbis virus was cleared from the circulation by the liver within minutes, in contrast to recombinant large-plaque viruses, which had longer circulating half-lives. These findings indicate that a decreased ability to bind to heparan sulfate allows more efficient viral production in vivo, which may in turn lead to increased mortality. Because Sindbis virus is only one of a growing number of viruses from many families which have been shown to bind to heparan sulfate, these results may be generally applicable to the pathogenesis of such viruses
- 16. Isolation of a virulent bacteriophage from a Propionibacterium species in the sheep rumen. Cheong, J. P. E., Brooker, J. D. (2000). Australian Journal of Agricultural Research 51:119-123. Propionibacterium is a facultative anaerobe associated with the rumen epithelium, the presence of which may influence the anaerobic environment through oxygen scavenging, as well as providing a source of propionate. Factors such as bacteriophages that influence Propionibacterium populations may therefore be important regulators of rumen function. This study describes the isolation and identification of a ruminal Propionibacterium bacteriophage. Sheep rumen fluid was screened for Propionibacterium species and 3 isolates were identified and characterised. One isolate, PA1, was used as an indicator strain to screen for the presence of Propionibacterium-specific virulent bacteriophages. A virulent bacteriophage, PB2, was isolated from clear plaques on a lawn of PA1 cells and was shown by transmission electron microscopy to be a siphovirus-like particle comprising an icosahedral head 50 nm in diameter and a tail 140 nm in length. The bacteriophage was visibly attached to and within PA1 cells, and was shown to infect all 3 ruminal isolates of Propionibacterium and 4 of 6 clinical isolates of P. acnes. Restriction mapping of bacteriophage PB2 demonstrated a 30.8 kb genome
 - 17. Effect of deleterious mutation-accumulation on the fitness of RNA bacteriophage MS2. de la Pena, M., Elena, S. F., Moya, A. (2000). Evolution 54:686-691. RNA viruses show the highest mutation rate in nautre. It has been extensively demonstrated that, in the absence of purifying selection, RNA viruses accumulate deleterious mutations at a high rate. However, the parameters describing this accumulation are, in general, poorly understood. The present study reports evidences for fitness declines by the accumulation of deleterious mutations in the bacteriophage MS2. We estimated the rate of fitness decline to be as high as 16% per bottleneck transfer. In addition, our results agree with an additive model of fitness effects
 - 18. Present and potential applications of genetic engineering in agronomy and agro-food. Desmazeaud, M. (2000). Comptes Rendus de l'Academie d'Agriculture de France 86:97-102. In agronomy, genetically engineered Rhizobium can improve plant growth. In the case of food productions, a bakery yeast was modified for increasing maltose utilization. Brewery and wine yeasts were modified for better properties than wild strains about alcohol or pH or sulfite resistance. New flavor profiles are obtained also: Kluyveromyces lactis can produce chymosin A used in cheese-making; lactic acid bacteria (mainly Lactococcus lactis) are modified for a controlled action during cheese ripening; genetic engineering can produce bacteriophage resistant strains
- Pathogenicity islands and phage conversion: Evolutionary aspects of bacterial pathogenesis. Dobrindt, U., Reidl, J. (2000). *IJMM International Journal of Medical Microbiology* 290:519-527. Horizontal gene transfer plays a key role in the generation of novel bacterial pathogens. Besides plasmids and bacteriophages, large genomic regions termed pathogenicity islands (PAIs) can be transferred horizontally. All three mechanisms for DNA exchange or transfer may be important for the evolution of bacterial pathogens
- 20. Control of bacterial spot on tomato in the greenhouse and field with h-mutant bacteriophages. Flaherty, J. E., Jones, J. B., Harbaugh, B. K., Somodi, G. C., Jackson, L. E. (2000). Hortscience 35:882-884. A mixture of host-range mutant (h-mutant) bacteriophages specific for tomato race 1 (T1) and race 3 (T3) of the bacterial spot pathogen, Xanthomonas campestris pv. vesicatoria (Doidge) Dye was evaluated for biological control of bacterial spot on 'Sunbeam' tomato (Lycopersicon esculentum Mill.) transplants and field-grown plants for two seasons (Fall 1997 and Fall 1998). Foliar applications of bacteriophages were compared with similar applications of water (control) and of copper/mancozeb bactericides, the commonly used chemical control strategy for tomato seedling and field production. In 1997, the incidence of bacterial spot on

greenhouse-grown seedlings was reduced from 40.5% (control) to 5.5% or 0.9% for bactericide- or bacteriophage-treated plants, respectively. In 1998, the incidence of bacterial spot was 17.4% on control plants vs. 5.5% and 2.7% for bactericide- and bacteriophage-treated plants, respectively, although these differences were not statistically significant at P ltoreq 0.05. Applications of bacteriophages to field-grown tomatoes decreased disease severity as measured by the area under the disease progress curve (AUDPC) by 17.5% (1997) and 16.8% (1998) compared with untreated control plants. Preharvest plant vigor ratings, taken twice during each field season, were higher in the bacteriophage-treated plants than in either bactericide-treated plants or nontreated controls except for the early vigor rating in 1998. Use of bacteriophages increased total weight of extra-large fruit 14.9% (1997) and 24.2% (1998) relative to that of nontreated control plants, and 37.8% (1997) and 23.9% (1998) relative to that of plants treated with the chemical bactericides. Chemical names used: manganese, zinc, carboxy-ethylene his dithiocarbamate (mancozeb)

- 21. The origins and ongoing evolution of viruses. Hendrix, R. W., Lawrence, J. G., Hatfull, G. F., Casjens, S. (2000). Trends in Microbiology 8:504-508. Genome analyses of double strand DNA tailed bacteriophages argue that they evolve by recombinational reassortment of genes and by the acquisition of novel genes as simple genetic elements termed morons. These processes suggest a model for early virus evolution, wherein viruses can be regarded less as having derived from cells and more as being partners in their mutual co-evolution.
- 22. Phage infection of the obligate intracellular bacterium, Chlamydia psittaci strain Guinea Pig Inclusion Conjunctivitis. Hsia, R. C., Ohayon, H., Gounon, P., Dautry-Varsat, A., Bavoil, P. M. (2000). Microbes and Infection 2:761-772. The infectious cycle of phiCPG1, a bacteriophage that infects the obligate intracellular pathogen. Chlamydia psittaci strain Guinea Pig Inclusion Conjunctivitis, was observed using transmission electron microscopy of phage-hyperinfected, Chlamydia-infected HeLa cells. Phage attachment to extracellular, metabolically dormant, infectious elementary bodies and cointernalisation are demonstrated. Following entry, phage infection takes place as soon as elementary bodies differentiate into metabolically active reticulate bodies. Phage-infected bacteria follow an altered developmental path whereby cell division is inhibited, producing abnormally large reticulate bodies, termed maxi-reticulate bodies, which do not mature to elementary bodies. These forms eventually lyse late in the chlamydial developmental cycle, releasing abundant phage progeny in the inclusion and, upon lysis of the inclusion membrane, into the cytosol of the host cell. Structural integrity of the hyperinfected HeLa cell is markedly compromised at late stages. Released phage particles attach avidly to the outer leaflet of the outer membranes of lysed and unlysed Chlamydiae at different stages of development, suggesting the presence of specific phage receptors in the outer membrane uniformly during the chlamydial developmental cycle. A mechanism for phage infection is proposed, whereby phage gains access to replicating chlamydiae by attaching to the infectious elementary body, subsequently subverting the chlamydial developmental cycle to its own replicative needs. The implications of phage infection in the context of chlamydial infection and disease are discussed
- 23. Control of the eel (Anguilla japonica) pathogens, Aeromonas hydrophila and Edwardsiella tarda, by bacteriophages. Hsu, C. H., Lo, C. Y., Liu, J. K., Lin, C. S. (2000). Journal of the Fisheries Society of Taiwan 27:21-31. Aeromonas hydrophila and Edwardsiella tarda are the two major pathogens of the eel, Anguilla japonica. The prevalent method to control the diseases is antibiotics. Long term and large scale application of the drugs results in resistance which makes disease control difficult. In the nature, bacteriophages are an important factor in controling bacterial population. The purpose of this research is to study the capability of the phages to control the pathogens in pond water. Several bacteriophages of *A. hydrophila* and *E. tarda* were isolated from the water samples of southern Taiwan. In pure culture, the phages could reduce the host 3 orders of magnitude in 2 hr when the multiplicity of infection (moi) was above 11.5 at 25°C. In the pond water with added *A. hydrophila* to 6 X 10⁵ / ml, the number dropped 250 folds at phage moi of 0.23 in 8 hr with accompanying phage multiplication to the level of 106 PFU/ml in the water. Most (85%) of the surviving hosts were still vulnerable to the phage. The resistant strains (15%) appeared to be lysogens since the culture broth of the strains could form phage plaques on *A. hydrophila*. In the case of *E. tarda*, the bacteria subsided rapidly even in the absence of phage in 48 hr in the pond water
 - 24. Temperature influences induction of a J7W-1-related phage in Bacillus thuringiensis serovar indiana. Kanda, K., Kayashima, T., Kato, F., Murata, A. (2000). Acta Virologica 44:183-187. Induction of a plasmid-integrative J7W-1-related phage in Bacillus thuringiensis serovar indiana by ethidium bromide was influenced by the temperature at which the host cells were cultured. Under optimal growth conditions, the maximum titer of the phage produced by the serovar indiana reached 1.2 X 10⁶ PFU/ml at 37°C while at 27°C it was lower by an order of magnitude (1.3 X 10⁵ PFU/ml). The temperature-sensitive period was estimated to occur early during the phage induction. However, the temperature effect observed with the serovar indiana did not occur with the serovar israelensis. In the latter case, the phage induction was the same at 37°C or 27°C. Thus we assume that the temperature sensitive phage induction observed with the serovar indiana as host was not a phenomenon caused by the phage genome but rather by product(s) encoded by certain host gene(s)
- 25. Mating in Bacillus thuringiensis can induce plasmid integrative prophage J7W-1. Kanda, K., Takada, Y., Kawasaki, F., Kato, F., Murata, A. (2000). Acta Virologica 44:189-193. Bacillus thuringiensis serovar israelensis, a bacterium which possesses plasmid transfer ability after mating, has been lysogenized by plasmid integrative phage J7W-1. The induction of phage in this J7W-1 lysogen was observed after mating with phage-insensitive strains, such as *B. thuringiensis* serovar thuringiensis, *B. cereus* and *B. subtilis*, as well as the phage-sensitive strain serovar israelensis. The phage induction was not observed after mating with *B. thuringiensis* strains AF101, serovar dendrolimus and serovar indiana. Because these strains are naturally associated with J7W-1 or its related phage, the data strongly suggest a constitutive expression of the repressor encoded by the prophage in these strains. However, the phage induction was observed in *B. thuringiensis* serovar aizawai, although it contained the J7W-1 DNA homologous region(s)
- 26. Rapid titration of multiple samples of filamentous bacteriophage (M13) on nitrocellulose filters. Koch, J., Breitling, F., Duebel, S. (2000). *BioTechniques* 29:1196-1202.
 - 27. Inactivation of coliphages by chitosan derivatives. Kochkina, Z. M., Surgucheva, N. A., Chirkov, S. N. (2000). *Mikrobiologiya* 69:261-265. The effect of chitosan fragments with different degrees of polymerization and the chemical derivatives of chitosan differing in the number of amino groups and total molecule charge on phages T2, T4, and T7 was studied. The interaction of chitosan with bacteriophage particles inactivated them to the extent dependent on the chemical properties of chitosan and its concentration. Phage T2 was found to be most susceptible to inactivation by chitosan. The polycationic nature of chitosan plays an important role in the inactivation of phages. It is assumed that the abnonnal rearrangement of the basal plate of phages, the loss of long tail fibers, and, probably, modification of the receptor-recognizing phage proteins may be responsible for the inactivation of coliphages by chitosan

- 28. Effect of simulated gastric fluid and bile on survival of Vibrio vulnificus and Vibrio vulnificus phage. Koo, J., Depaola, A., Marshall, D. L. (2000). Journal of Food Protection 63:1665-1669. Bacteria and phages may be exposed to acid conditions in the stomach and to bile in the intestine. Survival of three strains of Vibrio vulnificus and three strains of its phages was examined at 37°C after exposure to simulated gastric fluid at pH 3 to 4 or to 0, 1, and 2% bile in broth or buffer. Mean D-values (decimal reduction times) at pH 4 and 3 were 3.3 and 1.3 min for V. vulnificus and 97.8 and 0.7 min for its phages. No V. vulnificus survivors were found at pH 2.0. There were few survival differences among strains of V. vulnificus or its phages. Numbers of V. vulnificus increased 1 log in tryptic soy broth containing 1 or 2% bile after 3 h. Numbers of V. vulnificus bacteria and phages that survive stomach acidity may proliferate in the small intestine, since they are resistant to bile
 - 29. High-frequency interconversion of turbid and clear plaque strains of bacteriophage f1 and associated host cell death. Kuo, M. Y., Yang, M. K., Chen, W. P., Kuo, T. T. (2000). Canadian Journal of Microbiology 46:841-847. Under normal cultivation conditions, a mixture of turbid and clear plaques is often apparent in cultures of bacterial cells infected with filamentous bacteriophages. Beginning with a culture of wild-type filamentous phage f1, which itself produces turbid plaques, a clear plaque strain (c1) was isolated. From c1, the turbid plaque strain t1 was isolated; from t1, the clear plaque strain c2 was isolated; and from c2, the turbid plaque strain t2 was isolated. Each of these strains was generated with a frequency of approximately 1 X 10-4. Although filamentous phages have been thought not to induce host cell death, both turbid and clear plaque strains of f1 killed host bacteria. Plating of bacterial cells 1 h after infection revealed that colonies produced by cells infected with either wild-type f1 or strain c2 were smaller than those derived from uninfected cells, and that colony formation by infected cells was reduced by 15% and 38%, respectively. The time course of bacterial growth revealed that, at 4 h after infection, the number of CFU per milliliter of culture of cells infected with wild-type f1 or with strain c2 was reduced by 27% and 95%, respectively, compared with that for uninfected cells. Microculture analysis also revealed that the percentages of nondividing cells in f1 or c2 infected were 19% and 52%, respectively, 4 h after infection with wild-type f1 or with strain c2; no such cells were detected in cultures of uninfected cells. Negative staining and electron microscopy showed that 20% and 61% of cells infected with wild-type f1 or with strain c2 were dead 4 h postinfection. Finally, although the rates of DNA synthesis were similar for infected and uninfected cells, the rates of RNA and protein synthesis were markedly reduced in infected cells
- 30. Yersinia pestis variants, resistant to diagnostic bacteriophage, and problems connected with them. Lebedeva, S. A. (2000). Zhurnal Mikrobiologii Epidemiologii i Immunobiologii 99-104. The data of literature on the pleiotropic variability of the resistance of Y.pestis mutants to diagnostic phage are presented. The conditions of reversion to the initial phenotype are characterized. The mechanisms of the appearance of such variability of Y.pestis, as well as problems arising in connection with this variability and linked with the pathogenic activity of Y.pestis, low effectiveness of the diagnostic methods used in the inspection of the natural foci of plaque, the reservation of microbes in nature during the periods between epidemics, are discussed
 - 31. Broad-range bacteriophage resistance in Streptococcus thermophilus by insertional mutagenesis. Lucchini, S., Sidoti, J., Brussow, H. (2000). Virology 275:267-277. Streptococcus thermophilus is a lactic acid bacterium used in industrial milk fermentation. To obtain phage-resistant starters, S. thermophilus strain Sfi1 was submitted to mutagenesis with the thermolabile insertional vector pG+host9:ISS1 followed by a challenge with the lytic S. thermophilus phage Sfi19. Vector insertions into four distinct sites led to a phage-resistance phenotype. Three mutants were characterized further. They were protected against the homologous challenging phage and 14 heterologous phages. All three mutants adsorbed phages. No intracellular phage DNA synthesis was observed in mutants R7 and R71, while mutant R24 showed a delayed and diminished phage DNA synthesis compared to the parental Sfi1 strain. In mutant R7 a short deletion occurred next to the insertion site which removed the upstream sequences and the 15 initial codons from orf 394, encoding a likely transmembrane protein. Analogy with other phage systems suggests an involvement of this protein in the phage DNA injection process. In mutant R24 the vector was inserted into orf 269 predicting an oxido-reductase. When the vector sequence was removed via homologous recombination across the duplicated insertion elements, mutant R24 returned to the phage susceptibility of the parental strain. This observation suggested that inactivation of orf 269 was not crucial for the resistance phenotype. A gene encoding a likely restriction subunit of a type 1 restriction-modification system was located directly downstream of the insertion site in mutant R24. hsdM and hsdS gene encoding the modification and specificity subunits of a type 1 R-M system and biological evidence for an active R-M system were detected in strain Sfi1, suggesting involvement of a type 1 R-M system in the resistance phenotype of R24
- 32. The life cycles of the temperate lactococcal bacteriophage phiLC3 monitored by a quantitative PCR method. Lunde, M., Blatny, J. M., Kaper, F., Nes, I. F., Lillehaug, D. (2000). FEMS Microbiology Letters 192:119-124. We present here a new and general approach for monitoring the life cycles of temperate bacteriophages which establish lysogeny by inserting their genomes site-specifically into the bacterial host chromosome. The method is based on quantitative amplification of specific DNA sites involved in various cut-and-join events during the life cycles of the phages (i.e. the cos, attP, attB, attL and attR sites) with the use of sequence-specific primers. By comparing the amounts of these specific DNA sites at different intervals, we were able to follow the development of the lytic and lysogenic life cycles of the temperate lactococcal bacteriophage phiLC3 after infection of its bacterial host Lactococcus lactis ssp. cremoris IMN-C18

33. Distribution and evolution of bacteriophage WO in Wolbachia, the endosymbiont causing sexual alterations in arthropods. Masui, S., Kamoda, S., Sasaki, T., Ishikawa, H. (2000). Journal of Molecular Evolution 51:491-497. Wolbachia are obligatory intracellular and maternally inherited bacteria, known to infect many species of arthropod. In this study, we discovered a bacteriophage-like genetic element in Wolbachia, which was tentatively named bacteriophage WO. The phylogenetic tree based on phage WO genes of several Wolbachia strains was not congruent with that based on chromosomal genes of the same strains, suggesting that phage WO was active and horizontally transmitted among various Wolbachia strains. All the strains of Wolbachia used in this study were infected with phage WO. Although the phage genome contained genes of diverse origins, the average G+C content and codon usage of these genes were quite similar to those of a chromosomal gene of Wolbachia. These results raised the possibility that phage WO has been associated with Wolbachia for a very long time, conferring some benefit to its hosts. The evolution and possible roles of phage WO in various reproductive alterations of insects caused by Wolbachia are discussed

34. Viral contamination of shellfish: Evaluation of methods and analysis of bacteriophages and human viruses. Muniain-Mujika, I., Girones, R., Lucena, F. (2000). Journal of Virological Methods 89:109-118. Viral outbreaks attributed to the consumption of contaminated shellfish have been clearly demonstrated. Thirty-five samples of mussels collected from areas with two different levels of faecal pollution were analysed for somatic coliphages, F-RNA phages and bacteriophages infecting Bacteroides fragilis HSP40 and RYC2056 following standardised protocols, and for enterovirus, human adenovirus and hepatitis A virus by nucleic acid amplification (Nested-PCR and RT-PCR). Four methods for viral recovery from shellfish have been compared. The first method is based on the borate buffer at pH 9.5 as eluent, the second is based on glycine buffer at pH 10 as eluent, a third method is based on glycine buffer at pH 7.5 and changes in conductivity and the fourth method on nutritive broth with Tween 80 as eluent. The results obtained were analysed statistically and the method based in glycine buffer at pH 10 seems to be the most efficient and useful for the recovery of phages and human viruses. The results also show a different pattern in the proportions between the viral parameters when the source of the faecal pollution is close to or distant from the shellfish growing area

- 35. The R-type pyocin of *Pseudomonas aeruginosa* is related to P2 phage, and the F-type is related to lambda phage. Nakayama, K., Takashima, K., Ishihara, H., Shinomiya, T., Kageyama, M., Kanaya, S., Ohnishi, M., Murata, T., Mori, H., Hayashi, T. (2000). *Molecular Microbiology* 38:213-231. *Pseudomonas aeruginosa* produces three types of bacteriocins: R-, F- and S-type pyocins. The S-type pyocin is a colicin-like protein, whereas the R-type pyocin resembles a contractile but nonflexible tail structure of bacteriophage, and the F-type a flexible but non-contractile one. As genetically related phages exist for each type, these pyocins have been thought to be variations of defective phage. In the present study, the nucleotide sequence of R2 pyocin genes, along with those for F2 pyocin, which are located downstream of the R2 gene cluster on the chromosome of *P. aeruginosa* PAO1, was analysed in order to elucidate the relationship between the pyocins and bacteriophages. The results clearly demonstrated that the R-type pyocin is derived from a common ancestral origin with P2 phage and the F-type from lambda phage. This notion was supported by identification of a lysis gene cassette similar to those for bacteriophages. The gene organization of the R2 and F2 pyocin gene cluster, however, suggested that both pyocins are not simple defective phages, but are phage tails that have been evolutionarily specialized as bacteriocins. A systematic polymerase chain reaction (PCR) analysis of *P. aeruginosa* strains that produce various subtypes of R and F pyocins revealed that the genes for every subtype are located between trpE and trpG in the same or very similar gene organization as for R2 and F2 pyocins, but with alterations in genes that determine the receptor specificity
- 36. Phytoremediation of domestic wastewater for reducing populations of Escherichia coli and MS-2 coliphage. Neralla, S., Weaver, R. W. (2000). Environmental Technology 21:691-698. Sub-surfaceflow constructed wetlands enhance water quality of domestic wastewater by decreasing biochemical oxygen demand and the survival of enteric pathogens. Wetlands contain plants for aesthetic reasons and for phytoremediation. Glasshouse experiments were conducted during February and May to evaluate the role of four aquatic plant species, *Cyperus alternifolius*, *Cyperus isocladus*, *Typha latifolia*, and *Iris* sp. in reducing the populations of *E. coli* and MS-2 coliphage, indicators of bacterial and viral pathogens, respectively over 2-3 days retention. Plants were grown in 5-1 buckets containing washed gravel and domestic wastewater. Phytoremediation reduced populations of *E. coli* and MS-2 coliphage in February, but not in May. In February, plants reduced populations of *E. coli* by approximately 1.5 log 100ml-1 in 2 d, compared to 0.2 log 100 ml-1 reduction by the control without plants. In February, *C. alternifolius*, *C. isocladus* and *T. latifolia* decreased populations of MS-2 coliphage by approximately 1.5 log ml⁻¹ in 3 d

compared to 0.4-log ml⁻¹ reduction by the control without plants and Iris sp. During May, C. isocladus reduced E. coli populations from 4.5 log 100 ml⁻¹ to 1.85 log 100 ml⁻¹ in 2 d while populations in other treatments remained approximately 0.5 log 100 ml⁻¹ higher. Survival of *E. coli* and MS-2 in May was confounded by high temperature in microcosms without plants because of lack of shading, which led to poorer survival of the indicators. Phytoremediation may be one mechanism through which populations of enteric microorganisms are reduced in wetlands and may be enhanced by selection of plants for the wetland

- 37. A new bacteriophage, VHML, isolated from a toxin-producing strain of Vibrio harveyi in tropical Australia. Oakey, H. J., Owens, L. (2000). Journal of Applied Microbiology 89:702-709. Some strains of Vibrio harveyi are known to be pathogenic for fish and many invertebrates including crustaceans. Despite their importance, their modes of virulence have yet to be fully elucidated. Here, we present a previously unreported bacteriophage extracted from a toxin-producing strain of V. harveyi isolated from moribund prawn larvae in tropical Australia. Classification into the family Myoviridae was based upon morphological characteristics (an icosahedral head, a neck/collar region and a sheathed rigid tail) and nucleic acid characteristics (double-stranded linear DNA). We have termed the bacteriophage VHML (Vibrio Harveyi Myovirus Like). VHML is a temperate bacteriophage that has a narrow host range and shows an apparent preference for V. harveyi above other vibrios (63 Vibrio isolates tested) and other genera (10 other genera were tested). The conventional methods for phage concentration and extraction of nucleic acids from phage particles were not efficient and the alternative methods that were used are discussed
- 38. Protection against bacteriophage contamination in industrial fermentation processes: Investigation and applications of phage resistance mechanisms in bacteria. Ogata, S., Eguchi, T., Doi, K. (2000). *Virus (Nagoya)* 50:17-26.
- 39. Analysis of two-stage continuous operation of Escherichia coli containing bacteriophage lambda vector. Park, S. H., Park, T. H. (2000). Bioprocess Engineering 23:557-563. Bacteriophage lambda containing a cloned-gene is stably maintained in Escherichia coli in the lysogenic state while it is replicated and it overproduces a recombinant protein product in the lytic state. The host cell is eventually lysed in the lytic state. The kinetics of cell lysis and production induction were studied and are reported in this article through model equations. In two-stage continuous operation, the first tank is maintained in the lysogenic state for cell growth and cloned-gene stability while the second tank is in the lytic state for the overproduction of cloned-gene product. Individual cells in the second tank have different extent of the induction for product formation, since each has a different residence time. The different residence time for individual cells was taken into account using a population model. The numerical results show good agreement with the experimental data for the prediction of dilution rate in the second tank which gives the maximum product concentration
- 40. Transmission of viruses via contact in a household setting: Experiments using bacteriophage phiX174 as a model virus. Rheinbaben, F., Schuenemann, S., Gross, T., Wolff, M. H. (2000). Journal of Hospital Infection 46:61-66. Contamination of the environment with pathogens is the prerequisite for contact infections. The aim of this study was to elucidate how viruses can be transmitted from a primary contact person to further individuals. Bacteriophage phiX174 was chosen as a model virus. In its stability phiX174 is comparable with the most resistant human pathogenic viruses, e.g. polio- or parvoviruses. About 107 pfu were applied to exposed contact points such as door handles or the hands of volunteers. After touching of these handles and common social contacts like hand shaking, re-isolation rates were determined from the hands of our test persons. Contaminated door handles and skin surfaces were found to be efficient sources for potential infection. At least 14 persons could be contaminated by horizontal spread, one after the other by touching the same door handle. Successive transmission from one person to another could be followed up to the sixth contact person. These results were confirmed under everyday life conditions in a flat shared by four students. The transmission could not be prevented by the usual standards of hand hygiene, practised in this household. phiX174 could be reisolated after 24 h from the hands of all persons tested even after normal use and cleaning of

their hands. This might be improved by the use of liquid soap dispensers

41. Dynamics of bacterial community composition and activity during mesocosm diatom blooms. Riemann, L., Steward, G. F., Azam, F. (2000). Applied and Environmental Microbiology 66:578-587. Bacterial community composition, enzymatic activities, and carbon dynamics were examined during diatom blooms in four, 200 liter laboratory seawater mesocosms. The objective was to determine whether the dramatic shifts in growth rates and ectoenzyme activities, which are commonly observed during the course of phytoplankton blooms and their subsequent demise, could result from shifts in bacterial community composition. Nutrient enrichment of metazoan-free seawater resulted in diatom blooms dominated by Thalassiosira sp. which peaked nine days after enrichment (24 g chl a l⁻¹). At this time bacterial abundance abruptly decreased from 2.8 to 0.75 x 10⁶ ml⁻¹ and analysis of bacterial community composition, by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified, 16S rRNA gene fragments, revealed a disappearance of three dominant phylotypes. Increased viral and flagellate abundance suggested that both lysis and grazing could have played a role in the observed phylotype-specific mortality. Subsequently, new phylotypes appeared and bacterial production, abundance and enzyme activities shifted from being predominantly associated with the <1.0 m size-fraction towards the >1.0 m size-fraction indicating a pronounced microbial colonization of particles. Sequencing of DGGE bands suggested that the observed rapid and extensive colonization of particulate matter was mainly by specialized ??Proteobacteria and Cytophagales-related phylotypes. These particle-associated bacteria had high growth rates as well as high cell specific aminopeptidase, ??glucosidase and lipase activities. Rate measurements as well as bacterial population dynamics were almost identical among the mesocosms indicating that the observed bacterial community dynamics were systematic and repeatable responses to the manipulated conditions.

- 42. Progressive specific immune attrition after primary, secondary and tertiary immunizations with bacteriophage PHI X174 in asymptomatic HIV-1 infected patients. Rubinstein, A., Mizrachi, Y., Bernstein, L., Shliozberg, J., Golodner, M., Liu, G. Q., Ochs, H. D. (2000). AIDS (Hagerstown). 14:F55-F62. Background: Antibody responses to immunization are often compromised in patients infected by HIV-1, and the use of childhood immunization in affected children is controversial. We investigated whether multiple immunizations with a T cell-dependent neoantigen, bacteriophage PHI X174, induce selective immune attrition and post-vaccination viremia. Methods: Seventeen asymptomatic, antiretroviral therapy-naive HIV-1-infected patients with a CD4 cell count of 450 cells/mul or greater were immunized in 1990/1991 with three intravenous doses of bacteriophage PHI X174. Group 1 received zidovudine (ZDV) during the primary and secondary immunization. Group 2 received ZDV exclusively during the tertiary immunization. Bacteriophage-specific antibodies of the IgM and IgG class, lymphocyte phenotypes (CD4+, CD8+, CD4+DR+, CD8+DR+, CD4+CD45RO+ and CD4+45RA+, CD4+CD45RO+DR+) and HIV-1 plasma viremia were measured sequentially. Results: In both patient groups the primary, secondary and tertiary antibody responses, as expressed by geometric mean antibody titres and IgM to IgG switch, were impaired. Booster immunizations resulted in a progressive attrition of specific antibody responses to bacteriophage. Antibodies to tetanus toxoid remained stable. The HIV-1 viral loads, which were evaluated in archived specimens from eight patients, increased after immunization but returned to baseline appoximately 4 weeks later. The humoral immune attrition and increases in plasma viremia were blunted by concomitant short courses of ZDV. Discussion: Multiple boosters of immunizations in asymptomatic treatment-naive HIV-1infected patients may result in a specific immune attrition and vaccine-induced viremia. Short-term monotherapy with ZDV may have blunted these adverse effects. Hyperimmunization of HIV-1-infected patients may be detrimental unless accompanied by antiretroviral therapy
- 43. Removal efficiencies of indicator micro-organisms in the Al-Khobar wastewater treatment plant. Saleem, M., Bukhari, A. A., Al-Malack, M. H. (2000). Environmental Engineering Science 17:227-232. Increasing population and developmental needs of Saudi Arabia underline the need for an increase in the reuse of treated wastewater. However, treated wastewater contains a large number of pathogens that requires proper treatment before reuse. Little information is available on the treatment efficiency of wastewater treatment plants operating in this region. A 1-year study was conducted at the Al-Khobar wastewater treatment plant to investigate the removal efficiency of five indicator micro-organisms, namely, Standard Plate Count, total coliform, fecal coliform, coliphage, and Clostridium perfringens. The raw sewage, secondary effluent, and chlorinated effluent were analyzed weekly for the detection and enumeration of these indicator micro-organisms. High-percent removal of Standard Plate Count, total coliform, and fecal coliform (98 to 99%) was observed after secondary treatment compared to coliphage removal of 83.6% and Clostridium perfringens removal of 55.5%, whereas, after chlorination, Standard Plate Count, total coliform, and fecal coliform were removed up to 99.7% compared to coliphage reduction of 52% and Clostridium perfringens removal of coliphage removal of only 42%, showing a high resistance against chlorination. The insight gained from this study may be applied to other similar treatment plants
- 44. Removal of microorganisms by deep well injection. Schijven, J. F., Medema, G., Vogelaar, A. J., Hassanizadeh, S. M. (2000). Journal of Contaminant Hydrology 44:301-327. The removal of bacteriophages MS2 and PRD1, spores of Clostridium bifermentans (R5) and Escherichia coli (WR1) by deep well injection into a sandy aquifer, was studied at a pilot field site in the southeast of the Netherlands. Injection water was seeded with the microorganisms for 5 days. Breakthrough was monitored for 93 days at 4 monitoring wells with their screens at a depth of about 310 m below surface. Within the first 8 m of soil passage, concentrations of MS2 and PRD1 were reduced by 6 log10, that of R5 spores by 5 log10 and that of WR1 by 7.5 log10. Breakthrough of MS2 and R5 could also be followed at greater distances from the injection well. Concentrations of MS2 were reduced only by about 2 log10 in the following 30 m, and reduction of concentrations of R5 was negligible. Apparently, attachment was greater during the first 8 m of aquifer passage. At the point of injection, the inactivation rate coefficient of free MS2 was found to be 0.081 day-1, that of free PRD1 0.060 day-1, and that of E. coli strain WR1 0.063 day-1. In injection water that had passed 8 m of soil, inactivation of MS2 phages was found to be less than in water from the injection well: 0.039 day-1. Probably, the higher inactivation rate of MS2 in water from the injection well may be ascribed to the activity of aerobic bacteria. Inactivation of the R5 spores was not significant. From geochemical mass balances, it could be deduced that within the first 8 m distance from the injection well, ferric oxyhydroxides precipitated as a consequence of pyrite oxidation, but not at larger distances. Ferric oxyhydroxides provide positively charged patches onto which fast attachment of the negatively charged microorganisms may take place. The non-linear logarithmic reduction of concentrations with distance may therefore be ascribed to preferable attachment of microorganisms to patches of ferric oxyhydroxides that are present within 8 m distance from the injection point, but not thereafter. Declogging of the injection well introduced hydrodynamic shear that remobilized MS2, which was then transported farther downstream
- 45. Discovery, purification, and characterization of a temperate transducing bacteriophage for *Bordetella avium*. Shelton, C. B., Crosslin, D. R., Casey, J. L., Ng, S., Temple, L. M., Orndorff, P. E. (2000). *Journal of Bacteriology* 182:6130-6136. We discovered and characterized a temperate transducing bacterlophage (Ba1) for the avian respiratory pathogen *Bordetella avium*. Ba1 was initially identified along with one other phage (Ba2) following screening of four strains of *B. avium* for lysogeny.

Of the two phage, only Ba1 showed the ability to transduce via an allelic replacement mechanism and was studied further. With regard to host range, Ba1 grew on six of nine clinical isolates of B. avium but failed to grow on any tested strains of Bordetella bronchiseptica, Bordetella hinzii, Bordetella pertussis, or Bordetella parapertussis. Ba1 was purified by CsCl gradient centrifugation and was found to have an icosahedral head that contained a linear genome of approximately 46.5 kb (contour length) of double-stranded DNA and a contractile, sheathed tail. Ba1 readily lysogenized our laboratory B. avium strain (197N), and the prophage state was stable for at least 25 generations in the absence of external infection. DNA hybridization studies indicated the prophage was integrated at a preferred site on both the host and phage replicons. Ba1 transduced five distinctly different insertion mutations, suggesting that transduction was generalized. Transduction frequencies ranged from approximately 2 X 10-7 to 1 X 10-8 transductants/PFU depending upon the marker being transduced. UV irradiation of transducing lysates markedly improved transduction frequency and reduced the number of transductants that were lysogenized during the transduction process. Ba1 may prove to be a useful genetic tool for studying *B. avium* virulence factors

46. The interactions of peptides with the innate immune system studied with use of T7 phage peptide display [see comments]. Sokoloff, A. V., Bock, I, Zhang, G., Sebestyen, M. G., Wolff, J. A. (2000). Mol Ther 2:131-139. The icosahedral T7 phage (diameter approximately 65 nm) displaying random peptides at the carboxy-terminus of the phage coat proteins was used as a model for drug and gene delivery vehicles containing peptide ligands. We found that displayed peptides were recognized by natural antibodies and induced complement activation. Strikingly, the phage inactivation by complement was peptide-specific that implied the existence of numerous natural antibodies with different peptide specificity. Selection of phage that avoided inactivation by complement allowed the identification of peptides that protected the phage against complement-mediated inactivation by binding C-reactive protein. In human serum, a number of protective peptides with tyrosine residues were selected. The recognition of displayed peptides by natural antibodies appears to represent a universal mechanism for activation of complement at sites that contain identical or homologous proteins with exposed carboxy-termini

47. Genome size distributions indicate variability and similarities among marine viral assemblages from diverse environments. Steward, G. F., Montiel, J. L., Azam, F. (2000). *Limnology and Oceanography* 45:1697-1706. Pulsed field gel electrophoresis (PFGE) was used to determine the size distributions of virus-like DNA in seawater from diverse environments (Arctic Ocean, Ross Sea, Coastal Pacific Ocean, and Northern Adriatic Sea). Changes in DNA banding patterns indicated that shifts in the viral assemblage composition occurred on the order of = 2 d during an intense dinoflagellate bloom in coastal Pacific waters. Different DNA banding patterns from diverse locations also indicated spatial variability in composition, but all of the samples analyzed had similar features. Size frequency distributions for virus-like genomes (VLGs) were multi-modal with major peaks occurring around 31-36 kilobases (kb) and 58-63 kb. The smallest discrete band resolved was 26 kb and the largest was >200 kb and the overall mean virus-like genome size was 50 ± 4 kb (mean ± sd, n = 30). On average, in surface seawater, > 90% of the VLGs occurred in the 26-69 kb size range and at least half were between 28 to 45 kb. This first extensive survey of viral genome sizes in seawater indicates that most marine viruses have physical properties similar to other known viruses. The distributions revealed that the vast majority of the detected VLGs have sizes typical of bacteriophages while only a few percent were in the size range of known algal viruses.

48. Analysis of marine viral assemblages. Steward, G. F., Azam, F. (2000). pp. 159-165 in Bell, C. R., Brylinski, M., Johnson-Green, P. (eds.) *Microbial Biosystems: New Frontiers.*. Atlantic Canada Society for Microbial Ecology, ??? Viruses are the numerically dominant microbes in every oceanic environment from the surface into the sediments. A liter of

surface seawater from a typical mesotrophic area contains 10¹⁰ of them, about ten times more than bacteria. While total counts of viruses are becoming easier to make, we still know very little about the viruses that comprise a given assemblage. Infectivity assays are extremely useful and still the best way to assay for infectious viruses for any particular host. However, this approach requires that each potential host organism be cultured, making it impractical if not impossible to completely characterize natural assemblages. Morphological studies have been enlightening, but are time consuming and difficult to do quantitatively. Here we report a fingerprinting approach to characterize natural viral assemblages. In this approach, viruses are concentrated and intact viral genomes are separated based on their size via pulsed-field gel electrophoresis. The number of distinguishable bands provides a minimum estimate of the number of different viruses, while band position and staining intensity reveal the genome size distribution within the assemblage. With this technique we have detected spatial and temporal differences, as well as many similarities, in viral assemblages among a variety of marine habitats. Current efforts are directed toward combining this technique with other methods of fractionation and sequence analysis to allow both morphological and genetic description of uncultivated marine viruses. Direct investigation of dominant or particularly widespread viruses may ultimately provide clues as to which marine organisms contribute most to the viral pool, and which organisms are likely to be significantly influenced by viral mortality.

49. Use of bioluminescent Salmonella for assessing the efficiency of constructed phage-based biosorbent. Sun, W., Brovko, L., Griffiths, M. (2000). Journal of Industrial Microbiology & Biotechnology 25:273-275. A bacteriophage-based biosorbent for Salmonella enteritidis was constructed, and bacterial bioluminescence was used for assessment of the efficiency of cell capture. A strain of *S. enteritidis* with bioluminescent phenotype was constructed by transformation with plasmid pT7 carrying the entire lux operon from *Photorhabdus luminescens*. The relation between relative light output (RLU) and colony-forming units (CFU/ml) of the bioluminescent strain was established. The bacteriophage specific to *S. enteritidis* was biotinylated, and the biotinylation procedure was optimized based on the maximum retention of phage infectivity. The biotinylated phages were then coated onto streptavidin-labeled magnetic beads, and were used to capture the bioluminescent *S. enteritidis* cells. Our preliminary results showed that the number of cells captured by constructed biosorbent was five times higher than that of the control, magnetic beads coated with nonbiotinylated phage, indicating the capture is specific

50. Fate of indigenous bacteriophage in a membrane bioreactor. Ueda, T., Horan, N. J. (2000). Water Research 34:2151-2159. Indigenous bacteriophage was isolated from a conventional sewage treatment plant treating a largely domestic wastewater. The isolated phage was T-even-like with a mean size of 200 nm and was used to indicate the viral removal efficiency achieved in a bench-scale membrane bioreactor (MBR). The MBR incorporated three flat microfiltration membrane modules which were polyethylene with a pore size of 0.4 mum. When treating settled domestic sewage, the MBR achieved an overall removal rate of phage of 2.3-5.9 log across the treatment process. The membrane alone demonstrated a poor phage removal efficiency, but removal efficiency increased as the filtration resistance was increased. It was proposed therefore, that the biofilm accumulating on the surface of the membrane made a major contribution to phage removal. The MBR demonstrated an almost complete removal of faecal coliforms and faecal streptococci (up to 7 log). By comparison a full-scale treatment plant treating the same settled sewage and incorporating tertiary treatment, achieved only up to 2 log removal of the same excreted phage and bacteria

- 51. Killing of flies in electrocuting insect traps releases bacteria and viruses. Urban, J. E., Broce, A. (2000). Current Microbiology 41:267-270. Electrocuting insect traps (EIT) are popular devices frequently used by homeowners and food handlers attempting to localize the control of flying insects, including the ubiquitous house fly (Musca domestica L.). The traps contain a visual attractant and a high-voltage metal grid. Upon contact with the grids, the insects are disintegrated by the high voltage. As part of a systematic evaluation of EITs and their role in infectious disease spread, we quantitated spread of bacteria and a bacterial virus during electrocution of house flies. We loaded flies with Serratia marcescens or with the Escherichia coli phage PHIX174 and placed sprayed or fed flies into a room containing an EIT. While flies were being electrocuted, liberated particles and bacteria or viruses, and fed flies released one of every 1,000,000 of the consumed bacteria or viruses. Results of our studies suggest EITs could play a role in the spread of infectious disease agents, but the potential is influenced by the insect's route of contamination
- 52. Models of experimental evolution: The role of genetic chance and selective necessity. Wahl, L. M., Krakauer, D. C. (2000). Genetics 156:1437-1448. We present a theoretical framework within which to analyze the results of experimental evolution. Rapidly evolving organisms such as viruses, bacteria, and protozoa can be induced to adapt to laboratory conditions on very short human time scales. Artificial adaptive radiation is characterized by a list of common observations; we offer a framework in which many of these repeated questions and patterns can be characterized analytically. We allow for stochasticity by including rare mutations and bottleneck effects, demonstrating how these increase variability in the evolutionary trajectory. When the product Np, the population size times the per locus error rate, is small, the rate of evolution is limited by the chance occurrence of beneficial mutations; when Np is large and selective pressure is strong, the rate-limiting step is the waiting time while existing beneficial mutations sweep through the population. We derive the rate of divergence (substitution rate) and rate of fitness increase for the case when Np is large and illustrate our approach with an application to an experimental data set. A minimal assumption of independent additive fitness contributions provides a good fit to the experimental evolution of the bacteriophage phiX174
- 53. Experimental evolution recapitulates natural evolution. Wichman, H. A., Scott, L. A., Yarber, C. D., Bull, J. J. (2000). *Philosophical Transactions of the Royal Society of London B Biological Sciences* 355:1677-1684. Genomes of the closely related bacteriophages phiX174 and S13 are 5386 bases long and differ at 114 nucleotides, affecting 28 amino acids. Both parental phages were adapted to laboratory culture conditions in replicate lineages and analysed for nucleotide changes that accumulated experimentally. Of the 126 experimental substitutions, 90% encoded amino-acid changes, and 62% of the substitutions occurred in parallel in more than one experimental line. Furthermore, missense changes at 12 of the experimental sites were at residues differing between the parental phages; in ten cases the phiX174 experimental lineages were convergent with the S13 parent, or vice versa, at both the nucleotide and amino-acid levels. Convergence at a site was even obtained in both directions in three cases. These results point to a limited number of pathways taken during evolution in these viruses, and also raise the possibility that much of the amino-acid variation in the natural evolution of these viruses has been selected
- 54. Effect of alcohols on Escherichia coli phages. Yamashita, M., Murahashi, H., Tomita, T., Hirata, A. (2000). Biocontrol Science 5:9-16. Escherichia coli T even phages were ethanol sensitive, whereas T odd phages were ethanol tolerant. The phagecidal activities of alcohols on ethanol sensitive phages (T even, lambda) were revealed to be in the order n-propanol>ethanol>methanolmchgtn-butanolmchgtn-hexanolmchgtn-octanol . However, the those(?) of alcohols on ethanol tolerant phages (T odd) were revealed to be in the order methanol>ethanol>n-propanol. The phagecidal activity of n-butanol (hydrophobic) increased when small amounts of hydrophilic methanol (10 and 20%, v/v), ethanol (10 and 20%, v/v) were added. The relative solubility curves of glucose plus Sudan III (hydrophobicity-hydrophilicity indication) were similar to the lambda phagecidal activity curves of ethanol solution. The denaturation of ethanol-sensitive phage proteins with ethanol solution (50 or 70%, v/v) on SDS-polyacrylamide gel electrophoresis was associated with a loss of the plaque-forming activity of the phage. These phenomena were not observed for the ethanol-tolerant phages treated with ethanol solutions. The head proteins of ethanol-sensitive phage have a greater number of hydrophilic amino acids and fewer hydrophilic amino acids, whereas those of ethanol sensitive phage have a greater number of hydrophilic amino acids and fewer hydrophilic amino acids. From these data, we suggest that the hydrophobicity-hydrophilicity balance of the alcohol solution and the phage surface proteins affects the loss of the plaque-forming activity of *E. coli* phages by alcohol
- 55. High-frequency transduction (HFT) of resistance to ceftazidime and other antibiotics by a wild-type *Pseudomonas* aeruginosa phage. Blahová, J., Králiková, K., Krcméry, V., Sr., Bartoníková, N., Mikovicova, A (1999). *Zentralblatt Fuer* Bakteriologie 289:179-183.
- 56. High-frequency transduction of antibiotic resistance in *Pseudomonas aeruginosa* by a wild-type bacteriophage with restricted specificity for recipient strains. Blahová, J., Králiková, K., Krcméry, V., Sr., Bartoníková, N. (1999). *European Journal of Clinical Microbiology & Infectious Diseases* 18:152-154.
 - 57. Alternative mechanism of cholera toxin acquisition by Vibrio cholerae: Generalized transduction of CTXPHI by bacteriophage CP-T1. Boyd, E. F., Waldor, Matthew K. (1999). Infection and Immunity 67:5898-5905. Horizontal transfer of genes encoding virulence factors has played a central role in the evolution of many pathogenic bacteria. The unexpected discovery that the genes encoding cholera toxin (ctxAB), the main cause of the profuse secretory diarrhea characteristic of cholera, are encoded on a novel filamentous phage named CTXPHI, has resulted in a renewed interest in the potential mechanisms of transfer of virulence genes among *Vibrio cholerae*. We describe here an alternative mechanism of cholera toxin gene transfer into nontoxigenic *V. cholerae* isolates, including strains that lack both the CTXPHI receptor, the toxin coregulated pilus (TCP), and attRS, the chromosomal attachment site for CTXPHI integration. A temperature-sensitive mutant of the *V. cholerae* generalized transducing bacteriophage CP-T1 (CP-T1ts) was used to transfer a genetically marked derivative of the CTX prophage into four nontoxigenic *V. cholerae* strains, including two *V. cholerae* vaccine strains. We demonstrate that CTXPHI transduced by CP-T1ts can replicate and integrate into these nontoxigenic *V. cholerae* strains with high efficiency. In fact, CP-T1ts transduces the CTX prophage preferentially when compared with other chromosomal markers. These results reveal a potential mechanism by which CTXPHI+ *V. cholerae* strains that lack the TCP receptor may have arisen. Finally, these findings indicate an additional pathway for reversion of live-attenuated *V. cholerae* vaccine strains
- 58. Induction and characterization of *Pediococcus acidilactici* temperate bacteriophage. Caldwell, S. L., McMahon, D. J., Oberg, C. J., Broadbent, J. R. (1999). Systematic and Applied Microbiology. 22:514-519. Mitomycin C was used to induce temperate bacteriophage from three strains of *Pediococcus acidilactici*. The new bacteriophage, designated pa97, pa40, and pa42, were characterized based on morphology, DNA homology, and major protein profiles. Morphological attributes (small

isometric heads with non-contractile tails) place these bacteriophages within the B1 group of the family Siphovirdae. Restriction endonuclease digests suggested that the bacteriophage genomes were linear molecules without cohesive ends, and between 33 and 37 kilobases in length. All three bacteriophages possessed one major protein with an estimated mass of 30 to 35 kilodaltons. Bacteriophage pa42 also contained a second major protein of approximately 47 kilodaltons. DNA-DNA hybridization showed bacteriophages pa40 and pa42 were homologous to each other, but not to pa97, suggesting that *Pediococcus acidilactici* bacteriophage fall into at least two different species

- 59. Adsorption of bacteriophages on clay minerals. Chattopadhyay, S., Puls, R. W. (1999). Environmental Science & Technology 33:3609-3614. The ability to predict the fate of microorganisms in soil is dependent on an understanding of the process of their sorption on soil and subsurface materials. Presently, we have focused on studying the thermodynamics of sorption of bacteriophages (T-2, MS-2, and variant phiX-174) on clays (hectorite, saponite, kaolinite, and clay fraction of samples collected from a landfill site). The thermodynamic study not only determines the feasibility of the process but also provides information on the relative magnitudes of the different forces under a particular set of conditions. The total free energy of interaction during sorption of bacteriophages on clays (DELTAG) has been assumed to be the summation of DELTAGH (DELTAG due to hydrophobic interactions) and DELTAGEL (DELTAG due to electrostatic interactions). The magnitude of DELTAGH was determined from the different interfacial tensions (gamma) present in the system, while DELTAGEL was calculated from zeta-potentials of the colloidal particles. Calculated results show that surface hydrophobicities of the selected sorbents and sorbates dictate sorption. Among the selected bacteriophages, maximum sorption studies also corroborated those obtained from the theoretical study
- 60. Inactivation of faecal indicator microorganisms in waste stabilisation ponds: Interactions of environmental factors with sunlight. Davies-Colley, R. J., Donnison, A. M., Speed, D. J., Ross, C. M., Nagels, J. W. (1999). Water Research 33:1220-1230. Sunlight exposure is considered to be the most important cause of "natural" disinfection in waste stabilisation ponds (WSPs). We examined the influence of dissolved oxygen (DO), pH, and particulate and dissolved constituents in WSP effluent, on sunlight inactivation of faecal micro-organisms, using small reactors operated under controlled physico-chemical conditions. Inactivation of both enterococci and F-RNA phages increased strongly as DO was increased, and also depended on light-absorbing pondwater constituents, but pH was not influential over the range investigated (7.5 to 10). Inactivation of *E. coli* increased strongly when pH increased above 8.5, as well as being strongly dependent on DO. Inactivation of F-DNA phage was independent of the factors investigated. These results are consistent with the F-DNA phages being inactivated as a result of direct DNA damage by UVB in sunlight, whereas the other three microbiological indicators are inactivated as a result of photo-oxidative damage, although the target of damage is apparently different. Our findings of diverse influences of physico-chemical conditions suggest difficulties in interpreting data for a single micro-organism to indicate WSP effluent quality. However, sunlight remains the factor of over-riding importance, and disinfection in WSPs may be enhanced by increasing sunlight exposure
- 61. Rapid transport of viruses in a floodplain aquifer. DeBorde, D. C., Woessner, W. W., Kiley, Q. T., Ball, P. (1999). Water Research 33:2229-2238. An unconfined floodplain aquifer near Missoula, MT, was instrumented with 89 monitoring wells and 20 four-port multilevel samplers. Bromide, bacteriophages MS2, PRD1 and phiX174 and the attenuated enterovirus, polio virus (type-1 CHAT strain), were seeded into the aquifer as slug injections. Bromide transport rates ranged between 22-29 m/d. Input concentrations of the tracers and the placement of monitoring wells limited detection of bromide and polio virus to 19.4 m and the detection of three bacteriophage to 40.5 m downgradient from the injection point. After 7.5 m of transport, the calculated relative attenuations (Harvey R. W and Garabedian S. P. (1991) Env. Sci. Tech. 25, 178-185) for MS2, PRD-1, phiX174 and attenuated polio virus were 49, 71, 65 and 99%, respectively. During the 72-h experiment, die-off was negligible (less than 1%) and attachment of virus to sediment surfaces resulted in the overall differences in bromide and virus behavior. Although relative attenuations at downgradient monitoring wells at rates similar to the bromide peak. The high collision efficiency of the attenuated polio virus resulted in breakthrough curve truncation. Natural attenuation of slug input virus over a "typical" source-supply set-back distance of 30.5 m would most likely not reduce virus concentrations to proposed acceptable risk levels in this or a similar cold-water high-velocity groundwater system
- 62. Presence of bacteriophages in different stages of wastewater treatment. Donia, D., Divizia, M., Pana, A., Gabrieli, R., Gasbarro, M., Capuani, L., Morelli, A. L. (1999). *Igiene Moderna* 111:239-251. The presence and correlation among somatic coliphages, F-specific phages and the phage of *Bacteroides fragilis* in a wastewater treatment plant was evaluated. The efficiency of the treatment for bacteriological parameters was confirmed with a reduction of total coliforms of 99,9%, fecal coliforms of 96,0% and fecal streptococci of 97,9%. A similar abatement was present for F-specific phages and the phage of *Bacteroides fragilis* (2.0 log) whereas the somatic coliphages appeared stable. Cytopathogenic enteric viruses were also recovered in the different withdrawal points with different efficiency according to the methods used. A positive correlation was found in the inlet samples among coliphages, phage of *Bacteroides fragilis* and enteric viruses
 - 63. Horizontal gene transfer among bacteria in terrestrial and aquatic habitats as assessed by microcosm and field studies. Droege, M., Puehler, A., Selbitschka, W. (1999). Biology and Fertility of Soils 29:221-245. Genetic interactions among bacteria are mediated by one of the three distinct gene-exchange mechanisms: conjugation, transformation or transduction. Conjugative gene exchange relies on mobile elements, such as plasmids, which transfer between donor and recipient cells. In natural transformation, competent cells take up DNA and incorporate it into their genome. Gene transfer via transduction is mediated by bacteriophages which accidentally package donor DNA in their phage head and transfer it to recipient cells. Driven mainly by biosafety research and research into the rapid dissemination of antibiotic resistance, the evaluation of gene flux among bacteria in their natural habitats has become a focus of scientific interest in recent years. Accordingly, gene transfer has been assessed in laboratory-based studies employing model ecosystems, as well as in field experiments. Conjugative gene exchange has been shown to occur under a wide range of environmental conditions. Factors identified as conducive for conjugation include the presence of nutrients provided by the rhizosphere of plants. Studies addressing gene transfer via transformation have demonstrated that naturally transformable bacteria develop competence and take up DNA under in situ conditions. Moreover, DNA has been shown to persist to some extent in the environment, and thus be available for uptake by naturally competent cells. Gene exchange via transduction has been demonstrated under conditions of nutrient depletion and low densities of host cells. Whereas gene transfer is readily observed in the laboratory, more importantly, field studies have provided direct evidence that all three gene transfer mechanisms also occur in nature. DNA transfer frequencies observed in the environment in some cases differed considerably from those obtained under laboratory conditions. Transfers of low frequency observed in laboratory-based experiments have been readily detected in the environment in the presence of selective forces

64. Development of reduced acridines as antiprophage agents. El-Bermawy, M. A., Kadry, A., El-Didamony, G., Amin, M. (1999). Chinese Pharmaceutical Journal (Taipei) 51:191-200. A set of 9,10-diphenyldecahydroacridine-1, 8-diones 3-7 with different electronic characteristics were synthesized and tested as antiprophages, curing and as antimicrobial agents. All compounds except the 4-nitrophenyl 7 showed inhibition of prophage lambda (lambda) induction. These compounds appeared to have different levels of antiprophage activity. The 4-methyl derivative 5 has the highest inhibition of the prophage lambda induction. The prepared acridines were tested against standard strains of microorganisms. The minimum inhibitory concentrations (MICs) were 0.5, 0.6, 3.0, 3.5 and 4.0 mg/mL for compounds 7, 5, 6, 3 and 4 respectively. The mutagenic activity of the prophage inducing agent 7 was also investigated. Compound 7 has a curing activity on the plasmids of clinical isolates of *E. coli*

65. Lysogenic Conversion of Environmental Vibrio mimicus Strains by CTXF. Farugue, S. M., Rahman, M. M., Asadulghani, K. M., Islam, N., Mekalanos, J. J. (1999). Infection and Immunity 67:5723-5729. The filamentous bacteriophage CTXF, which encodes cholera toxin (CT) in toxigenic Vibrio cholerae, is known to propagate by infecting susceptible strains of V. cholerae by using the toxin coregulated pilus (TCP) as its receptor and thereby causing the origination of new strains of toxigenic V. cholerae from nontoxigenic progenitors. Besides V. cholerae, Vibrio mimicus strains which are normally TCP negative have also been shown to occasionally produce CT and cause diarrhea in humans. We analyzed nontoxigenic V. mimicus strains isolated from surface waters in Bangladesh for susceptibility and lysogenic conversion by CTXF and studied the expression of CT in the lysogens by using genetically marked derivatives of the phage. Of 27 V. mimicus strains analyzed, which were all negative for genes encoding TCP but positive for the regulatory gene toxR, 2 strains (7.4%) were infected by CTX-KmF, derived from strain SM44(P27459 ctx::km), and the phage genome integrated into the host chromosome, forming stable lysogens. The lysogens spontaneously produced infectious phage particles in the supernatant fluids of the culture, and high titers of the phage could be achieved when the lysogens were induced with mitomycin C. This is the first demonstration of lysogenic conversion of V. mimicus strains by CTXF. When a genetically marked derivative of the replicative form of the CTXF genome carrying a functional ctxAB operon, pMSF9.2, was introduced into nontoxigenic V. mimicus strains, the plasmid integrated into the host genome and the strains produced CT both in vitro and inside the intestines of adult rabbits and caused mild-to-severe diarrhea in rabbits. This suggested that in the natural habitat infection of nontoxigenic V. mimicus strains by wildtype CTXF may lead to the origination of toxigenic V. mimicus strains which are capable of producing biologically active CT. The results of this study also supported the existence of a TCP-independent mechanism for infection by CTXF and showed that at least one species of Vibrio other than V. cholerae may contribute to the propagation of the phage

66. Phage typing of Campylobacter jejuni and Campylobacter coli and its use as an adjunct to serotyping. Frost, J. A., Kramer, J. M., Gillanders, S. A. (1999). Epidemiology and Infection 123:47-55. Campylobacter is the most commonly reported cause of gastro-intestinal infection in England and Wales, with over 50000 reported cases in 1997. The majority of human campylobacter isolates in England and Wales are *C. jejuni* (c. 90%) with most of the remainder being *C. coli*. We describe the use of phage typing as an extension to serotyping for more detailed characterization within these two species. The scheme was piloted during a study of 2407 *C. jejuni* and 182 *C. coli* strains isolated in Wales between April 1996 and March 1997. Fifty-seven *C. jejuni* phage types were identified, with the ten most prevalent phage types accounting for 60% of isolates tested; 16% of isolates were untypable. The most common phage type was PT 1 which represented c. 20% of isolates. A further 7% of isolates reacted with the phages but did not conform to a designated type (RDNC). Only 12 phage types were identified among *C. coli*, with the two most common types, PT 2 and PT 7 accounting for 75.2% of isolates. When used in conjunction with serotyping, the ability of phage typing to identify between 6 and 29 subtypes within each of the predominant HS types has enabled a further level of discrimination to be achieved that enhances the epidemiological typing of *C. jejuni* and *C. coli*

67. Removal of viruses by microfiltration membranes at different solution environments. Herath, G., Yamamoto, K., Urase, T. (1999). Water Science and Technology 40:331-338. Rejection change by nuclepore microfiltration membranes with solution environment was investigated by using the RNA coliphages Qbeta, MS2, fr and DNA coliphage T4. The obtained rejection results showed a higher rejection at lower pH than at higher pH for all viruses. The highest rejection for all viruses were obtained at pH closer to their isoelectric points and also the rejection variation indicates a similar pattern of behavior. This phenomenon of higher virus rejection at lower pH is explained with a possible viruses aggregation with each other due to their own electrostatic charge and isoelectric points. Also it was observed that the virus rejection was enhanced when they are in mixed environment. Finally the effect of protein was studied where, the virus rejection below pH 5.0 showed protein influence

68. Changing consumer water-use patterns and their effect on microbiological water quality as a result of an engineering intervention. Jagals, P., Bokako, T. C., Grabow, W. O. K. (1999). Water S A (Pretoria) 25:297-326. A previous study done during 1994-1995 in a section of a large, low socio-economic urban development with limited sanitary facilities and drinking-water provision indicated that the community was exposed to water-related health risks when consuming the water supplied. The study indicated that, although the public supplied water was of a good quality, the stored water, once fetched from the standpipes, deteriorated to a quality often not safe for human consumption. Based on the findings of this previous study, the local authority decided to install standpipes for each individual family in the area concerned and these were placed in the house yards. The closer proximity of the standpipes immediately altered the water-fetching and storing patterns of the community. The consequent study, on which this abstract is based, assessed the potential risk of infection posed to health by the altered water-use pattern. Weekly water samples were collected from standpipes outside as well as from containers kept inside houses of selected families. Total coliforms, faecal coliforms, heterotrophic plate counts, *Clostridium perfringens* and somatic coliphages were used as microbiological indicators. Although the improvement of water accessibility enhanced the microbiological quality of stored water, the results indicated that hygienic quality still deteriorated. This situation indicated that a suitable education and information programme to enhance the quality gains of such engineering interventions should accompany engineering improvement of water accessibility

69. Development of lytic *Lactococcus lactis* bacteriophages in a Cheddar cheese plant. Josephsen, J., Petersen, A., Neve, H., Nielsen, E. W. (1999). *International Journal of Food Microbiology* 50:163-171. The mixed TK5 starter culture was used in a Danish factory as the only starter for production of Cheddar cheese for more than 11 years before the factory experienced serious bacteriophage attacks with inhibition of the acid production in the curd. The cheese whey contained some phages from the beginning, and gradually new phages appeared able to infect an increasing number of isolates. Three bacteriophages jw30, jw31 and jw32 were isolated from the factory whey collected in 1989-94 and compared with lytic bacteriophages isolated in the period 1982-86 (Josephsen et al., 1994) DNA hybridisation showed that the type phage P008 had high homology to the new phages jw30, jw31 and jw32 as had the phages isolated in 1982-84. The new phages had broader host ranges and higher burst sizes than the previously isolated phages, showing that the lytic phages had become more virulent

- 70. Cryostabilization of biological properties of Yersinia pestis phages. Kadetov, V. V., Kudryakova, T. A., Terentyev, A. N., Kachkina, G. V., Borodina, T. N., Sayamov, S. R. (1999). Voprosy Virusologii 44:136-139. Conditions of kryostabilization of Yersinia pestis phages preserving their biological properties at very low temperature are studied
- 71. A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. Karaolis, D. K. R., Somara, S., Maneval, D. R., Jr., Johnson, J. A., Kaper, J. B. (1999). Nature (London) 399:375-379. The virulence properties of many pathogenic bacteria are due to proteins encoded by large gene clusters called pathogenicity island, which are found in a variety of human pathogens including *Escherichia coli*, *Salmonella*, *Shigella*, Yersinia, *Helicobacter pylori*, *Vibrio cholerae*, and animal and plant pathogens such as *Dichelobacter nodosus* and *Pseudomonas syringae*. Although the presence of pathogenicity islands is a prerequisite for many bacterial diseases, little is known about their origins or mechanism of transfer into the bacterium. The bacterial agent of epidemic cholera, *Vibrio cholerae*, contains a bacteriophage known as cholera-toxin phage (CTXPHI), which encodes the cholera toxin, and a large pathogenicity island called the VPI (for *V. cholerae* pathogenicity island) which itself encodes a toxin-coregulated pilus that functions as a colonization factor and as a CTXPHI receptor. We have now identified the VPI pathogenicity island as the genome of another filamentous bacteriophage, VPIPHI. We show that VPIPHI is transferred between *V. cholerae* strains and provide evidence that the TcpA subunit of the toxin-coregulated type IV pilus is in fact a coat protein of VPIPHI. Our results are the first description of a phage that encodes a receptor for another phage and of a virus-virus interaction that is necessary for bacterial pathogenicity
- 72. Biological projectiles (phage, yeast, bacteria) for genetic transformation of plants. Kikkert, J. R., Humiston, G. A., Roy, M. K., Sanford, J. C. (1999). In Vitro Cellular & Developmental Biology Plant 35:43-50. Bacteriophage lambda particles, yeast cells, and bacterial cells were tested as projectiles to deliver marker/reporter genes into plant cells via the biolistic process. When phage particles were complexed to tungsten or gold particles and used to bombard tobacco cells, fewer than 15 cell clusters per plate transiently expressed beta-glucuronidase (GUS). Cells of wild-type Saccharomyces cerevisiae were too large to be effective projectiles, but use of a reduced-size mutant resulted in a small number of transformants. Escherichia coli cells complexed with tungsten were the most effective projectile for plant transformation. Various methods to prepare E. coli were tested to reduce particle size, improve binding of bacteria to metal particles, and/or minimize particle clumping. In maize, the number of transformants was highest when bacteria/tungsten particles were air-dried onto macrocarriers from an aqueous solution. When maize cells were bombarded with bacteria/tungsten projectiles, rates of transient gene expression (2000 per plate) and stable transformation (50 per plate) were only two- to threefold lower than when purified DNA was used. Transformation of tobacco with E. coli projectiles was improved when the bacteria were treated with a series of ethanol and ether washes, then dried into a powder. Nevertheless, tobacco transformation was still 24- (transient) and 200-fold (stable) less than when purified DNA was used. Biological projectiles can be effective for plant transformation and are advantageous because once a DNA construct is made and put into the appropriate microorganism, the need to isolate and purify DNA for the biolistic process is eliminated, which saves time and lessens DNA shear. Such projectiles may be especially well suited where high molecular weight DNA constructs are needed
 - 73. Comparative genomics of Streptococcus thermophilus phage species supports a modular evolution theory. Lucchini, S., Desiere, F., Brussow, H. (1999). Journal of Virology 73:8647-8656. The comparative analysis of five completely sequenced Streptococcus thermophilus bacteriophage genomes demonstrated that their diversification was achieved by a combination of DNA recombination events and an accumulation of point mutations. The five phages included lytic and temperate phages, both pac site and cos site, from three distinct geographical areas. The units of genetic exchange were either large, comprising the entire morphogenesis gene cluster, excluding the putative tail fiber genes, or small, consisting of one or maximally two genes or even segments of a gene. Many indels were flanked by DNA repeats. Differences in a single putative tail fiber gene correlated with the host ranges of the phages. The predicted tail fiber protein consisted of highly conserved domains containing conspicuous glycine repeats interspersed with highly variable domains. As in the T-even coliphage adhesins, the glycine-containing domains were recombinational hot spots. Downstream of a highly conserved DNA replication region, all lytic phages showed a short duplication; in three isolates the origin of replication was repeated. The lytic phages could conceivably be derived from the temperate phages by deletion and multiple rearrangement events in the lysogeny module, giving rise to occasional selfish phages that defy the superinfection control systems of the corresponding temperate phages
 - 74. The genetic relationship between virulent and temperate Streptococcus thermophilus bacteriophages: Whole genome comparison of cos-site phages Sfi19 and Sfi21. Lucchini, S., Desiere, F., Brussow, H. (1999). Virology 260:232-243. The virulent cos-site Streptococcus thermophilus bacteriophage Sfi19 has a 37,392-bp-long genome consisting of 44 open reading frames all encoded on the same DNA strand. The genome of the temperate cos-site S. thermophilus phage Sfi21 is 3.3 kb longer (40,740 bp, 53 orfs). Both genomes are very similarly organized and differed mainly by gene deletion and DNA rearrangement events in the lysogeny module; gene replacement, duplication, and deletion events in the DNA replication module, and numerous point mutations. The level of point mutations varied from 15% (DNA packaging and head morphogenesis modules). A dotplot analysis showed nearly a straight line over the left 25 kb of their genomes. Over the right genome half, a more variable dotplot pattern was observed. The entire lysogeny module from Sfi21 comprising 12 genes was replaced by 7 orfs in Sfi19, six showed similarity with genes from temperate pac-site S. thermophilus phages. Noneof the genes implicated in the establishment of the lysogenic state (integrase, superinfection immunity, repressor) or remnants of it were conserved in Sfi19, while a Cro-like repressor was detected. Downstream of the highly conserved DNA replication module 11 and 13 orfs were found in Sfi19 and phiSfi21, respectively: Two orfs from Sfi21 were replaced by a different gene and a duplication of the phage origin of replication in Sfi19; a further orf was only found in Sfi21. All other orfs from this region, which included a second putative phage repressor, were closely related between both phages. Two noncoding regions of Sfi19 showed sequence similarity to pST1, a small cryptic plasmid of S. thermophilus
 - 75. History of the discovery and study of brucellar bacteriophages. Lyapustina, L. V., Lyamkin, G. I., Taran, I. F. (1999). Zhurnal Mikrobiologii Epidemiologii i Immunobiologii 123-124.
 - 76. Enteroviruses in the recreational waters of Lake Orta. Maiello, A., Guidetti, A., Poncetta, D., Ossola, O., Guidetti, L., Buttinelli, G., Fiore, L., Ruggenini, A. M. (1999). Lakes Reservoirs Research and Management 4:93-99. The water quality of Lake Orta was evaluated for recreational use. This lake was the only Italian lake to undergo a 'liming treatment' which neutralizes water acidity by adding carbonates. Chemical, bacteriological and virological parameters were monitored for 3 years after the treatment ended. Chemical and bacteriological studies were performed according to national standard methods (DPR 470/82) whereas F' specific bacteriophage were enumerated according to an international standard (ISO 10705-1). Enteroviruses were detected by the observation of a cytophatic effect on buffalo green monkey cultures. Also, the possibility of

applying molecular biological techniques for enterovirus detection directly to waters concentrated without previous isolation of viruses in cell culture was verified. The sensitivity, specificity and feasibility of both methods were evaluated. The association between the direct detection of enteroviruses and the indirect indication of their presence by the presence of bacteriophages, and the relationship between bacteriophage presence and bacteriological contamination were also evaluated. In the course of surveillance, none of the samples infringed the law with respect to the physical-chemical parameter (pH < 6). As far as bacteriological characteristics were concerned, 16.6% of the samples taken in 1993 infringed the law, as well as 19.4% of samples in 1994 and 19.4% in 1995. The level of faecal coliforms most frequently exceeded the given limits. The detection test for enteroviruses was positive in 5.1% of the samples using the traditional method with cell culture, and it was positive in 7.6% using the antigen capture polymerase chain reaction (AC-PCR) method directly applied to concentrated waters, indicating the feasibility and a higher sensitivity of the AC-PCR method compared to cell culture. Bacteriophages were present in all the samples that were positive in the virological analysis, as well as in 46.7% of negative samples

77. Prophages inserted in archaebacterial genomes. Makino, S. I., Amano, N., Koike, H., Suzuki, M. (1999). Proceedings of the Japan Academy Series B Physical and Biological Sciences 75:166-171. By analyzing archaebacterial genomic DNA sequences, three new prophages have been found inserted in tRNA genes of the host genomes: proPOF1 and proPOF2 in the genome of Pyrococcus sp. OT3, and proMJF1 in that of Methanococcus jannaschii. The three prophages possess a gene coding for site-specific tyrosine integrase, and are characterized with pairs of elements of the same 46-65 base sequences, that are positioned on the borders to the host genomes (i.e. the attachments). If the two ends of proPOF1 are connected to form a circle by overlapping the two attachments, proPOF1 possesses 33 ORFs in 9 putative transcription units (i.e. 5 operons and 4 independently transcribed ORFs) in 4 clusters inside each of which the direction of transcription is kept the same. This prophage is more likely to inherit a potential to be activated to a phage than is proPOF2, which has only 3 ORFs. In proPOF1 and proPOF2 the attachments are designed, so that their nucleotide sequences are complementary to the 3'-terminal halves of the host tRNA genes. These attachments into two segments and are positioned at the two ends of the prophages. In proMJF1 the integrase gene is undivided, and is positioned between the two attachments whose nucleotide sequence is the same as that of the 3'-terminal half of the host tRNA gene. Thus, the type of phage-integration that created proMJF1 is different from that created proPOF1 and proPOF2

78. Seasonal changes in densities of cyanophage infectious to *Microcystis aeruginosa* in a hypereutrophic pond. Manage, P., Kawabata, Z., Nakano, S. (1999). *Hydrobiologia*. 211-216. Seasonal changes in densities of cyanophages infectious to *Microcystis aeruginosa* were studied in a hypereutrophic pond from March 1997 to January 1998 to elucidate the potential impact of the cyanophage on *M. aeruginosa* mortality. Densities of *M. aeruginosa* ranged between 1.8 X 10⁴ and 9.4 X 10⁵ cells ml-1, while those of the cyanophages were between 2.0 X 10² and 4.2 X 10⁴ PFU ml⁻¹. Sharp decreases in densities of *M. aeruginosa* were detected on 10 June and 24 September, as densities of the cyanophages may have a substantial effect on cyanobacterial succession in the pond. Densities of cyanophages became undetectable when those of *M. aeruginosa* were at low levels during winter. We suggest that there is a tight host-pathogen relationship between *M. aeruginosa* and the cyanophage in the pond

- 79. Bacterial and viral indicators of fecal pollution in Mexico City's southern aquifer. Mazari-Hiriart, M., Torres-Beristain, B., Velazquez, E., Calva, J. J., Pillai, S. D. (1999). Journal of Environmental Science and Health Part A Toxic-Hazardous Substances & Environmental Engineering 34:1715-1735. Mexico City with a population of about 18 million people relies on groundwater to supply about 70% of its water needs. In order to understand the extent of microbial pathogen contamination of these reserves, a 10 month long monitoring study of the southern aquifer was undertaken. Groundwater samples were collected from five different locations and analyzed (100 mL) for total coliforms, fecal coliforms, and fecal streptococci. Larger volume samples (5 L) were collected and concentrated for quantitative and qualitative (presence/absence) determination of microorganisms including bacteriophages. Gene amplification (PCR) approaches were employed to screen for *Escherichia colifShigella* specific (uid) sequences. Laboratory microcosms were conducted to evaluate the potential survival of pathogenic viruses in the groundwater using MS-2 and PRD-1 as model viruses. Coliphage as a single indicator, or in conjunction with fecal coliforms and fecal streptococci were found to have value as an indicator of fecal pollution in this geographical region. The results indicate that the southern aquifer underlying metropolitan Mexico City can pose a significant risk to public health when water is distributed and used without adequate disinfection. The pumping wells located in the transition and mountain areas indicated the presence of extensive microbial pathogen contamination. There was surprisingly, no difference between the dry and rainy seasons in terms of the presence of fecal pollution microbial indicators
- 80. Virulence evolution in a virus obeys a trade-off. Messenger, S. L., Molineux, I. J., Bull, J. J. (1999). Proceedings of the Royal Society of London Series B Biological Sciences 266:397-404. The evolution of virulence was studied in a virus subjected to alternating episodes of vertical and horizontal transmission. Bacteriophage f1 was used as the parasite because it establishes a debilitating but non-fatal infection that can be transmitted vertically (from a host to its progeny) as well as horizontally (infection of new hosts). Horizontal transmission was required of all phage at specific intervals, but was prevented otherwise. Each episode of horizontal transmission was followed by an interval of obligate vertical transmission, followed by an interval of obligate horizontal transmission etc. The duration of vertical transmission was eight times longer per episode in one treatment than in the other, thus varying the relative intensity of selection against virulence while maintaining selection for some level of virus production. Viral lines with the higher enforced rate of infectious transmission evolved higher virulence and higher rates of virus production. These results support the trade-off model for the evolution of virulence
- 81. Quantitative assessment of the inactivation of pathogenic and indicator viruses in natural water sources. Nasser, A. M., Oman, S. D. (1999). Water Research 33:1748-1752. Suitable monitoring of drinking water sources may prevent the occurrence of waterborne disease. Furthermore, determining the environmental factors which affect virus inactivation may help in predicting the fate of pathogenic microorganisms in water sources. This study was conducted to evaluate the suitability of F+ bacteriophages as indicators for microbial contamination of water sources and to determine the effects of water quality, temperature and virus type on virus inactivation. The inactivation of hepatitis A virus (HAV), poliovirus 1, F+ bacteriophages and *E. coli* was compared in groundwater and wastewater effluents at various temperatures. Similar inactivation patterns were observed for HAV and poliovirus 1 under various experimental conditions. Male-specific bacteriophages persisted for the longest time in the various water types, whereas *E. coli* inactivation was the fastest in groundwater at 4 and 37degreeC. F+ bacteriophages have been found more suitable than *E. coli* to predict the inactivation of pathogenic viruses in natural water sources. The effects of temperature and microorganism type was highly significant in groundwater and phosphate buffered saline

and less significant in raw wastewater, indicating that wastewater may enhance virus persistence. The difference between the inactivation of F+ bacteriophages and *E. coli* was significant under all experimental conditions, indicating their suitability to signal the persistence of pathogenic viruses in natural water sources

82. A possible role of temperate phage in the regulation of Trichodesmium biomass. Ohki, K. (1999). Bulletin de l'Institut Oceanographique (Monaco) 0:287-291.

83. Microbiological studies in the Dead Sea: Future challenges toward the understanding of life at the limit of salt concentrations. Oren, A. (1999). Hydrobiologia 1-9. no abstract

84. Induction of the phage resistance in the progeny of an infected bacterial cell. Pererva, T. P. (1999). Biopolimery i Kletka 15:63-66. Using bacteriophages MS2, P17 and lambda-vir infecting Escherichia coli AB 259 (Hfr 3000), E. coli M17 and E. coli C600 cells appropriately the author demonstration that both RNA- and DNA-containing phages induce the development of phage-resistant forms appearing with high frequency in the progeny of infected cells

85. [Evaluation of the usefulness of new international experimental phages for typing methicillin resistant *Staphylococcus aureus* (MRSA)]. Piechowicz, L., Wisniewska, K., Galinski, J. (1999). *Medycyna Doswiadczalna i Mikrobiologia* 51:31-36. The aim of the study was to determine the usefulness of the set of experimental phages obtained from the Central Public Health Laboratory in London for typing of MRSA strains in Poland. The study was performed on 150 MRSA strains isolated from various clinical materials in various regions of the country. The set of 10 experimental phages and the international basic set of 23 phages were used for typing. The results of the study showed that 76.8% of MRSA strains were typing with the experimental set of phages. The frequency of inhibition reactions was 19.9%. Only 3.3% of the strains were nontypable with the new phages while nearly half of the studied strains were nontypable with the basic set of phages. There was a high frequency of typable strains among MRSA typable and nontypable strains and those inhibited by the basic set of phages (71.4%-85.7%). These data indicate that the set of 10 experimental phages is useful for typing of MRSA strains isolated in Poland except for phage M3 which failed to react with almost all the strains and should be excluded from the proposed set

- 86. An evaluation of the efficacy of a wastewater treatment plant. Poncetta, D., Maiello, A., Guidetti, L., Moiraghi, R. A. (1999). Igiene Moderna 112:1-15. Effectiveness of wastewater treatment systems for microbiological pollution removal is a significant hygienic-sanitary problem, principally for the evaluation of effects on basins where the depuration final effluents flow. This study aimed to verify, from a microbiological viewpoint, the effectiveness of the activated sludge depuration process of Verbania and the environmental impact that its effluent has in comparison with the Lago Maggiore waters it flows into. The bacteric pollution extent has been evaluated quantifying the presence of fecal pollution indicators (total and fecal coliforms, fecal streptococci), sulfite-reducing clostridia spores and the presence of pathogens bacteria (salmonella) through classic colony techniques (membrane filter, MPN and pour plate method). The presence of enterovirus and bacteriophages have also been detected through isolation on cell cultures and lysis plaques. The results indicate a substantial reduction of all detected microorganisms of at least 2 log after the activated sludge treatment, and at least 3 log after chloration, with the disappearance of bacteriophages and salmonella in many samples. Enterovirus detection, based on qualitative methods, gave positive results in 95% of samples collected at the entry to the plant, whereas at the exit 63,2% of the tested samples resulted positive for ECP and this percentage fell to 15,8% after disinfection treatment. The positive samples were confirmed by biological molecular techniques. The comparison of the microbiological features surveyed in the coastal waters where the treatment plant effluent flows before and after its coming into operation shows an improvement of hygienic conditions. This situation is probably was due to the discharged effluent quality and to the convey to the plant of built-up area wastewater, which, in the past, directly reached the lake without passing through any depuration treatment
- 87. Prevention of Clostridium difficile-induced ileocecitis with bacteriophage. Ramesh, V, Fralick, Joe A., Rolfe, Rial D. (1999). Anaerobe 5:69-78. A bacteriophage specific for *Clostridium difficile* was examined for its ability to prevent ileocecitis in a hamster model. This species- and strain-specific bacteriophage was isolated from a lysogenic strain of C. difficile. Hamsters were maintained in sterile isolation cages to prevent the acquisition of *C. difficile* from the environment. Bicarbonate neutralization of gastric acidity was necessary for bacteriophage survival in the hamster's gastrointestinal tract. Bacteriophage recovery from the hamster cecum was 2 X 10⁴ plaque forming units/mL of cecal contents 24 h after orogastric challenge with 10⁸ plaque forming units/mL of bacteriophage. However, there was no bacteriophage recovery 48 h post challenge, indicating dissipation of bacteriophage from the hamster intestinal tract within this time frame. Twenty-four hours after being challenged with clindamycin, one group of hamsters was challenged with *C. difficile* followed by a single dose of bacteriophage (108 plaque forming units/mL). Two additional groups of hamsters received phage doses immediately after *C. difficile* challenge and subsequently thereafter every 8 h up to 48 and 72 h, respectively. The gastric acidity was neutralized with bicarbonate buffer preceding every bacteriophage treatment. Control animals that received only clindamycin and *C. difficile* died within 96 h after challenge while the majority of bacteriophage treatment. C. *difficile*. All the hamsters died within 96 h indicating susceptibility of the surviving hamsters to *C. difficile* disease in the absence of bacteriophage treatment
- 88. Physicochemical mechanisms responsible for the filtration and mobilization of a filamentous bacteriophage in quartz sand. Redman, J. A., Grant, S. B., Olson, T. M., Adkins, J. M., Jackson, J. L., Castillo, M. S., Yanko, W. A. (1999). Water Research 33:43-52. This study examines the influence of pore water chemistry on the filtration and physicochemical properties of a mate-specific filamentous bacteriophage isolated from chlorinated effluent of the San Jose Creek Water Reclamation Plant in Los Angeles County, California. The isolate belongs to a class of bacteriophage that are naturally present in sources of sewage, and hence may be an indicator of fecal contamination in groundwater. Furthermore, there is some evidence that this class of bacteriophage are mobilized in the subsurface following rainfall events, although the mechanism responsible for this process is not yet clear. Using a model filtration system consisting of packed columns of quartz sand, we found that the filtration of this isolate was strongly dependent on the concentration and valence of the dominant cation in the pore fluid. In one set of experiments involving columns 19 cm in length, virus retention in the column increased from 0% to 99.999% when the electrolyte composition of the pore fluid was changed from 10 mM NaCl to 10 mM CaCl2. With one exception, filtration efficiencies calculated from the column experiments were inversely proportional to the electrophoretic mobility of the virus, implying that electrostatic interactions between the virus and the quartz surface dominate the filtration dynamics of this particular bacteriophage. From a practical perspective, these results indicate that small changes in the hardness and total dissolved solids of pore fluids - as might occur following a rainfall event - can dramatically affect both the filtration and mobilization of filamentous bacteriophage in subsurface systems

- 89. Analysis of bacteriophage inactivation and its attenuation by adsorption onto colloidal particles by batch agitation techniques. Rossi, P., Aragno, M. (1999). Canadian Journal of Microbiology 45:9-17. A batch agitation technique was designed to specify the different parameters that influence the inactivation and adsorption mechanisms of viruses in water. The advantage of this method over the classical procedures is that the kinetic reactions of the different subfractions of the virus population can be described simultaneously. A first set of experiments with phage T7 showed that this phage is rapidly inactivated in a constantly agitated liquid medium. This inactivation rate is highly influenced by temperature, but variation of the pH (from 5 to 9) and increase in salt concentration have no effect on it. The addition of colloidal clay particles (CCPs) of montmorillonite and attapulgite into the liquid medium considerably modifies this behavior, even at very low concentrations (0.025 mg/mL). The experiments show that the viruses react quickly with the particles and that bonding is not permanent. Viruses establish a dynamic equilibrium, which is strongly dependent on physicochemical parameters such as pH, ionic concentrations, and the presence of proteins or protein hydrolysates. A major environmental consequence is that the presence of CCPs seems to effectively protect the coliphage T7 from rapid inactivation
- 90. Bacteriological evaluation of composting systems in sludge treatment. Shaban, A. M. (1999). Water Science and Technology 40:165-170. Sludge disposal was considered as a serious problem to the authorities. Thus, the treatment of sludge resulting from sewage treatment plants to remove pathogenic microorganisms and to improve its impact on the environment was considered as the main objective of several investigators. Composting is one of the methods of sludge treatment. Different systems of composting (static pile, windrow and natural draft) were applied and evaluated bacteriologically. Faecal coliform and salmonellae were removed completely during the first two weeks in case of forced aeration, but the former are still present till near the end of experiment with natural aeration. For the natural draft system with sawdust base, faecal coliform reduction increased up to 100% after 7 weeks, while faecal streptococci and coliphage decreased gradually and were removed completely at the end of treatment. Salmonellae disappeared after a few days from starting treatment. In case of alkaloids addition (cement and lime), the tested organisms reached acceptable levels with any concentration of alkaloids. Coliphage and faecal streptococci survived till the end of treatment. So, from the previous results it is clear to say, coliphage and faecal streptococci were more resistant to the composting processes than other organisms
- PPL1c, a virulent mutant bacteriophage useful for identification of *Paenibacillus larvae* subspecies larvae. Stahly, D. P., Alippi, A. M., Bakhiet, N., Campana, C. F., Novak, C. C., Cox, R. (1999). *Journal of Invertebrate Pathology* 74:295-296. *Paenibacillus larvae* subspecies *larvae* is a pathogen of honey bee (*Apis mellifera*) larvae, causing American foulbrood. We isolated a virulent mutant bacteriophage, PPL1c, from *P. larvae* subsp. *larvae* NE that should be of value for rapid identification of *P. larvae* subsp. *larvae*.
- 92. The panda and the phage: Compensatory mutations and the persistence of small populations. Whitlock, M. C., Otto, S. P. (1999). Trends in Ecology & Evolution 14:295-296. Mutation is the ultimate source of all the genetic variation necessary for evolution by natural selection; without mutation evolution would soon cease. Unfortunately, this comes at a cost: most mutations that affect fitness are deleterious. For most large sexual populations, these less fit alleles are eventually eliminated from the population by natural selection. In small populations, however, new deleterious mutations can sometimes increase in frequency and even fix within the population. If harmful mutations fix repeatedly, the fitness of a population might eventually reach such a low level that the population is not capable of sustaining itself and may go extinct, the socalled 'mutational meltdown'. The most important genetic threat to small endangered populations is thought to be this accumulation of new deleterious mutations by genetic drift... But there is hope. Back mutations are only one type of beneficial mutation, and they are a limited subset of the variety of ways in which a genome can mutate to repair the effects of a new deleterious mutation. A fascinating new paper by Christina Burch and Lin Chao has demonstrated, using the bacteriophage f6 as a model system, the ready availability of novel mutations that are capable of compensating for the fitness effects of a fixed deleterious allele rather than simply reverting to the original genotype...
 - 93. Purification of MS2 bacteriophage from complex growth media and resulting analysis by the integrated virus detection system (IVDS). Wick, C. H., McCubbin, P. E. (1999). Toxicology Methods 9:253-263. Purification and concentration of viruses from the background material is required whatever subsequent analysis methods are used. For the analysis of viruses it is essential and detection methods depend on this solution. This report demonstrates a methodology for the removal of growth media from a virus preparation. A sample of MS2 was purified using a new ultrafiltration (UF) technique with hollow fibers. A typical MS2 virus sample with a nominal stated concentration of 1.4 X 10¹² plaque-forming units (pfu)/mL in the original growth media was used to demonstrate this method. After UF, the growth media was removed and the virus counted using the integrated virus detection system (IVDS) instrument. This report further describes the use of this ultrafiltration procedure to remove other impurities, such as cesium chloride and albumin, from solutions containing a purified solution of MS2 bacteriophage. These solutions were also analyzed using the IVDS instrument
- 94. Characterization of purified MS2 bacteriophage by the physical counting methodology used in the integrated virus detection system (IVDS). Wick, C. H., McCubbin, P. E. (1999). Toxicology Methods 9:245-252. A new physically based methodology-the integrated virus detection system (IVDS)-was used to characterize a high-concentration, 10.2 mg protein/mL, sample preparation of MS2 bacteriophage with a reported 10¹⁴ plaque-forming units (pfu)/mL (DPM14) virus count in a common TNME buffer. Virus counts were made using the IVDS instrument following serial dilution. Results indicated virus counts of 1.5 X 105 for the neat sample (DPM14), followed by 6.5 X 10⁴ viruses (DPM13), 1.2 X 10⁴ viruses (DPM12), 9.3 X 10² viruses (DPM11), 88 viruses (DPM10), and 5 viruses (DPM9), respectively. Lower concentrations displayed a consistent multiplier and were consistent with target dilutions. Increases in virus concentration appear to decrease the multiplier, probably through aggregation. The results demonstrate a consistent and simple-to-use methodology. The results further indicate that the IVDS instrument can be used for characterization of other virus preparations with equal ease and similar results
 - 95. Passage of MS2 bacteriophage through various molecular weight filters. Wick, C. H., McCubbin, P. E. (1999). *Toxicology Methods* 9:265-273. MS2 bacteriophage has a reported nominal molecular weight of 2M daltons. It would be expected that this phage would not pass through filters of various sizes with low molecular weight cutoff (MWCO) values of less than 1M daltons. It was discovered that MS2 bacteriophage will pass through filters with 750K-, 500K-, and 300K-dalton MWCO values. MS2 was retained on the 100K-dalton filter. A cross-flow hollow fiber apparatus was used for the 750K- and 500K-dalton analysis. Centrifuge filters of 1M and 300K and 100K daltons were used. The rate of passage of MS2 through the cross-flow filters is dependent on the tangential flow rate and pressure. Passage through the centrifuge filters depended on the gravitational force applied

96. Analysis of cyanophage diversity and population structure in a south-north transect of the Atlantic ocean. Wilson, W. H., Fuller, N. J., Joint, I. R., Mann, N. H. (1999). Bulletin de l'Institut Oceanographique (Monaco). 0:209-216. Cyanophages (viruses which infect cyanobacteria) are abundant in the marine environment and are thought to be a significant factor in determining the dynamics of Synechococcus spp. populations. In an effort to use molecular techniques to characterise cyanophage populations, we designed cyanophage-specific (CPS) PCR primers based on a gene found in three genetically distinct marine cyanophages (Fuller et al., 1998). CPS primers were used to amplify cyanophage DNA extracted from viral communities concentrated from sea-water samples obtained during a cruise transect between the Falkland Islands, in the south Atlantic ocean, to the UK. Following phylogenetic analysis of cloned and sequenced PCR products, it was revealed that genetic diversity of marine cyanophage clones within a single water sample was as great as clones and cyanophage isolates collected between different oceans. Denaturing gradient gel electrophoresis (DGGE) analysis confirmed this high diversity. DGGE analysis also revealed changes in cyanophage population structure in surface seawater over the south-north transect and throughout depth profiles in the water column. Maximum Synechococcus spp. concentrations, in a stratified water column, correlated with maximum cyanophage diversity

- 97. An unexpected temporal pattern of coliphage isolation in groundwaters sampled from wells at varied distances from reclaimed water recharge sites. Yanko, W. A., Jackson, J. L., Williams, F. P., Walker, A. S., Castillo, M. S. (1999). Water Research 33:53-64. Potable and monitoring wells located in close proximity to a large groundwater recharge project which utilizes a blend of surface water and reclaimed wastewater for recharge were tested for coliphage over a period of 6 months to assess the potential for virus migration. During the first 3 months FRNA phage were detected once at a shallow monitoring well. In late summer, an unexpected pulse of phage was detected in all wells, including control sites, suggesting an ecological phenomenon independent of recharge operations. Cubic and filamentous F-specific coliphage, consistent with the Leviviridae and Inoviridae groups, and a noncontractile tailed phage consistent with the Siphoviridae family were detected. There was no discernible relationship between recharge operations and the pattern of phage populations detected. Phage were detected using a host designated HS12, a variant of HS(pFamp)R (Debartolomeis, J. and Cabelli, V. J. (1991). Evaluation of an Escherichia coli host strain for enumeration of f male-specific bacteriophages. Appl. Environ. Microbiol. 57, 1301). During the study it was found that HS12 contained a temperate Myoviridae phage; Myoviridae phage were subsequently excluded from the results. A total of 26 production wells, including 3 control sites, were sampled monthly and 6 monitoring wells were sampled every two weeks. Water reclamation plant effluents and river water upstream of effluent discharges were randomly sampled. The concentration and distribution of phage isolated was guite different in chlorinated effluent compared to river water. The majority of isolates from reclaimed water were filamentous DNA F-specific phage suggesting this group was more resistant to chlorine. Groundwater samples were analyzed using a novel large volume enrichment technique that proved very sensitive for detecting low concentrations of phage
- 98. Characterization of a Vibrio parahaemolyticus phage isolated from marine. Yoon, S. O., Ju, S. A., Heo, M. S., Jung, C. R., Ju, J. W. (1999). Journal of the Korean Society for Microbiology 34:423-433. A novel bacteriophage, designated as VPP97, that infects the strains of Vibiro parahaemolyticus (hallophilic, Gram-negative bacterium) isolated most commonly from marine environments, has been discovered, and several of its properties have been determined. The plaques were clear and sized 0.6-1.0 mm in diameter. The virion forms a single band on 70% sucrose gradient and p1.50 CsCl gradient by sucrose gradient centrifugation and CsCl gradient centrifugation respectively. It has a hexagonal head and a relatively long tail, as shown by electron microscopy. Vibrio alginolyticus, Vibrio fluvialis and Vibrio furnissii were also sensitive to this phage It was almost totally inactivated at 70°C and at pH below 5 or over 10. The nucleic acid of VPP97 is composed of DNA. The VPP97 had 9 specific structural proteins sized between 21.5 kDa and 97.4 kDa on SDS-PAGE. When V. parahaemolyticus cultures were treated with either phage VPP97 or one of the several antibiotics for 2 hours, the viable number of V. parahaemolyticus treated with tetracycline. Mice that have responded to the phage treatment revealed the lower numbers of V. parahaemolyticus in small intestine and less damage on small intestine compared to the untreated mice. Therefore, we suggest that the phage treatment appears effective to the infection by V. parahaemolyticus

99. Microbiological and chemical quality of sludges from domestic wastewater plants. Aulicino, F. A., Colombi, A., Calcaterra, E., Carere, M., Mastrantonio, A., Orsini, P. (1998). International Journal of Environmental Health Research 8:137-144. Digested sludge samples from domestic wastewater treatment plants located in Northern Italy were tested as far as the presence of viruses (enteric viruses and coliphages), bacteria (faecal coliforms, salmonella) and helminth eggs is concerned. Heavy metals were also analysed. Escherichia coli bacteriophages and faecal coliforms were isolated from all samples, while salmonellae and helminth eggs were isolated only from four and three out of 27 total samples, respectively. The 66% of sludge samples, 46 and 82% of aerobic and anaerobic digested sludges respectively, showed the presence of enteric viruses (enteroviruses). The virus concentrations ranged from 0.6 to 123 MPNCU/g. Results of this study suggest that significant concentrations of pathogens such as enteric viruses can be present in digested sludge. which showed compliance with Italian legislation. As suggested by other authors, there is the need for surveillance and reference indications both in EU (European Union) and Italian regulations concerning the use of sludge in agriculture

100. Transduction of imipenem resistance by wild-type bacteriophages carried by three strains of *Pseudomonas* aeruginosa isolated from a single source. Blahova, J., Kralikova, K., Krcmery, V, Mlynarcik, D., Trupl, J. (1998). Journal Of Antimicrobial Chemotherapy 41:660-662. letter (no abstract)

101. Filamentous bacteriophages of *Vibrio parahaemolyticus* as a possible clue to genetic transmission. Chang, B., Taniguchi, H., Miyamaoto, H., Yoshida, S. I. (1998). *Journal of Bacteriology* 180:5094-5101. We have previously reported the isolation and characterization of two filamentous bacteriophages of *Vibrio parahaemolyticus*, designated Vf12 and Vf33. In this study, to understand the potential of these phages as tools for genetic transmission, we investigated the gene structures of replicative-form (RF) DNAs of their genomes and the distribution of these DNAs on chromosomal and extrachromosomal DNAs. The 7,965-bp nucleotide sequences of Vf12 and Vf33 were determined. An analysis of the overall gene structures revealed that Vf12 and Vf33 had conserved regions and distinctive regions. The gene organization of their conserved regions was similar to that of CTX phage of Vibrio cholerae and coliphage Ff of *Escherichia coli*, while their distinctive regions were characteristic of Vf12 and Vf33 phage genomes. Southern blot hybridization testing revealed that the filamentous phage genomes integrated into chromosomal DNA of *V. parahaemolyticus* at the distinctive region of the phage genome and were also distributed on some plasmids of *V. parahaemolyticus* and total cellular DNAs of one *Vibrio damsela* and one nonagglutinable *Vibrio* strain tested. These results strongly suggest the possibilities of genetic interaction among the bacteriophage Vf12 and Vf33 genomes and chromosomal and plasmid-borne DNAs of *V. parahaemolyticus* strains and of genetic transmission among strains through these filamentous phages

- 102. The fate and transport of viruses through surface water constructed wetlands. Chendorian, M., Yates, M., Villegas, F. (1998). Journal of Environmental Quality 27:1451-1458. Coliphage removal efficiency and the effects of wetland hydrology on virus transport were determined for constructed wetlands in San Jacinto, CA. Mathematical models were used to further characterize virus transport. MS2, an F-specific RNA (FRNA) coliphage was used as a model for human enteric viral behavior. Two wetland types were studied, a one-phase cell and three-phase cell. These wetlands received unchlorinated secondary effluent at a constant rate. The mean residence time in the wetlands was 9 +- 3 d as determined using bromide as a conservative tracer. Assuming 100% porosity, a plug flow model predicts this mean residence time within the experimental standard deviation (8 d). This suggests that a negligible volume was occupied by vegetation and settled solids. The convection-dispersion equation adequately simulated the residence time distribution of the conservative tracer. MS2 removal in the wetlands was experimentally determined to be 97 +- 3%. There was no distinction between the two wetland types in terms of removal efficiency. The average coliphage decay rate was calculated to be 0.44 per day. However, the error involved with using the first order decay rate was high, 83 +- 12%. Therefore, first order decay does not adequately describe removal processes within the wetland. Most virus removal occurred within the first 3 m (k = 4.0 +- 1.8 d-1) with a removal efficiency of 85.3 +- 0.6%. The remainder had a decay rate of 0.20 +- 0.17 d-1 with a removal efficiency of 56 +- 33%
- 103. Bacteriophages and bacteria as indicators of enteric viruses in oysters and their harvest waters. Chung, H., Jaykus, L. A., Lovelace, G., Sobsey, M. D. (1998). Water Science and Technology 38:37-44. Reliable indicators are needed to detect enteric virus contamination of bivalve molluscan shellfish and their harvest waters. Concentrations of male-specific (F+) coliphages, Bacteroides fragilis phages, Salmonella phages and several indicator bacteria in wastewater, estuarine receiving water and its oysters were examined for their ability to predict the presence and levels of faecal contamination and enteric viruses in oysters. Enteric viruses in oysters were detected by cell culture and RT-PCR methods. F+ coliphages, Salmonella phages, B. fragilis phages and faecal indicator bacteria (faecal coliforms, E. coli, enterococci and Clostridium perfringens) were generally positively associated and were highest in raw sewage and progressively lower in sewage effluent and in receiving waters at increasing distance from the wastewater discharge. Indicator levels in oysters were highest for F+ coliphages and C. perfringens. One F+ RNA coliphage serotype (Group II) predominated in the wastewater, receiving water and oysters. Human enteric viruses were detected in 17/31 oyster samples. The levels of most indicators in oysters and water were higher when oysters were enteric virus-positive and lower when oysters were enteric virus-negative. F+ coliphages and C. perfringens were the only indicators significantly associated with the presence of enteric viruses in oysters. F+ coliphages and their serotypes are promising indicators of human enteric virus contamination in oysters and their harvest waters
- 104. Coliphage prevalence in high school septic effluent and associated ground water. DeBorde, D. C., Woessner, W. W., Lauerman, B., Ball, P. (1998). Water Research 32:3781-3785. At the present time, somatic and male-specific coliphage and human enterovirus groups are being considered as indicators of possible pathogenic human enteric virus contamination from fecal contamination. A primary attribute for any indicator of fecal contamination is its prevalence at the source and in associated ground water. It must be consistently found in the source material at concentrations that are measurable with available techniques. Over a period of ten months, male-specific and somatic coliphage ranged from apprx7000 to apprx4,000,000 PFU/L in the effluent from a multi-user septic-tank. Unlike the values determined for septic-tank effluent, coliphage concentrations measured in ground water over this same period only varied by five-fold. Coliphage concentration in ground water under the down-gradient edge of the drainfield contained apprx1000 PFU/L. This concentration decreased at -1 log10/5 m during 17.4 m of ground-water transport. From these data, coliphage concentrations in septic-tank effluent seem sufficient to allow their use as indicators of fecal contamination in ground water
- 105. The development of management strategies for control of virological quality in oysters. Dore, W. J., Henshilwood, K., Lees, D. N. (1998). Water Science and Technology 38:29-35. This laboratory has previously described the development of a PCR method for the detection of small round structured viruses (SRSVs) in shellfish and the use of male-specific RNA bacteriophages as "viral" indicators to predict the occurrence and behaviour of such viruses in shellfish. We now describe the application of these procedures to monitor oyster harvesting areas, shellfish treatment processes and products sold to the consumer. Oysters are traditionally consumed raw and can be treated to enable them to meet the legislative end-product standard of < 0.4), correlations with all microbiological parameters. Somatic coliphages also revealed highly significant (0.32 < r < 0.66) correlations (P = < 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 (R2 = < 5 mg/L iodine doses, losing 6 logs (99.9999%) of infectivity within less than 3 min contact time. The effect of pH on MS2 inactivation within the range of 6 to 8 was not statistically significant. However, in the presence of dissolved organic substances, such as detergents and proteins, the inactivation of MS2 viruses decreased significantly to less than 4 logs (99.99%). Of special interest was that in the presence of beef extract proteins, an apparent reversal of MS2 inactivation, dubbed rebound, was observed. It was observed that after an initial 5 to 6 log reduction in infectivity, a consistent and statistically significant increase in the number of plaque forming units (PFU), as much as 2 logs, was measured. MS2 rebound occurred only when the oxidized iodine residual had been quickly consumed by beef extract proteins in solution. Neither virus particle aggregation nor water salinity were found to account for the increase in PFU values. Based on other investigators' suggestions that iodine disinfection caused changes to viral protein coats, it was hypothesized that conformational changes in MS2's protein coat caused by iodine would result in a change in the isoelectric focusing point of whole MS2 virions. A shift in isoelectric focusing point from an acidic pH value of 3.9 to more basic values, and a dispersion of the virus band after exposure to high levels of iodine was observed, supporting the hypothesis that iodine caused changes in the charge distribution characteristics of the protein coat
 - 106. Effectiveness of membrane-filtration for phage technique for the detection of Xanthomonas campestris pv. citri. Ebisugi, H., Ooishi, S., Goda, T., Kubo, H., Sakiyama, K. (1998). Research Bulletin of the Plant Protection Service Japan 113-115. The plaque count method test for the detection of Xanthomonas campestris pv. citri was reexamined. The membrane-filter (pore size 0.22 mum) was used after centrifugation process of test solution in the phage count method in order to exclude the contaminated bacteria in phage suspension. The use of membrane filter was effective in plaque-counting
 - 107. Characterization of Lactobacillus fermentum bacteriophage Z63-B2. Foschino, R., Castiglioni, E., Galli, A. (1998). Annali di Microbiologia ed Enzimologia 48:151-159. This communication reports physiologic and structural characteristics of phage Z63-B2 active on Lactobacillus fermentum strain ATCC 9338; a comparison with phage Z63-B1, previously isolated from the same kind of environment (sourdoughs for bread-making) was discussed. Z63-B2 showed a multiplicative cycle with a burst size of 100 PFU per infectious centre and a latent period of 150 min. Its electrophoretical profile of proteins differed from that of phage Z63-B1 only for one minor protein. DNA fragments obtained with different restriction enzymes resulted very similar and a high homology between the viruses was confirmed by hybridization tests. Two phages prove to be variants each other. Resistant

clones to phage Z63-B2 isolated after lysis of the host strain, showed costantly a different cell morphology and colony growth in comparison with the original host strain; however resistance was not imputable to a lysogenic state

- 108. Bacteriophage and associated polysaccharide depolymerases: Novel tools for study of bacterial biofilms. Hughes, K. A., Sutherland, I. W., Clark, J., Jones, M. V. (1998). Journal of Applied Microbiology 85:583-590. Bacteriophage for three representative strains of Gram-negative biofilm bacteria have proved to be of widespread occurrence. Lytic bacteriophage have been isolated from local sewage for the bacterium 1.15, an exopolysaccharide (EPS)-producing pseudomonad found originally as a component of biofilms in a local river, and for two *Enterobacter agglomerans* strains from industrial biofilms. Representative examples of all three bacteriophage possess a relatively low burst size and on solid media, exhibit very large plaques surrounded by a wide halo (5-20 mm) indicative of polysaccharide depolymerase action. The bacteriophage are thus similar to other viruses for EPS-producing bacteria in inducing the synthesis of enzymes degrading the polymers which occlude the bacterial cell surface. In each preparation, the polysaccharase activity was associated both with sedimented phage particles and with the supernate of bacterial lysates. The enzymes have been partially purified and used to prepare polysaccharide digests in which the major products from each polysaccharide are the presumed repeat units of the polymers or oligomers of these. The soluble phage enzymes each degrade their substrate by acting as endo-glycanohydrolases. The phage and their associated enzymes thus provide very useful highly specific tools for studies of biofilms incorporating the bacterial host strains. Their potential applications in studies on bacterial biofilms are discussed
- 109. Biofilm susceptibility to bacteriophage attack: The role of phage-borne polysaccharide depolymerase. Hughes, K. A., Sutherland, I. W., Jones, M. V. (1998). *Microbiology (Reading)* 144:3039-3047. Biofilm bacteria *Enterobacter agglomerans* 53b and Serratia marcescens Serr were isolated from a food processing factory. A bacteriophage (SF153b), which could infect and lyse strain 53b, was isolated from sewage. This has been shown to possess a polysaccharide depolymerase enzyme specific for the exopolysaccharide (EPS) of strain 53b. Using batch culture and chemostat-linked Modified Robbins Device systems it was observed that SF153b could degrade the EPS of a mono-species biofilm (strain 53b) and infect the cells. The disruption of the biofilm by phage was a combination of EPS degradation by the depolymerase and infection and subsequent cell lysis by the phage. Strain Serr biofilms were not susceptible to the phage and the biofilm EPS was not degraded by the phage glycanase, with the result that the biofilm was unaffected by the addition of SF153b phage. Scanning electron microscopy confirmed that specific phage could extensively degrade susceptible biofilms and continue to infect biofilm bacteria whilst EPS degradation was occurring
- 110. Virus removal by advanced membrane filtration for wastewater reclamation. Iranpour, R. (1998). Water Environment Research 70:1198-1204. Measurements of indigenous and seeded male-specific (MS2) bacteriophages were made in an effort to gain insight into the response of membrane filtration systems to varying virus concentrations and varying flow rates at the Terminal Island Treatment Plant, operated by the City of Los Angeles Bureau of Sanitation. Bacteriophages were seeded into secondary effluent that had been filtered through a trimedia filter. The seeded effluent was then processed by microfiltration (MF) and reverse osmosis (RO) pilot units for which the Department of Water and Power was evaluating effluent water quality parameters in conjunction with a water reclamation project. The samples were assayed for virus. The seeded tests utilized higher concentrations of MS2 viruses and sampled the process streams with higher time resolution than those used by other researchers in comparable experiments. As expected from the physics of RO process, the RO unit reduced the virus concentration below the threshold of detection, but the MF membranes consistently reduced virus concentrations by less than one log unit (order of magnitude). This MF performance differs from most results of similar tests carried out elsewhere, but it was consistently observed, despite substantial variability in the virus removal factor, as revealed by the high time resolution of the measurements. Budgetary limits prevented extending this research to clarify the indications in the data that removal efficiency may be affected by the MF unit's backwash cycle, or the membrane flux, but if these results can be verified, they may provide valuable insight for improved membrane technology and planning for large-scale membrane-based water reclamation
- 111. Inactivation of bacteriophage lambda, Escherichia coli, and Candida albicans by ozone. Komanapalli, I. R., Lau, B. H. S. (1998). Applied Microbiology and Biotechnology 49:766-769. The effects of ozone (O₃) on three types of microbes were studied. Test suspensions were exposed to 600 ppm O₃ at room temperature. Control experiments were performed under

identical conditions using oxygen gas. Bacteriophage lambda was completely inactivated at 10 min while *Escherichia coli* and *Candida albicans* were only inactivated by factors of 10⁵ and 10⁴ respectively at 40 min. Exposure of a mixed microbial suspension to O₃ for 5 min resulted in 100% killing of bacteriophages while the viability of *E. coli* remained unchanged. Various body fluids containing phages were exposed to O₃. Compared to buffered solution, the decrease in phage titers was significantly slower in whole blood, plasma, and albumin. Both *E. coli* and *C. albicans* had increased production of thiobarbituric-acid-reactive substances with increased O₃ exposure. ³H-labelled amino acids were incorporated into *E. coli* O₃ treatment resulted in a loss of radioactivity, indicating leakage of cytoplasmic contents. The data indicate that microbes are present determines the

effectiveness and outcome of O_3 treatment

112. Advanced wastewater disinfection technologies: Short and long term efficiency. Lazarova, V, Janex, M. L., Fiksdal, L., Oberg, C., Barcina, I, Pommepuy, M. (1998). Water Science and Technology 38:109-117. Advanced disinfection processes (peracetic acid, UV irradiation and ozonation) have been tested and evaluated through bench and pilot scale studies. 3 log removals of total coliforms, faecal coliforms and faecal streptococci were achieved by 10mg/L peracetic acid at a 10 min contact time, by UV radiation at 35mW.s/cm2 and by ozone at 5mg/L for 10min contact time. Higher doses are required for virus removal by UV and PAA and especially for highly resistant viruses such as F-specific bacteriophage MS2. Ozonation has the advantage of having a strong effect on all types of bacteriophages and protozoa cysts even when low treatment doses and short contact times are applied. The results of this study demonstrated that evaluation of disinfection efficiency of ozone, UV and PAA depends on the criteria and methods employed. Standard method (plate count) results showed an important disinfection effect on culturability, while results from nonstandard methods (respiratory activity and beta-galactosidase activity assay) indicated less reduction of viable cells. Moreover, the results confirm that disinfectants act on bacteria in different ways. It has been clearly demonstrated that b-galactosidase activity is affected by PAA while UV treatment has no or very limited effect on the enzyme activity. Even without sunlight reactivation, bacterial regrowth in seawater was observed after disinfection of sewage effluents. This study also shows that the biodegradability of sewage effluent for an *E. coli* strain was affected differently by the oxidative disinfectants ozone and PAA. Biodegradability should therefore be considered when evaluating the total disinfection efficiency.

A. S., Vassal, L., Cesselin, B., Delacroix-Buchet, A., Gripon, J. C., Chapot-Chartier, M. P. (1998). International Dairy Journal 8:667-674. Lactococcus lactis subsp. cremoris AM2 strain was previously shown to lyse early and extensively during cheese ripening. This strain is lysogenic and contains a prophage named PHIAM2. Lysis of strain AM2 and its prophage-cured derivative AM2-C in Saint-Paulin pressed-type cheese was monitored by the following parameters: cell viability, morphological changes of bacteria observed by electron microscopy and release of intracytoplasmic peptidases. Proteolysis was quantified by measuring soluble nitrogen (SN), phosphotungstic acid soluble nitrogen (PTA-N) and free amino acids. By contrast to the wild type strain AM2 which lyses early and extensively, its prophage-cured derivative AM2-C lyses only slowly and to a limited extent in cheese. These results indicate that the prophage PHIAM2 is involved in the lytic behaviour of *L. lactis* AM2 during cheese ripening. In addition, the comparison of two isogenic strains with similar enzymatic potential but different ability to lyse demonstrates that starter strain lysis results in a higher free amino acids production rate and a decrease of bitter taste

114. Reconstruction of the presumptive mechanisms of bacteriophage speciation and morphological evolution. Letarov, A. V. (1998). Genetika 34:1461-1469. The problem of the origin and evolution of viruses and, in particular, the origin and evolution of bacteriophages is of considerable interest. However, so far, this problem has not been solved with quantitative methods of molecular systematics. In the present study, an attempt to reconstruct the possible paths of appearance and evolution of bacteriophages based on their structural features and morphogenesis, as well as general characteristics of their life cycles and genome organization, was carried out. A scheme describing phylogeny of the main bacteriophage groups and evolution of their life cycles is suggested. Existence of two independently evaluating types of morphogenesis ("budding outward" and "budding inward") is postulated

115. Defective phage as an antagonistic factor of closely related bacilli. Lotareva, O. V., Prozorov, A. A. (1998). *Mikrobiologiya* 67:788-791. The antagonistic effect produced by the defective phage PBSX during cocultivation of the mutant strain *B. subtilis* 168, in which this phage is heat-inducible, and strain *B. subtilis* NRS231, which also bears a defective phage, was investigated. As soon as in the first hours of cocultivation under conditions of PBSX induction, the number of viable cells of strain NRS231 decreased by two orders of magnitude. However, the effect was not observed if the temperature of cocultivation was noninducing. The results confirm the supposition that defective phages may play a role in the competition between closely related bacilli

- 116. 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
 - 117. Bacteriophage PM2 nomenclature revision. Merino, S., Tomas, J. M., Maniloff, J. (1998). Archives of Virology 143:1852-1853. Two taxonomically different bacteriophages have unintentionally been given the same name. Alteromonas phage PM2, isolated in 1968, is the type species of the family Corticoviridae, genus Corticovirus: lipid-containing, icosahedral viruses containing circular dsDNA. Aeromonas phage PM2, isolated in 1990, is a tailed virus, with contractile tail, and presumably a member of the family Myoviridae: phages with contractile tails, containing linear dsDNA. To avoid confusion, the name of Aeromonas phage PM2 is now changed to phage AehPM2
 - 118. Long term use of a Cheddar starter and development of phages with homology to its bacteria. Nielsen, E. W. (1998). International Dairy Journal 8:1003-1009. A mixed, homofermentative, mesophilic starter was used for more than 11 years as the only starter for production of Cheddar cheese at a Danish cheese factory. The activity of the starter at the factory was carefully recorded, and whey samples were regularly tested for bacteriophages. During the years bacteriophages, homologous to an increasing number of the strains of the starter culture, gradually evolved. After 4 years bacteriophages, homologous to more than 50%-and after 6 years to more than 90%-of 62 bacterial isolates from the starter, had appeared. For most of the strains the virulence of their phages was low at first and thereafter increased over several years. It was, however, only after more than 11 years that the cheese factory first began to observe cases of reduced activity of the bulk starter; presumably because the virulence of the bacteriophages had developed to such a degree that very low levels of contamination of the milk for bulk starter could impair the subsequent activity of the bulk starter in the curd

119. Virus removal in a membrane separation process. Otaki, M., Yano, K., Ohgaki, S. (1998). Water Science and Technology 37:107-116. Recently, membrane technology has been considered an alternative to conventional water purification. To study the fate of viruses in membrane processes, indigenous coliphages in pilot scale membrane processes located in the eastern part of Tokyo Metropolitan area have been surveyed for 6 months. This plant used river water as its resource and had two microfiltration membrane processes which had different pore sizes (0.2 mum and 0.1 muM) and one ultrafiltration process which had 13,000 nominal molecular weight cut off. To detect indigenous coliphages, E. coli K12 F+(A/lambda) and E. coli C were used as host bacteria. E. coli K12 F+(A/lambda) can detect both DNA and RNA phages and E. coli C can only DNA phage. The resource water contained E. coli K12 phages at 200-1500 PFU/100 mL and the removal ratio of these DNA and RNA phages was lower than that of DNA phage by E. coli C in both MF membrane processes through 6 months. It is thought to be caused by difference of phage size, because DNA phage is bigger than RNA phage in general. The removal ratio of E. coli K12 and E. coli C phages reached 100% in the UF membrane process. According to the comparison of the concentration of phages in solution and eluted from suspended solid in resource and drain, it is thought that most phages concentrated in the drain were absorbed in suspended solids. To make certain of the removal ratio in UF and NF (nanofiltration) processes, high concentrations of coliphage Qbeta and poliomyelitis virus vaccine were fed into these processes. The removal ratio of coliphage Qbeta in UF and NF processes are $10^{-8.3}$ and $10^{-6.3}$ respectively, and the ratio of poliomyelitis virus vaccine in UF and NF are < $10^{-6.7}$ and < $10^{-7.3}$ respectively

120. Coliphages and indicator bacteria in birds around Boston Harbor. Ricca, D. M., Cooney, J. J. (1998). Journal of

Industrial Microbiology & Biotechnology 21:28-30. Droppings from feral populations of pigeons, geese and herring gulls from the urban/suburban environment around Boston Harbor, MA, USA contained up to 10⁶ somatic coliphages, 10⁸ enterococcl, 10⁹ thermotolerant coliforms and 10² F-specific coliphages per gram of feces. Somatic collphages, enterococci and thermotolerant coliforms were common in the feces of all three kinds of birds but F-specific collphages were found in droppings from only three of 32 gulls. Thus these sources of bacterial and viral Indicators should be considered when dealing with the ecology of fecal pollution Indicators. Moreover, microbial indicators of fecal or sewage pollution originating from bird droppings may be mistaken for indicators that come from humans. This may cause an overestimate of the hazard from human pathogens in water and confound attempts to locate sources of fecal or sewage pollution

- 121. Reduction of FRNA-bacteriophages and faecal indicator bacteria by dune infiltration and estimation of sticking efficiencies. Schijven, J. F., Hoogenboezem, W., Nobel, P. J., Medema, G. J., Stakelbeek, A. (1998). Water Science and *Technology* 38:127-131. A field study was performed to investigate reduction by dune infiltration and to estimate sticking efficiencies of F-specific RNA bacteriophages, total and thermotolerant coliforms, faecal streptococci and spores of sulphite-reducing clostridia. Reduction was considered as a beta-binomially distributed process and a Monte Carlo simulation wag applied for estimating sticking efficiencies. Reduction of F-specific RNA bacteriophages within the first 2m was 3.8 log10 and the sticking efficiency was about 0.002. The faecal indicator bacteria were removed only 0.9 log10 within 2m and sticking efficiency was 0.007. Concentrations of spores of sulphite reducing clostridia were reduced 1.9 log10 and their sticking efficiency was about 0.009
- 122. Reduction of Norwalk virus, poliovirus 1 and coliphage MS2 by monochloramine disinfection of water. Shin, G. A., Sobsey, M. D. (1998). Water Science and Technology 38:151-154. The reduction of Norwalk virus (NV) by a 2 mg/L dose of pre-formed monochloramine was determined at pH 8 and 5degreeC in bench-scale, batch disinfection experiments using quantitative RT-PCR for NV assays. Two other enteric viruses, poliovirus 1 (PV1) and coliphage MS2, were included for comparison and assayed by infectivity as well as RT-PCR. After 3h, reductions of PV1 and MS2 by infectivity assays were about 1 log10 but there were no reductions of these viruses by RT-PCR assays. Hence, RT-PCR underestimated virus inactivation by monochloramine. However, NV reduction by monochloramine was about 1 log10 by RT-PCR assay, suggesting that it is more susceptible to monochloramine than the other two viruses tested. Based on RT-PCR titre reduction, the CT99 value for NV was about 775 mg-min/L. If the reduction of NV infectivity by monochloramine is ever greater than the reduction of RT-PCR signals, the CT99 value would be smaller. However, the results of this study indicate that NV and the other enteric viruses tested are not rapidly and extensively reduced by disinfection with preformed monochloramine
- 123. Time dose reciprocity in UV disinfection of water. Sommer, R., Haider, T., Cabaja., Pribil, W., Lhotsky, M. (1998). Water Science and Technology 38:145-150. The microbicidal effect of UV light depends on the dose in both, disinfection processes and natural inactivation by the sunlight in surface water. Deviations of the time dose reciprocity are well known from chemical water disinfection whereas no data are available about this effect in UV inactivation in water. In a previous study we found that the UV inactivation behaviour of yeast strains does not follow the time dose reciprocity, insofar that longer exposure led to higher reduction of cultivable cells. In contrast, an earlier study about E. coli B/r claimed a higher inactivation with single exposure compared with fractionated UV irradiation. To investigate this guestion we selected water-relevant microorganisms and studied their UV inactivation behaviour (253.7nm) by means of a specially designed UV irradiation apparatus (a) under standard irradiation conditions (2W/m2) and (b) with three levels of UV dose rate (2, 0.2 and 0.02W/m2). The test organisms were (i) three E. coli strains (ATCC 25922, ATCC 11229 and an isolate from sewage) representing the routinely used faecal indicator, (ii) three bacterial viruses (MS2, variant phiX174 and B40-8) proposed as indicators for viral contamination in water and (iii) spores of Bacillus subtilis because of their use as a biodosimeter in prototype testing of commercial UV plants for drinking water disinfection. We found, under standard inactivation conditions, that the E. coli strains and phage variant phiX174 are most UV susceptible, followed by B40-8 and finally MS2 and bacterial spores. The dose protraction experiments revealed for the E. coli strains a higher inactivation with high dose rates compared to low dose rates at the same UV doses (difference of about 1 log10 at 80-100J/m2). The other test organisms did not deviate from the time dose reciprocity in the proven range of dose
 - 124. Biological properties and classification of *Erwinia carotovora* bacteriocins. Tovkach, F. I. (1998). *Mikrobiologiya* 67:767-774. Study of 52 phytopathogenic strains of *Erwinia carotovora* showed that all of them could produce both colicin-like and macromolecular phage-tail-like bacteriocins. Colicin-like carotovoricins proved to lyse phytopathogenic agrobacteria, pseudomonads, and E. chrysanthemi, as well as the representatives of *E. herbicola* and *Klebsiella* sp. Macromolecular carotovoricins (MCTV) primarily lysed *E. carotovora* and *Escherichia coli* strains. A classification of MCTV was elaborated
- 125. Chemiluminescence patterns from bacterial cultures undergoing bacteriophage induced mass lysis. Vogel, R., Guo, X, Suessmuth, R. (1998). Bioelectrochemistry and Bioenergetics 46:59-64. Chemiluminescence from liquid culture media, as they are being used for the growth of microorganisms, is a common observation under aerobic conditions. During the growth of bacterial cells it is subjected to a process that leads to an almost complete elimination of the light emission. We report on a cancellation of this quenching process during mass lysis of cultures of the strains *Escherichia coli* B and *Lactococcus lactis* lactis 530-12 induced by the bacteriophages T7 and P530-12, respectively, leading to distinct light emission patterns. This cancellation does not occur with *E. coli* B when lysis occurs above a specific critical cell density. As cancellation can be induced by the addition of detergents to the lysate, this is probably due to the formation of membrane vesicles that continue quenching the chemiluminescence from the medium
 - 126. Biochemical and genetic analysis of lambdaW, the newly isolated lambdoid phage. Wrobel, B., Srutkowska, S., Wegrzyn, G. (1998). Acta Biochimica Polonica 45:251-259. Otherwise isogenic Escherichia coli CP78 (relA+) and CP79 (relA-) strains are commonly used in studies on the stringent control, the bacterial response to amino acid starvation. We found that these strains are lysogenic for a phage which is spontaneously induced with a low frequency, producing virions able to infect other *E. coli* strains. Genetic studies, restriction analysis of the phage DNA genome, and electron microscopy revealed that this phage is very similar to, but not identical with, bacteriophage gamma. We called the newly isolated phage lambdaW, and found that most of CP78/CP79 ancestor strains are lysogenic for this phage

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