Bacteriophage Ecology Group (BEG) News

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

October 1, 2002 issue (volume 14)

At this site you will find . . .

Editorial

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

Phage or Phages

by Hans-Wolfgang Ackermann

During the last 50 years the terms "bacteriophage" and "phage" had a plural in the English-language scientific literature. As usual, it was indicated by -s: bacteriophage**s**, phage**s**. Similar plurals exist in other major scientific languages such as German, French, and Russian. Japanese is an exception because it has no plurals.

This happy situation seems to be over. Suddenly one notices in recent papers or manuscripts that some authors are using "bacteriophage" and "phage" as invariable nouns: one bacteriophage, two bacteriophage rather than one bacteriophage, two bacteriophage**s** . These authors are generally raw newcomers to the field of virology. Where did they get their terminology from? What is correct? Presumably not from dictionaries or usage books. Dictionaries use singular forms of nouns and do not indicate plurals. Usage books do not even mention "bacteriophage" and "phage."

We love (or hate) bacterial viruses. We owe them our salaries and livelihood. We write papers on them (or are supposed to). We should strive to use optimal language. We thus should give the matter some thought. To do this, we shall go back to the history of the terms "bacteriophage" and "phage" and seek help in the books of Raettig [9, 10], which are indispensable guides to the older literature.

The terms "bacteriophage" and "phage" were coined by Félix d'Hérelle in 1918 [6]. He believed that there was only one bacteriophage with many races (hence no need for a plural) though he was convinced of the corpuscular, viral nature of his agents (which, of course, would suggest a need). d'Hérelle's ideas and terminology became widely known and almost

universally accepted, partly because his two most important books were translated into English in 1922 and 1926, respectively [7, 8]. However, already in 1923 an outsider put the word "bacteriophage" into plural [5].

The plural form was truly introduced in 1929, when Burnet and McKie [1] proved that viruses of staphylococci were indeed heterogeneous and could not be considered as a single entity. By the fifties the plural form became generalized, for example in the publications of Delbrück, Dulbecco, Elford, Jacob, Luria, Lwoff, Nicolle, Ruska, and Wyckoff. Some people, using "bacteriophage" as variable or invariable nouns in different papers, had it both ways. In the sixties, the variable form (one bacteriophage, two bacteriophage**s**) was almost universally accepted, although use of the singular form (two bacteriophage) lingered on.

Now, after 50 years of relative peace, the old invariable form is returning to the literature. We now have two usages:

1. **Bacteriophage(s)** and **phage(s)**. The noun is variable. The singular denotes an individual virus particle, a phage species, or a phage strain. The plural designates a population of phage particles, several phage species or strains, and the sum of all bacterial viruses: two types of phage**s** or the sum of all phage**s**.

2. **Bacteriophage** and **phage**. The noun is invariable. Singulars and plurals are indicated by pronouns or modifiers (it, this, these, few) and verb forms: these phage, those phage, and a few phage as well as two phage and the sum of all phages, rather than "phage**s**" for each of the above examples. Differentiation is generally impossible in the past tense.

Invariable nouns are infrequent in the English language and fall into several categories [2, 3, 4].

1. Nouns used only in the singular (e.g., physics).

2. Nouns used only in the plural (e.g., clothes or arms).

3. Nouns used in the singular or plural according to context:

    a. Collective or groups nouns, in which the singular form (without terminal -s) can take either a singular or plural verb (army, committee, family, majority). The choice depends on whether the group is considered as a single unit or a collection of individuals [2].

    b. Oddities without plural endings (e.g., sheep, aircraft, offspring, series, species, French, Japanese, Swiss).

    c. Ethnic names with facultative -s terminals (e.g., Haussa, Yoruba). The choice depends on the whim of the writer.

    d. Pidgins (e.g., "two book are" in Jamaican Creole) [4].

The invariable variety of the noun "bacteriophage" originated as a term without a plural and then became a group name. It is thus grammatically correct to say "bacteriophage is" and "bacteriophage was." The question is rather: is there any advantage to this? I see only one, of sorts, that it recalls an old misinterpretation. The disadvantages are many. In particular, invariable nouns are:

1. Relatively unusual.

2. Relatively rigid and unclear; indeed, they promote muddled writing.

3. Unwarranted and generally useless if there are plurals in good standing. In particular, "the bacteriophages" is an excellent collective name and there is no need for an invariable form.

4. Virtually nonexistent elsewhere in microbiology. I noticed indeed a title in an old paper that read "destruction of bacterial virus", but this would be inacceptable today.

5. Reminiscent of Pidgin English.

Finally, the invariable term "bacteriophage" is at variance with the use of this word in other scientific languages and the use of "-phage" in related compound words (e.g., anthropophage, macrophage, sarcophage). Thus, I do not understand when the old invariable term was resurrected and I side, definitely, in favor of pluralization and "bacteriophages and phages."

REFERENCES

1. Burnet, F.M., McKie, M. 1929. Type differences amongst staphylococcal bacteriophages. Austr. J. Exp. Biol. Med. Sci. 6, 21.

2. Chalker, S., Weiner, E. 1994. The Oxford Dictionary of English Grammar. Clarendon Press, Oxford, 1994, pp. 69, 180, 301.

3. Clark, J.O.E. 1990. Harrap's Dictionary of English Usage. Harrap, London, p. 317.

4. Crystal, D. 1995. The Cambridge Encyclopedia of the English Language. Cambridge University Press, Cambridge, p. 200.

5. Doerr, R. 1922. Die Bakteriophagen (Phänomen von Twort und d'Hérelle). Klin. Wschr. 1922, 1489 and 1537.

6. d'Hérelle, F. 1918. Sur le rôle du microbe filtrant bacteriophage dans la dysenterie bacillaire. C.R. Acad. Sci. 167, 970.

7. d'Hérelle, F. 1922. The bacteriophage; its role in immunity. Baltimore, Williams & Wilkins.

8. d'Hérelle, F. 1926. The bacteriophage and its behavior. Baltimore, Williams & Wilkins.

9. Raettig, H. 1958. Bakteriophagie 1917 bis 1956, part II. Gustav Fischer, Stuttgart.

10. Raettig, H. 1968. Bakteriophagie 1957-1965, part II. Gustav Fischer, Stuttgart.

Editor's Note: Though I will make no claims to consistency, nevertheless when in doubt I substitute "horse" for "phage" in my writing. If the resulting construct seems to call for "horses" rather than "horse," then I use "phages" rather than "phage," i.e., one horse, two horses, many horses, those horses, etc.

Editorial Archive

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

The BEG members page can be found at www.phage.org/beg_members.htm. There are two ways of "joining" BEG. One, the "traditional" way, is to have your name listed on the web page and on the list server. The second, the "non-traditional" way, is to have your name only listed on the list server. The latter I refer to as "non-members" on that list. Members, e.g., individuals listed on the BEG members list page, should be limited to individuals who are actively involved in science (research, instruction, outreach, industry) and who can serve as a phage ecology resource to interested individuals. If you have an interest in phage ecology but no real expertise in the area, then you should join as a non-member. To join as a member, please contact BEG using the following link: abedon.1@osu.edu. Include:

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

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

Please welcome our newest members

name (home page links)	status	e-mail	address

Deepak Bala	PI	phage_66 @hotmail.com	GangaGen Biotechnologies Pvt. Ltd., #6, 6th main, BDA Industrial Layout, Near SRS Road, Peenya, Bangalore 500058, INDIA
	interests:	My principal field of interest is Phage Biology particularly *Pseudomonas* phages. (contents | BEG members | top of page)	
Botond Balogh	---	bbalogh @mail.ifas.ufl.edu	1453 Fifield Hall, PO Box 110680, Gainesville, FL 32611-0680
	interests:	Control of tomato bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* with bacteriophage applications. In the future I will work on controlling citrus cancer, caused by *Xanthomonas axonopodis* pv. *citri*, with phages. (contents | BEG members | top of page)	
Bharat Bongale	---	bharatbongale @hotmail.com	211-41 Woodridge Crescent, Nepean, Ontario K2B 7T6, Canada
	interests:	Phage Therapy. Currently working on application of bacteriophages on *Campylobacter*. It is one of the major problem in beef, poultry and swine industry, in North America. My work includes collection, isolation and characterization of phages from environment. (contents | BEG members | top of page)	
Graeme Frith	---	graeme.frith @jenner.ac.uk	Carbohydrate Immunology Group, The Edward Jenner Institute for Vaccine Research, Compton, Berks. RG20 7NN. UK
	interests:	I have a background in phage-display of recombinant scFv molecules for both therapeutic and detection purposes. This has led to an interest in developing (i) recombinant phages expressing novel receptors for the use in prophylactic vaccines (I would be interested to hear if anyone has access to lytic phages against *Neisseia meningitides* or *Bacillus anthracis*) and (ii) applications for phage glycosidases in processing potential glycoprotein or LPS vaccine targets. (contents | BEG members | top of page)	
Kiarash Ghazvini	PI	k-ghazvini @mums.ac.ir	Mashhad university of medical science (mums), no31, kamal al molk 6, malek abad ave., mashhad,Iran
	interests:	Use of bacteriophage to control of bacteria in environment and in foods. (contents | BEG members | top of page)	
Michael W. Heuzenroeder	---	michael.heuzenroeder @imvs.sa.gov.au	Infectious Diseases Laboratories, Institute of Medical and Veterinary Science; Mail address: PO Box 14, Rundle Mall, Adelaide, SA, 5000, Australia; Street address: Gate 7, RAH Campus, Frome Road, Adelaide, South Australia
	interests:	Significance of temperate phage in *Salmonella* and their influence upon epidemiology and typing systems. (contents | BEG members | top of page)	
T. Toney Ilenchuk	PI	toney.ilenchuk @nrc.ca	Biophage Inc., 6100 Royalmount, Montreal, Quebec H4P 2R2, Canada
	interests:	Biophage Pharma Inc. VP and Chief Development Officer responsible for the development of phage therapy program. (contents | BEG members | top of page)	
Athol V. Klieve	PI	athol.klieve @dpi.qld.gov.au	Queensland Department of Primary Industries, Animal Research Institute, Locked Mail Bag, No. 4, Moorooka, Qld. 4105, AUSTRALIA
	interests:	Role of bacteriophages in the rumen ecosystem and the downsteam impact that phage-mediated bacterial lysis has on the nutrition of ruminants in agricultural systems. Use of bacteriophages and archaeal viruses to manipulate the rumen ecosystem. Phages that carry Shiga-toxin genes and their ecology in relation to *E. coli*. (contents | BEG members | top of page)	
Adeniran Koko	---	nirankoko @yahoo.com	Dept.of Botany and Microbiology, University of Lagos, Akoka, Nigeria,
	interests:	Isolation of phages from various marine sources; phage therapy particularly of *Staphylococcus*,*Psuedomonas* and *E.coli*; and electron microscopy and classification of phages in Nigeria. (contents | BEG members | top of page)	
Toshihiro Nakai	PI	nakaitt @hiroshima-u.ac.jp	Graduate School of Biosphere Science, Hiroshima University, Higashihiroshima 739-8528, Japan
	interests:	Phage therapy of fish. (contents | BEG members | top of page)	
Gregory R. Siragusa	PI	Siragusa @saa.ars.usda,gov	Agricultural Research Service - USDA, Russell Research Center, 950 College Station Road, P.O. Box 5677, Athens, GA 30605
	interests:	Ecology of bacteriophage populations in the poultry intestinal tract and rearing environment. Biocontrol using specific phage and phage mixtures for human and poultry pathogens. (contents | BEG members | top of page)	
Kui Wang	---	wangk @umbi.umd.edu	Center of Marine Biotechnology, University of Maryland Biotechnology Institute, University of Maryland, College Park
	interests:	I am interested in the ecology and evolution of cyanophage. I have been working on isolation of cyanophages infecting unicellular *Synechococcus* spp. (contents | BEG members | top of page)	

Meetings

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

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

Evergreen International Phage Meeting

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

Three molecular biologists in their native environment. Left to right: Paul Hyman, Steve Abedon, and Danny Munoa = senior scientist at NanoFrames LLC, visiting prof (from Ohio State), and Summer-intern undergrad (from Eddie Goldberg's lab at Tufts), respectively.

Jobs

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

Click here for International Society for Microbial Ecology Employment Listings.

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

Click here for AAAS "Microbial Ecology" Search.

Click here for AAAS "Ecology and Microbiology" Search.

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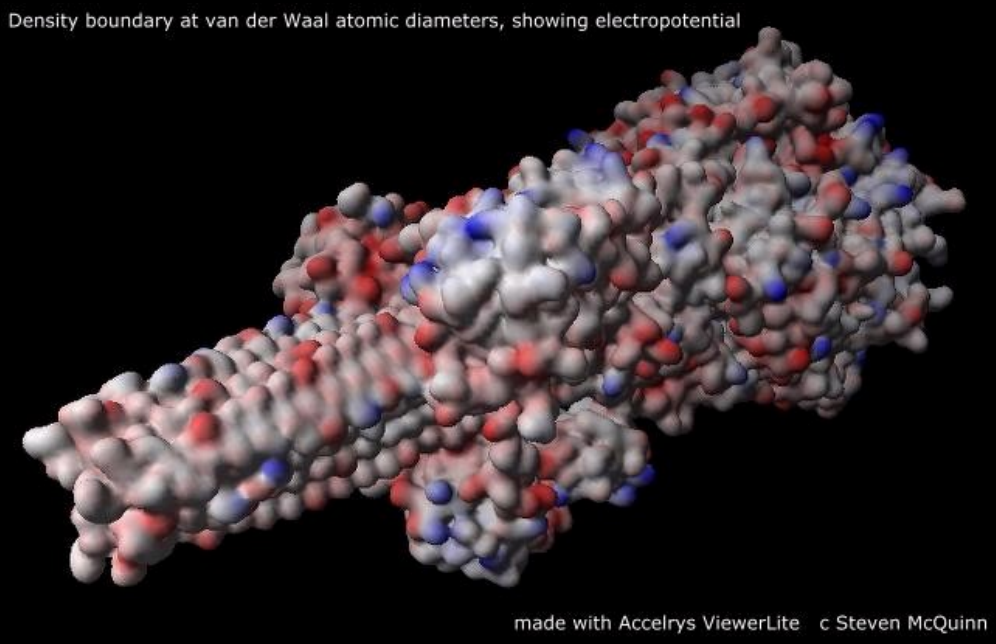
Submissions

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

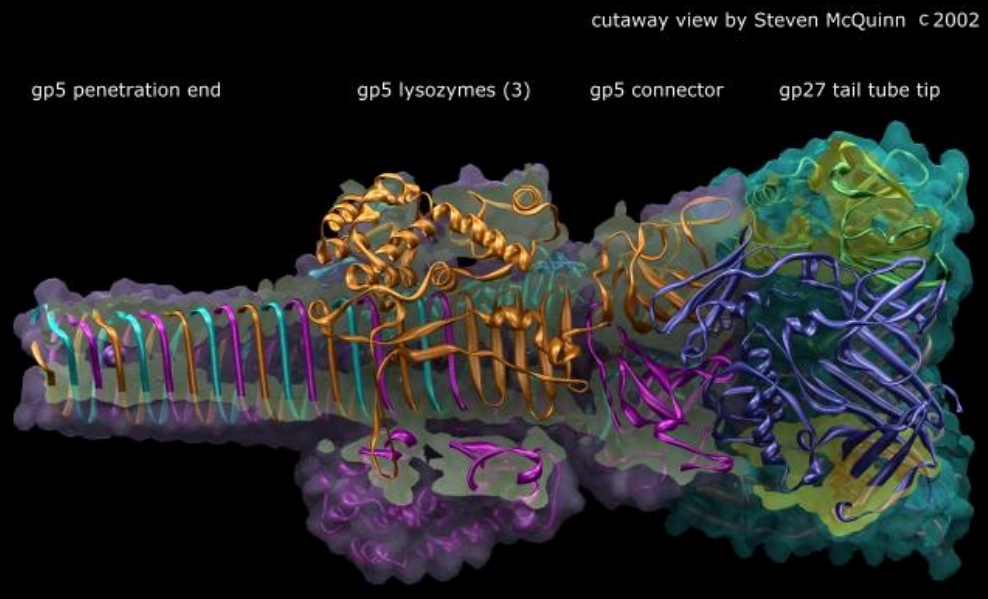
Eye On The Needle:
Phage T4 Puncturing Point May Answer Penetrating Questions

by Steven McQuinn



The elaborate infection mechanics of Bacteriophage T4 are often illustrated as Nature's nanoscale version of a hypodermic syringe. This misleading analogy has flourished in graphics used by introductory textbooks, biology lectures and the popular media, even though phage researchers have long known that "it ain't necessarily so."

The real story is infinitesimally more complicated and not fully understood. The recent revelation of structural detail in the cell-puncturing tip of T4 may eventually provide answers to the question, "How does T4 thread its long strand of DNA through the host cell wall into the cytoplasm?"



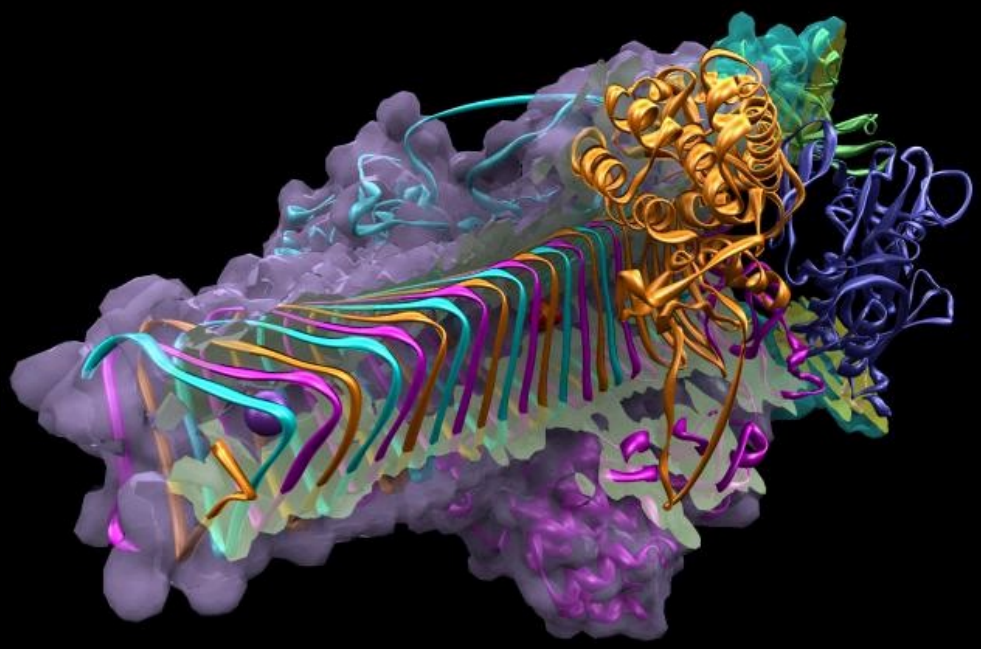
10 nanometers

Structure of cell wall puncturing device on tail tip of Bacteriophage T4

The syringe analogy pictures the phage tip plunging completely through the "skin" of the host, forcibly injecting phage DNA through a penetrating pipe that extends well into the cell interior. Evidence contradicts this model, while suggesting an alternative.

The cell envelope of the gram negative bacteria, *Escherichia coli*, is a laminate similar to a Kevlar flack vest, with an inner and outer membrane and a tough layer of fiber between. The T4 tail tip, pressed into the cell wall by tail sheath contraction, seems to be structured for pushing aside the lipid outer membrane, then cutting through the peptidoglycan fibers using the three lysozyme "scissors" loosely and flexibly deployed around its central barrel.

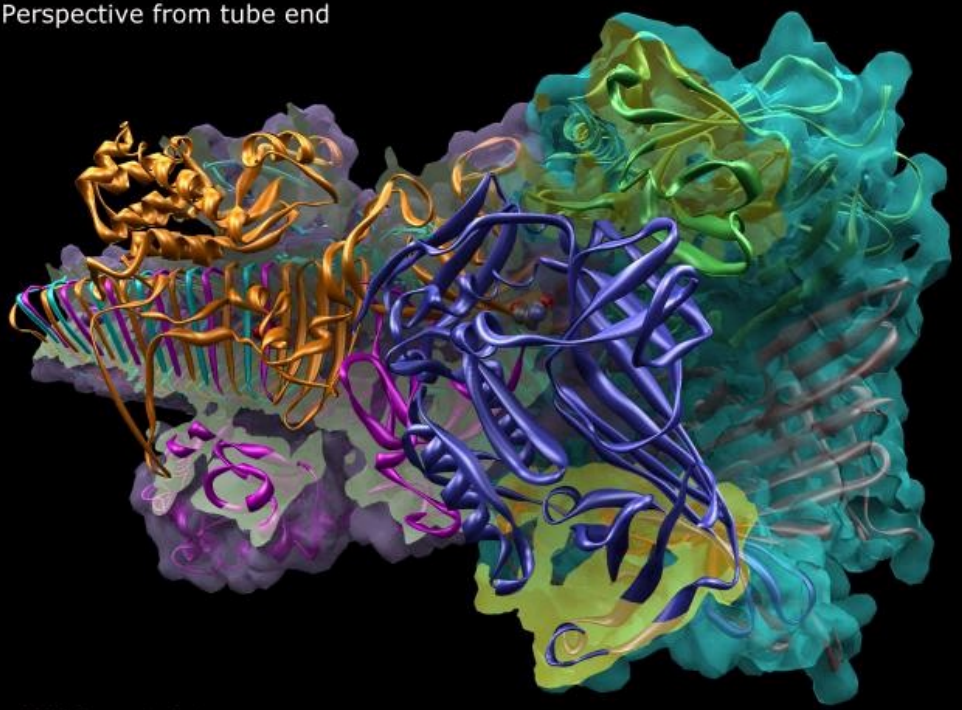
Perspective from tip end



While the literature is not completely clear about the penetration of the inner membrane, the inner and outer membranes apparently fuse across the puncture zone to create a channel through the cell envelope for the hollow tail tube pushing the tail tip. Electron micrographs show that the hollow tail tube usually extends down to, but not into, the cytoplasm.

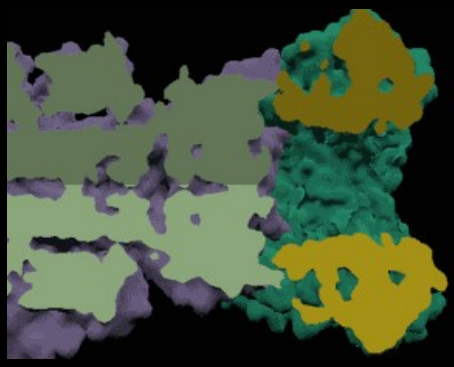
X-ray crystallography to 2.9 Angstrom resolution reveals that the penetrating point itself provides no hollow passageway for DNA. However, the penetrating tip is connected to the tail tube via a hollow ring, allowing a route for DNA, or a leader for the DNA, to reach the backside of the tail tip via the tail tube.

Perspective from tube end



Thus poised at the edge of the cytoplasm, what now draws the tail tip and the DNA into the guts of the cell? Again, the structure of the tail tip may provide clues. Researchers have speculated that a voltage difference across the inner membrane (the Proton Motive Force) may act upon both the tail tip and the DNA strand, moving them into the cytoplasm. The distribution of electro-potential on the surface of the tail tip might support that hypothesis.

It is clear that the tip, gene product 5 (gp5), is structured to break away from the hollow ring connecting it to the tail tube, gene product 27 (gp27). It is not clear whether the separated tip plays a role in DNA entry or is simply removed to make way for the DNA. In at least one electron micrograph, a string of expelled phage DNA seems to dangle a little bauble that looks suspiciously like a tail tip. One is tempted to make comparisons with a hooked trout spooling the line off a fishing reel, but as can be seen with hypodermic syringes, reasoning by analogy can be dangerous.



See Acknowledgements for an alternate version of this animated gif.

These images were constructed from data obtained from the web-based Atlas of Macromolecules via Protein Explorer, the web-based interface for the molecular visualization engine, Chime. The Atlas version of 1K28 provides a full model of the gp5/gp27 complex, whereas the Protein Data Bank version, 1K28.pdb, provides only one third of the full structure.

After saving the full structure from Chime as a .pdb file, I opened it in the free molecular visualization program, Accelrys ViewerLite, which I used for the electro-potential surface rendering. Exporting the surface and the ribbon as VRML files, I cut the surface mesh using Amapi 6, then assembled and rendered the cutaway view in Carrara Studio 2.

Click Here for One More (Spectacular) Image (about 500 kb)

Citations:

Kanamaru S, Leiman PG, Kostyuchenko VA, Chipman PR, Mesyanzhinov VV, Arisaka F, Rossmann MG. Structure of the cell-puncturing device of bacteriophage T4. Nature. 2002 Jan 31;415(6871):553-7. Abstract

Goldberg, E., Grinius, L. & Letellier, L. in Molecular Biology of Bacteriophage T4 (ed. Karam, J. D.) Chapter 34, p. 347-356 (American Society for Microbiology, Washington, DC, 1994).

Coombs, D. H. & Arisaka, F. in Molecular Biology of Bacteriophage T4, Chapter 21, p. 259-281.

Kutter, E., Guttmann, B., Carlson, K., in Molecular Biology of Bacteriophage T4, Chapter 33, p. 343-356.

Submissions Archive

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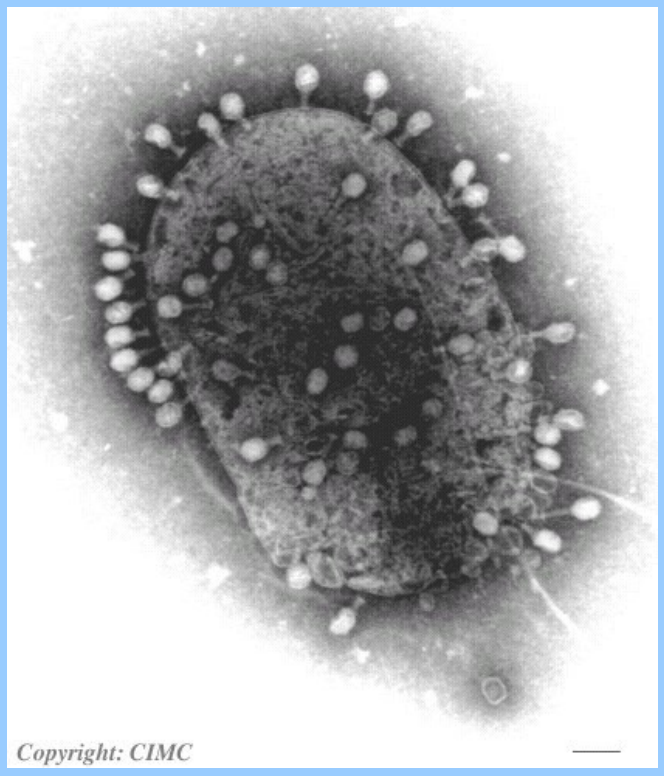
Phage Images

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

- BEG Phage Images Page
- The Face of the Phage
- Bacteriophage T2
- SSV1-Type Phage
- Saline Lake Bacteriophage
- Coliphage LG1
- Bacteriophage HK97
- Phage T4 (art)
- Phage T4 on the pedestal outside of Barker Hall at Berkeley
- Electron micrograph of phage P22
- Thin section of T4 phages hitting a microcolony of *E. coli* K-12
- T4 phage v1
- T4 Tail Model
- Gingerbread phage
- T4 adsorbing en mass

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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. Complete genomic sequence of SfV, a serotype-converting temperate bacteriophage of *Shigella flexneri*. Allison, G. E., Angeles, D., Tran-Dinh, N., Verma, N. K. (2002). *Journal of Bacteriology* 184:1974-1987. [PRESS FOR ABSTRACT]

2. The hyaluronan lyase of *Streptococcus pyogenes* bacteriophage H4489A. Baker, J. R., Dong, S., Pritchard, D. G. (2002). *Biochemical Journal* 365:317-322. [PRESS FOR ABSTRACT]

3. Genome sequence of a serotype M3 strain of group A Streptococcus: phage-encoded toxins, the high-virulence phenotype, and clone emergence. Beres, S. B., Sylva, G. L., Barbian, K. D., Lei, B., Hoff, J. S., Mammarella, N. D., Liu, M. Y., Smoot, J. C., Porcella, S. F., Parkins, L. D., Campbell, D. S., Smith, T. M., McCormick, J. K., Leung, D. Y. M., Schlievert, P. M., Musser, J. M. (2002). *Proceedings of the National Academy of Sciences, USA* 99:10078-10083. [PRESS FOR ABSTRACT]

4. The in vitro interaction of *Streptococcus pyogenes* with human pharyngeal cells induces a phage-encoded extracellular DNase. Broudy, T. B., Pancholi, V., Fischetti, V. A. (2002). *Infection and Immunity* 70:2805-2811. [PRESS FOR ABSTRACT]

5. Removal of bacterial and viral faecal indicator organisms in a waste stabilization pond system in Choconta, Cundinamarca (Colombia). Campos, C., Guerrero, A., Cardenas, M. (2002). *Water Science and Technology* 45:61-66. [PRESS FOR ABSTRACT]

6. Memory in bacteria and phage. Casadesus, J., D'Ari, R. (2002). *Bioessays* 24:512-518. [PRESS FOR ABSTRACT]

7. Genomic sequence and evolution of marine cyanophage P60: a new insight on lytic and lysogenic phages. Chen, F., Lu, J. (2002). *Applied and Environmental Microbiology* 68:2589-2594. [PRESS FOR ABSTRACT]

8. The antibody response to bacteriophage is linked to the lymphopenia gene in congenic BioBreeding rats. Clark, L., Greenbaum, C., Jiang, J., Lernmark, A., Ochs, H. (2002). *FEMS Immunology and Medical Microbiology* 32:205-209. [PRESS FOR ABSTRACT]

9. Est-ce que les bactériophages pourraient être une thérapie antimicrobienne efficace pour résoudre le problème de la résistance bactérienne aux antibiotiques ? Colbert, M., Guilmette, M. (2002). [NO ABSTRACT]

10. Sequence analysis of the lactococcal bacteriophage bIL170: insights into structural proteins and HNH endonucleases in dairy phages. Crutz-Le Coq, A.-M., Cesselin, B., Commissaire, J., Anba, J. (2002). *Microbiology* 148:985-1001. [PRESS FOR ABSTRACT]

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Postic, D., Baranton, G., Saint Girons, I (2001). *FEMS Microbiology Letters* 201:139-143. [PRESS FOR ABSTRACT]

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52. Removal of *Salmonella* and microbial indicators in constructed wetlands treating swine wastewater. Hill, V. R., Sobsey, M. D. (2001). *Water Science and Technology* 44:215-222. [PRESS FOR ABSTRACT]

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61. Susceptibility of *Staphylococcus epidermidis* biofilm in CSF shunts to bacteriophage attack. Wood, H. L., Holden, S. R., Bayston, R. (2001). *European Journal of Pediatric Surgery* 11 Suppl 1:S56-S57. [NO ABSTRACT]

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63. Horizontal gene transfer and the origin of species: lessons from bacteria. de la Cruz, F., Davies, J. (2000). *Trends in Microbiology* 8:128-133. [PRESS FOR ABSTRACT]

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66. Monitoring of environmental UV radiation by biological dosimeters. Ronto, G., Berces, A., Grof, P., Fekete, A., Kerekgyarto, T., Gaspar, S., Stick, C. (2000). *Advances in Space Research* 26:2021-2028. [PRESS FOR ABSTRACT]

67. A hypothesis for DNA viruses as the origin of eukaryotic replication proteins. Villarreal, L. P., DeFillips, V. R. (2000). *Journal of Virology* 74:7079-7084. [PRESS FOR ABSTRACT]

68. Diffusion of bacteriophage through alginate gels. Ibrahim, L. J., Denyer, S. P., Hanlon, G. W. (1999). *Journal of Pharmacy and Pharmacology* 51:38. [NO ABSTRACT]

69. Identification of two types of actionophage parasitic to potato common scab pathogens in Hokkaido, Japan. Ogiso, H., Akino, S., Ogoshi, A. (1999). *Soil Microorganisms* 53:37-43. [NO ABSTRACT]

70. Sequence of Shiga toxin 2 phage 933W from *Escherichia coli* O157:H7: Shiga toxin as a phage late-gene product. Plunkett III, G., Rose, D. J., Durfee, T. J., Blattner, F. R. (1999). *Journal of Bacteriology* 181:1767-1778. [PRESS FOR ABSTRACT]

71. Bacteriophages as surface and ground water tracers. Rossi, P., Doerfliger, N., Kennedy, K., Müller, I., Aragno, M. (1998). *Hydrology and Earth System Science* 2:101-110. [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. **Complete genomic sequence of SfV, a serotype-converting temperate bacteriophage of *Shigella flexneri*. Allison, G. E., Angeles, D., Tran-Dinh, N., Verma, N. K. (2002). *Journal of Bacteriology* 184:1974-1987.** Bacteriophage SfV is a temperate serotype-converting phage of *Shigella flexneri*. SfV encodes the factors involved in type V O-antigen modification, and the serotype conversion and integration-excision modules of the phage have been isolated and characterized. We now report on the complete sequence of the SfV genome (37,074 bp). A total of 53 open reading frames were predicted from the nucleotide sequence, and analysis of the corresponding proteins was used to construct a functional map. The general organization of the genes in the SfV genome is similar to that of bacteriophage lambda, and numerous features of the sequence are described. The superinfection immunity system of SfV includes a lambda-like repression system and a P4-like transcription termination mechanism. Sequence analysis also suggests that SfV encodes multiple DNA methylases, and experiments confirmed that orf-41 encodes a Dam methylase. Studies conducted to determine if the phage-encoded methylase confers host DNA methylation showed that the two S. flexneri strains analyzed encode their own Dam methylase. Restriction mapping and sequence analysis revealed that the phage genome has cos sites at the termini. The tail assembly and structural genes of SfV show homology to those of phage Mu and Mu-like prophages in the genome of *Escherichia coli* O157:H7 and *Haemophilus influenzae*. Significant homology (30% of the genome in total) between sections of the early, regulatory, and structural regions of the SfV genome and the e14 and KpLE1 prophages in the *E. coli* K-12 genome were noted, suggesting that these three phages have common evolutionary origins

2. **The hyaluronan lyase of *Streptococcus pyogenes* bacteriophage H4489A. Baker, J. R., Dong, S., Pritchard, D. G. (2002). *Biochemical Journal* 365:317-322.** Many pathogenic streptococci produce extracellular hyaluronan lyases which are thought to aid the spread of the organism in host tissues. In addition, several phages of group A streptococci are known to synthesize a bound form of hyaluronidase. It has been suggested that the function of this hyaluronidase is to facilitate penetration of the hyaluronan capsule by phage and thus to gain access for the phage to the cell surface of the host streptococcus [Hynes, Hancock and Ferretti (1995) Infect. Immun. 63, 3015-3020]. In the present work, the hyaluronidase of *Streptococcus pyogenes* bacteriophage H4489A, expressed in *E. coli*, has been purified and characterized. The enzyme was shown to be a lyase with a distributive action pathway. Unlike most bacterial hyaluronidases that have been characterized, the phage enzyme was found to specifically cleave hyaluronan, which adds credence to the view that its function is to digest the hyaluronan capsule of the host organism. This bacteriophage lyase may provide a practical alternative to the lyase from *Streptomyces hyalurolyticus* as a reagent for the specific cleavage of hyaluronan

3. **Genome sequence of a serotype M3 strain of group A Streptococcus: phage-encoded toxins, the high-virulence phenotype, and clone emergence. Beres, S. B., Sylva, G. L., Barbian, K. D., Lei, B., Hoff, J. S., Mammarella, N. D., Liu, M. Y., Smoot, J. C., Porcella, S. F., Parkins, L. D., Campbell, D. S., Smith, T. M., McCormick, J. K., Leung, D. Y. M., Schlievert, P. M., Musser, J. M. (2002). *Proceedings of the National Academy of Sciences, USA* 99:10078-10083.** Genome sequences are available for many bacterial strains, but there has been little progress in using these data to understand the molecular basis of pathogen emergence and differences in strain virulence. Serotype M3 strains of group A Streptococcus (GAS) are a common cause of severe invasive infections with unusually high rates of morbidity and mortality. To gain insight into the molecular basis of this high-virulence phenotype, we sequenced the genome of strain MGAS315, an organism isolated from a patient with streptococcal toxic shock syndrome. The genome is composed of 1,900,521 bp, and it shares approximately 1.7 Mb of related genetic material with genomes of serotype M1 and M18 strains. Phage-like elements account for the great majority of variation in gene content relative to the sequenced M1 and M18 strains. Recombination produces chimeric phages and strains with previously uncharacterized arrays of virulence factor genes. Strain MGAS315 has phage genes that encode proteins likely to contribute to pathogenesis, such as streptococcal pyrogenic exotoxin A (SpeA) and SpeK, streptococcal superantigen (SSA), and a previously uncharacterized phospholipase A(2) (designated Sla). Infected humans had anti-SpeK, -SSA, and -Sla antibodies, indicating that these GAS proteins are made in vivo. SpeK and SSA were pyrogenic and toxic for rabbits. Serotype M3 strains with the phage-encoded speK and sla genes increased dramatically in frequency late in the 20th century, commensurate with the rise in invasive disease caused by M3 organisms. Taken together, the results show that phage-mediated recombination has played a critical role in the emergence of a new, unusually virulent clone of serotype M3 GAS

4. **The in vitro interaction of *Streptococcus pyogenes* with human pharyngeal cells induces a phage-encoded extracellular DNase. Broudy, T. B., Pancholi, V., Fischetti, V. A. (2002). *Infection and Immunity* 70:2805-2811.** The role lysogenic bacteriophage play in the pathogenesis of the host bacterium is poorly understood. In a previous study, we found that streptococcal coculture with human pharyngeal cells resulted in the induction of lysogenic bacteriophage as well as the phage-associated streptococcal pyrogenic exotoxin C (SpeC). In this study, we have determined that in addition to SpeC induction, a number of other streptococcal proteins are also released by the bacteria during coculture with pharyngeal cells. Among these, we identified and characterized a novel 27-kDa secreted protein. Sequence analysis of this novel protein demonstrated it to be encoded by the same lysogenic bacteriophage which harbors speC. Protein sequence analysis revealed varied homologies with several streptococcal DNases. Further biochemical characterization of the recombinantly expressed protein verified it to be a divalent cation-dependent streptococcal phage-encoded DNase (Spd1). Although functionally distinct, SpeC and Spd1 are associated by a number of parameters, including genetic proximity and transcriptional regulation. Finally, we speculate on the induction of phage-encoded DNase (Spd1) enhancing the fitness of both bacteria and phage

5. **Removal of bacterial and viral faecal indicator organisms in a waste stabilization pond system in Choconta, Cundinamarca (Colombia). Campos, C., Guerrero, A., Cardenas, M. (2002). *Water Science and Technology* 45:61-66.** A major objective for domestic wastewater treatment using waste stabilization pond systems is the removal of pathogenic microorganisms. Traditional evaluation parameters for faecal contamination are the total and faecal coliforms. However, epidemiological studies, environmental resistance and the behaviour in the treatment systems, show that viruses are an important disease agent and even more resistant to disinfection than bacteria. Therefore, it is important to introduce viruses as a faecal indicator and to compare them with the traditional bacterial indicators. A waste stabilization pond system was evaluated in the municipality of Choconta, Cundinamarca (Colombia), for the removal of faecal indicators (such as *Escherichia coli*, *Streptococcus faecalis*, *Clostridium perfringens*) and viruses like F+, somatic and *Bacteroides fragilis* phages. The system includes two facultative ponds in series with a flow of 1555 m3/day. Samples were collected at the entrance of the system, in the two ponds and from the final effluent. Results show a decrease between 0.3 and 4.7 logarithmic units in the bacterial indicators and between 1 and 4.6 logarithmic units with viral indicators

6. **Memory in bacteria and phage. Casadesus, J., D'Ari, R. (2002). *Bioessays* 24:512-518.** Whenever the state of a biological system is not determined solely by present conditions but depends on its past history, we can say that the system has memory.

Bacteria and bacteriophage use a variety of memory mechanisms, some of which seem to convey adaptive value. A genetic type of heritable memory is the programmed inversion of specific DNA sequences, which causes switching between alternative patterns of gene expression. Heritable memory can also be based on epigenetic circuits, in which a system with two possible steady states is locked in one or the other state by a positive feedback loop. Epigenetic states have been observed in a variety of cellular processes, and are maintained by diverse mechanisms. Some of these involve alternative DNA methylation patterns that are stably transmitted to daughter molecules and can affect DNA-protein interactions (e.g., gene transcription). Other mechanisms exploit autocatalytic loops whereby proteins establish the proper conditions for their continued synthesis. Template polymers other than nucleic acids (e.g., components of the cell wall) may also propagate epigenetic states. Non-heritable memory is exemplified by parasitic organisms that bear a signature of their previous host, such as host-controlled modification of phage DNA or porin hitchhiking in predatory bacteria. The heterogeneous nature of the examples known may be indicative of widespread occurrence of memory mechanisms in bacteria and phage. However, the actual extent, variety and potential selective value of prokaryotic memory devices remain open questions, still to be addressed experimentally

7. **Genomic sequence and evolution of marine cyanophage P60: a new insight on lytic and lysogenic phages. Chen, F., Lu, J. (2002). *Applied and Environmental Microbiology* 68:2589-2594.** The genome of cyanophage P60, a lytic virus which infects marine *Synechococcus* WH7803, was completely sequenced. The P60 genome contained 47,872 bp with 80 potential open reading frames that were mostly similar to the genes found in lytic phages like T7, fYeO3-12, and SIO1. The DNA replication system, consisting of primase-helicase and DNA polymerase, appeared to be more conserved in podoviruses than in siphoviruses and myoviruses, suggesting that DNA replication genes could be the critical elements for lytic phages. Strikingly high sequence similarities in the regions coding for nucleotide metabolism were found between cyanophage P60 and marine unicellular cyanobacteria

8. **The antibody response to bacteriophage is linked to the lymphopenia gene in congenic BioBreeding rats. Clark, L., Greenbaum, C., Jiang, J., Lernmark, A., Ochs, H. (2002). *FEMS Immunology and Medical Microbiology* 32:205-209.** Congenic BioBreeding (BB) rats, homozygous for the autosomal lymphopenia (Lyp) gene (Lyp/Lyp), heterozygous (Lyp/+), or wild-type (+/+), were immunized with the T cell-dependent bacteriophage fX174 to determine effects of Lyp on primary and secondary antibody responses. The primary fX174 antibody response did not differ between the three different genotypes. In contrast, the secondary immune response, expressed as the peak neutralizing titer, was markedly reduced in Lyp/Lyp (9.9+/-3.2; mean value+/-S.E.M. for seven rats) compared to both Lyp/+ (51+/-12; n=13; P=0.006) and +/+ (100+/-20; n=7; P=0.004) BB rats. We suggest that the secondary antibody response to the T cell-dependent neoantigen fX174 is linked in a recessive manner to genetic factor(s) in the Lyp gene region

9. **Est-ce que les bactériophages pourraient être une thérapie antimicrobienne efficace pour résoudre le problème de la résistance bactérienne aux antibiotiques ? Colbert, M., Guilmette, M. (2002).**

10. **Sequence analysis of the lactococcal bacteriophage bIL170: insights into structural proteins and HNH endonucleases in dairy phages. Crutz-Le Coq, A.-M., Cesselin, B., Commissaire, J., Anba, J. (2002). *Microbiology* 148:985-1001.** The complete 31754 bp genome of bIL170, a virulent bacteriophage of *Lactococcus lactis* belonging to the 936 group, was analysed. Sixty-four ORFs were predicted and the function of 16 of them was assigned by significant homology to proteins in databases. Three putative homing endonucleases of the HNH family were found in the early region. An HNH endonuclease with zinc-binding motif was identified in the late cluster, potentially being part of the same functional module as terminase. Three putative structural proteins were analysed in detail and show interesting features among dairy phages. Notably, gpl12 (putative fibre) and gpl20 (putative baseplate protein) of bIL170 are related by at least one of their domains to a number of multi-domain proteins encoded by lactococcal or streptococcal phages. A 110- to 150-aa-long hypervariable domain flanked by two conserved motifs of about 20 aa was identified. The analysis presented here supports the participation of some of these proteins in host-range determination and suggests that specific adsorption to the host may involve a complex multi-component system. Divergences in the genome of phages of the 936 group, that may have important biological properties, were noted. Insertions/deletions of units of one or two ORFs were the main source of divergence in the early clusters of the two entirely sequenced phages, bIL170 and sk1. An exchange of fragments probably affected the regions containing the putative origin of replication. It led to the absence in bIL170 of the direct repeats recognized in sk1 and to the presence of different ORFs in the ori region. Shuffling of protein domains affected the endolysin (putative cell-wall binding part), as well as gpl12 and gpl20

11. **A satellite phage-encoded antirepressor induces repressor aggregation and cholera toxin gene transfer. Davis, B. M., Kimsey, H. H., Kane, A. V., Waldor, M. K. (2002). *EMBO Journal* 21:4240-4249.** CTXphi is a filamentous bacteriophage whose genome encodes cholera toxin, the principal virulence factor of *Vibrio cholerae*. We have found that the CTXphi-related element RS1 is a satellite phage whose transmission depends upon proteins produced from a CTX prophage (its helper phage). However, unlike other satellite phages and satellite animal viruses, RS1 can aid the CTX prophage as well as exploit it, due to the RS1-encoded protein RstC. RstC, whose function previously was unknown, is an antirepressor that counteracts the activity of the phage repressor RstR. RstC promotes transcription of genes required for phage production and thereby promotes transmission of both RS1 and CTXphi. Antirepression by RstC also induces expression of the cholera toxin genes, ctxAB, and thus may contribute to the virulence of *V.cholerae*. In vitro, RstC binds directly to RstR, producing unusual, insoluble aggregates containing both proteins. In vivo, RstC and RstR are both found at the cell pole, where they again appear to form stable complexes. The sequestration/inactivation process induced by RstC resembles those induced by mutant polyglutamine-containing proteins implicated in human neurodegenerative disorders

12. **Bacteriophages: potential treatment for bacterial infections. Duckworth, D. H., Gulig, P. A. (2002). *BioDrugs* 16:57-62.** Bacteriophages (phages) are viruses of bacteria that can kill and lyse the bacteria they infect. After their discovery early in the 20th century, phages were widely used to treat various bacterial diseases in people and animals. After this enthusiastic beginning to phage therapy, problems with inappropriate use and uncontrolled studies and ultimately the development of antibacterials caused a cessation of phage therapy research in the West. However, a few institutions in Eastern Europe continued to study and use phages as therapeutic agents for human infections. The alarming rise in antibacterial resistance among bacteria has led to a review of the Eastern European studies and to the initiation of controlled experiments in animal models. These recent studies have confirmed that phages can be highly effective in treating many different types of bacterial infections. The lethality and specificity of phages for particular bacteria, the ability of phages to replicate within infected animal hosts, and the safety of phages make them efficacious antibacterial agents. Although there are still several hurdles to be overcome, it appears likely that phage therapy will regain a role in both medical and veterinary treatment of infectious diseases, especially in the scenario of emerging antibacterial resistance

13. **Combined antimicrobial effect of nisin and a listeriophage against *Listeria monocytogenes* in broth but not in buffer**

or on raw beef. Dykes, G. A., Moorhead, S. M. (2002). *International Journal of Food Microbiology* 73:71-81. The effect of nisin and listeriophage LH7, alone and in combination, on the growth and survival of two strains of *Listeria monocytogenes* in broth and two model food systems, with appropriate controls, was determined. Growth curves for both bacterial strains in tryptic soy broth incubated at 7 or 30 degrees C, and with the addition of nisin and/or listeriophage at lag, mid-exponential or early stationary phase, were obtained by measuring absorbance at 550 nm. Numbers of mixed populations of both *L. monocytogenes* strains in phosphate buffered saline (pH 5.5) and on vacuum-packaged fresh beef, both stored for 4 weeks at 4 degrees C, and with the addition of nisin and/or listeriophage, were determined. This was achieved by plating appropriately diluted samples on both Tryptic Soy Agar and Modified Oxford Agar to determine both *L. monocytogenes* numbers and the presence of sub-lethal injury. In broth nisin alone, reduced levels or prevented growth of the two strains under the conditions studied, but regrowth to levels equivalent to those of untreated cells, occurred. Listeriophage LH7 alone, on the other hand, had no effect in broth under the conditions studied. Notably, however, a mixture of nisin and listeriophage displayed a combined effect in broth and reduced levels of cells substantially without regrowth under the conditions studied. In both model food systems only nisin appeared to be active, in a manner consistent with existing literature, and no combined action was apparent. The use of nisin and listeriophage has potential to control *L. monocytogenes* in foods but a further understanding of the interactions in this complex system needs to be achieved before it could be applied practically

14. ***Giardia* and *Cryptosporidium* removal from waste-water by a duckweed (*Lemna gibba* L.) covered pond. Falabi, J. A., Gerba, C. P., Karpiscak, M. M. (2002). *Letters in Applied Microbiology* 34:384-387.** AIMS: To determine the ability of duckweed ponds used to treat domestic waste-water to remove *Giardia* and *Cryptosporidium*. METHODS AND RESULTS: The influent and effluent of a pond covered with duckweed with a 6 day retention time was tested for *Giardia* cysts, *Cryptosporidium* oocysts, faecal coliforms and coliphage. *Giardia* cysts and *Cryptosporidium* oocysts were reduced by 98 and 89%, respectively, total coliforms by 61%, faecal coliforms by 62% and coliphage by 40%. There was a significant correlation between the removal of *Giardia* cysts and *Cryptospordium* oocysts by the pond ($P < 0.001$). Influent turbidity and parasite removal were also significantly correlated (*Cryptosporidium* and turbidity, $P=0.05$; *Giardia* and turbidity, $P=0.01$). CONCLUSIONS: The larger organisms (parasites) probably settled to the bottom of the pond, while removal of smaller bacteria and coliphages in the pond was not as effective. SIGNIFICANCE AND IMPACT OF THE STUDY: Duckweed ponds may play an important role in wetland systems for reduction of *Giardia* and *Cryptosporidium*

15. **Phages and other mobile virulence elements in gram-positive pathogens. Gentry-Weeks, C., Coburn, P. S., Gilmore, M. S. (2002). *Current Topics in Microbiology and Immunology* 264:79-94.**

16. **Control of *Brochothrix thermosphacta* spoilage of pork adipose tissue using bacteriophages. Greer, G. G., Dilts, B. D. (2002). *Journal of Food Protection* 65:861-863.** Adipose tissue discs were coinoculated with Brochothrix thermosphacta and homologous bacteriophages (phages) to determine the effects these had on phage multiplication, bacterial growth, and off-odor development during storage at 2 degrees C or under simulated retail display at 6 degrees C. In the presence of about 10(5) bacteria/cm2 and an equivalent number of phages, there was a 3-log increase in phage numbers and a 2-log decrease in bacterial numbers, and objectionable off-odors were suppressed during refrigerated storage. Up to 68% of the surviving bacterial population were resistant to phages. The storage life of adipose tissue could be increased from 4 days in controls to 8 days in phage-treated samples by preventing the development of off-odors associated with the growth of B. thermosphacta. Phages may provide a novel approach to extending the storage quality of chilled meats

17. **Use of signal-mediated amplification of RNA technology (SMART) to detect marine cyanophage DNA. Hall, M. J., Wharam, S. D., Weston, A., Cardy, D. L. N., Wilson, W. H. (2002). *BioTechniques* 32:604-611.** Here, we describe the application of an isothermal nucleic acid amplification assay, signal-mediated amplification of RNA technology (SMART), to detect DNA extracted from marine cyanophages known to infect unicellular cyanobacteria from the genus *Synechococcus*. The SMART assay is based on the target-dependent production of multiple copies of an RNA signal, which is measured by an enzyme-linked oligosorbent assay. SMART was able to detect both synthetic oligonucleotide targets and genomic cyanophage DNA using probes designed against the portal vertex gene (g20). Specific signals were obtained for each cyanophage strain (S-PM2 and S-BnMI). Nonspecific genomic DNA did not produce false signals or inhibit the detection of a specific target. In addition, we found that extensive purification of target DNA may not be required since signals were obtained from crude cyanophage lysates. This is the first report of the SMART assay being used to discriminate between two similar target sequences

18. **Comparative analysis of the genomes of the temperate bacteriophages phi 11, phi 12 and phi 13 of *Staphylococcus aureus* 8325. Iandolo, J. J., Worrell, V., Groicher, K. H., Qian, Y., Tian, R., Kenton, S., Dorman, A., Ji, H., Lin, S., Loh, P., Qi, S., Zhu, H., Roe, B. A. (2002). *Gene* 289:109-118.** The genomes of the three temperate bacteriophages contained in the chromosome of *Staphylococcus aureus* 8325 have been extracted from the sequence database and analyzed. phi 11, phi 12 and phi 13 are members of the same lytic group but different serogroups and consequently co-habitate the same host cell. Their genomes are approximately 42 kb to 45 kb and contain about 90 ORFs of at least 50 codons. Of these, about 50 have similarities to known genes or to genes of other staphylococcal phages. Each of the phages clusters within a homology group that share large regions of sequence identity while intergroup homology is comparatively low. The arrangement of genes on the chromosomes of the three phages is similar and consistent with current modular theory of phage gene organization. The replicated genomes appear to be packaged by different mechanisms. Phage phi 11 and phi 12 have been found to contain sequences consistent with pac-site phages while phi 13 has sequences consistent with cos-site phages. The attBsite for phi 11 is located in an intergenic region of the S. aureus chromosome while phi 12 and phi 13 integrate into specific genes. The phi 12 att-site is within an unknown gene, but the phi 13 att-site is within the beta-toxin gene. In contrast to the other two phages, phi 13 also introduces the staphylokinase gene (sak) and a second gene related to expression of fib

19. **Overcoming the phage replication threshold: a mathematical model with implications for phage therapy. Kasman, L. M., Kasman, A., Westwater, C., Dolan, J., Schmidt, M. G., Norris, J. S. (2002). *Journal of Virology* 76:5557-5564.** Prior observations of phage-host systems in vitro have led to the conclusion that susceptible host cell populations must reach a critical density before phage replication can occur. Such a replication threshold density would have broad implications for the therapeutic use of phage. In this report, we demonstrate experimentally that no such replication threshold exists and explain the previous data used to support the existence of the threshold in terms of a classical model of the kinetics of colloidal particle interactions in solution. This result leads us to conclude that the frequently used measure of multiplicity of infection (MOI), computed as the ratio of the number of phage to the number of cells, is generally inappropriate for situations in which cell concentrations are less than 10(7)/ml. In its place, we propose an alternative measure, MOI(actual), that takes into account the cell concentration and adsorption time. Properties of this function are elucidated that explain the demonstrated usefulness of MOI

at high cell densities, as well as some unexpected consequences at low concentrations. In addition, the concept of MOI(actual) allows us to write simple formulas for computing practical quantities, such as the number of phage sufficient to infect 99.99% of host cells at arbitrary concentrations

20. **Nucleotide sequence of a ssRNA phage from Acinetobacter: kinship to coliphages. Klovins, J., Overbeek, G. P., van, den Worm, Ackermann, H. W., van, Duin (2002). *Journal of General Virology* 83:1523-1533.** The complete nucleotide sequence of ssRNA phage AP205 propagating in Acinetobacter species is reported. The RNA has three large ORFs, which code for the following homologues of the RNA coliphage proteins: the maturation, coat and replicase proteins. Their gene order is the same as that in coliphages. RNA coliphages or Leviviridae fall into two genera: the allolevivirus, like Qb, which have a coat read-through protein, and the levivirus, like MS2, which do not have this coat protein extension. AP205 has no read-through protein and may therefore be classified as a levivirus. A major digression from the known leviviruses is the apparent absence of a lysis gene in AP205 at the usual position, overlapping the coat and replicase proteins. Instead, two small ORFs are present at the 5' terminus, preceding the maturation gene. One of these might encode a lysis protein. The other is of unknown function. Other new features concern the 3'-terminal sequence. In all ssRNA coliphages, there are always three cytosine residues at the 3' end, but in AP205, there is only a single terminal cytosine. Distantly related viruses, like AP205 and the coliphages, do not have significant sequence identity; yet, important secondary structural features of the RNA are conserved. This is shown here for the 3' UTR and the replicase-operator hairpin. Interestingly, although AP205 has the genetic map of a levivirus, its 3' UTR has the length and RNA secondary structure of an allolevivirus. Sharing features with both MS2 and Q(beta) suggests that, in an evolutionary sense, AP205 should be placed between Q(beta) and MS2. A phylogenetic tree for the ssRNA phages is presented

21. **The ability of the plasmid-encoded restriction and modification system LlaBIII to protect *Lactococcus lactis* against bacteriophages. Kong, J., Josephsen, J. (2002). *Letters in Applied Microbiology* 34:249-253.** AIMS: To investigate the potential of the plasmid-encoded restriction and modification (R/M) system LlaBIII to protect *Lactococcua lactis* against bacteriophages during milk fermentations. METHODS AND RESULTS: The R/M system LlaBIII on plasmid pJW566 was cloned with a chloramphenicol cassette, resulting in plasmid pJK1. When introduced into L. lactis strains, pJK1 conferred increased phage resistance against the three most common lactococcal phage species 936, c2, and P335 and three unclassified industrial phages. The growth of the strains in RSM was not affected by the presence of plasmid pJK1. CONCLUSIONS: The plasmid-encoded R/M system LlaBIII has great ability to protect L. lactis strains against bacteriophages in milk fermentations. SIGNIFICANCE AND IMPACT OF THE STUDY: This study evaluates the ability of the LlaBIII R/M system to function as a phage defence mechanism which is an essential step prior to considering utilizing it for improving starter cultures

22. **The activity of chosen bacteriophages on *Yersinia enterocolitica* strains. Kot, B., Bukowski, K., Jakubczak, A., Kaczorek, I. (2002). *Pol. J. Vet. Sci.* 5:47-50.** The aim of the present study was to evaluate the lytic activity of three bacteriophages on *Yersinia enterocolitica* strains isolated from humans and pigs. The *Y. enterocolitica* strains tested belonged to 0:3, 0:9 and 0:2 serogroups. The ZD5 phage was obtained from a water sample, but remaining phages were obtained from the lysogenic *Y. frederiksenii* 7291 and *Y. enterocolitica* 8684 strains. All the *Y. enterocolitica* strains tested which belonged to 0:9 serogroup did not show any susceptibility to the bacteriophages used. The bacteriophages tested showed different lytic activity on the *Y. enterocolitica* 0:3 strains investigated. The phage susceptibility of *Y. enterocolitica* 0:3 strains revealed 9 different phage patterns. ZD5 phage showed the highest lytic activity, because it produced confluent lysis of the most Y. enterocolitica 0:3 strains tested. The Y. enterocolitica 0:2 strains isolated from pigs showed the similar phage susceptibility. The *Y. kristensenii* and *Y. pseudotuberculosis* strains tested were not sensitive to the bacteriophages used

23. **A bacteriophage reagent for *Salmonella*: molecular studies on Felix 01. Kuhn, J., Suissa, M., Chiswell, D., Azriel, A., Berman, B., Shahar, D., Reznick, S., Sharf, R., Wyse, J., Bar-On, T., Cohen, I., Giles, R., Weiser, I., Lubinsky-Mink, S., Ulitzur, S. (2002). *International Journal of Food Microbiology* 74:217-227.** Felix 01 (F01) is a bacteriophage originally isolated by Felix and Callow which lyses almost all *Salmonella* strains and has been widely used as a diagnostic test for this genus. Molecular information about this phage is entirely lacking. In the present study, the DNA of the phage was found to be a double-stranded linear molecule of about 80 kb. 11.5 kb has been sequenced and in this region A + T content is 60%. There are relatively few restriction endonuclease cleavage sites in the native genome and clones show this is due to their absence rather than modification. A restriction map of the genome has been constructed. The ends of the molecule cannot be ligated although they contain 5' phosphates. At least 60% of the genome must encode proteins. In the sequenced portion, many open reading frames exist and these are tightly packed together. These have been examined for homology to published proteins but only 1 to 17 shows similarity to known proteins. F01 is therefore the prototype of a new phage family. On the basis of restriction sites, codon usage and the distribution of nonsense codons in the unused reading frames, a strong case can be made for natural selection that reacts to mRNA structure and function

24. **Detection of bacteria using foreign DNA: the development of a bacteriophage reagent for *Salmonella*. Kuhn, J., Suissa, M., Wyse, J., Cohen, I., Weiser, I., Reznick, S., Lubinsky-Mink, S., Stewart, G., Ulitzur, S. (2002). *International Journal of Food Microbiology* 74:229-238.** A phage-based reagent was developed for the detection of *Salmonella* in food samples. The parental phage was Felix 01, which lyses practically all *Salmonella*. Using data obtained about the molecular biology of the phage, a recombinant phage that carried the bacterial genes specifying luciferase was produced. The method involved the isolation of amber nonsense mutations and subsequent crosses to render doubly mutant phage with a very low reversion rate on strains lacking an amber suppressor. A plasmid was constructed that contained a segment of Felix 01 DNA with two adjacent genes, one dispensable and the other essential, and their flanking sequences. Recombinant DNA technology was used to remove the two genes and the luxA and luxB genes for luciferase, and a gene specifying a tRNA that recognizes amber codons (supF=tyrT) was put in their stead. This region could be transferred into the genome of the phage by homologous recombination. The recombinant phage cannot grow because it lacks an essential gene. However, it can grow in a host that synthesizes the missing protein. This technique allows the construction of "locked" recombinant phages that carry foreign DNA but which cannot propagate themselves in nature

25. **Complete genomic sequence of bacteriophage ul36: demonstration of phage heterogeneity within the P335 quasi-species of lactococcal phages. Labrie, S., Moineau, S. (2002). *Virology* 296:308-320.** The complete genomic sequence of the *Lactococcus lactis* virulent phage ul36 belonging to P335 lactococcal phage species was determined and analyzed. The genomic sequence of this lactococcal phage contained 36,798 bp with an overall G+C content of 35.8 mol %. Fifty-nine open reading frames (ORFs) of more than 40 codons were found. N-terminal sequencing of phage structural proteins as well as bioinformatic analysis led to the attribution of a function to 24 ORFs (41%). A lysogeny module was found within the genome of this virulent phage. The putative integrase gene seems to be the product of a horizontal transfer because it is more closely related to *Streptococcus pyogenes* phages than it is to *L. lactis* phages. Comparative genome analysis with six complete

genomes of temperate P335-like phages confirmed the heterogeneity among phages of P335 species. A dUTPase gene is the only conserved gene among all P335 phages analyzed as well as the phage BK5-T. A genetic relationship between P335 phages and the phage-type of the BK5-T species was established. Thus, we proposed that phage BK5-T be included within the P335 species and thereby reducing the number of lactococcal phage species to 11

26. **Evolution of bacteriophage in continuous culture: a model system to test antiviral gene therapies for the emergence of phage escape mutants. Lindemann, B. F., Klug, C., Schwienhorst, A. (2002). *Journal of Virology* 76:5784-5792.** The emergence of viral escape mutants is usually a highly undesirable phenomenon. This phenomenon is frequently observed in antiviral drug applications for the treatment of viral infections and can undermine long-term therapeutic success. Here, we propose a strategy for evaluating a given antiviral approach in terms of its potential to provoke the appearance of resistant virus mutants. By use of Q beta RNA phage as a model system, the effect of an antiviral gene therapy, i.e., a virus-specific repressor protein expressed by a recombinant *Escherichia coli* host, was studied over the course of more than 100 generations. In 13 experiments carried out in parallel, 12 phage populations became resistant and 1 became extinct. Sequence analysis revealed that only two distinct phage mutants emerged in the 12 surviving phage populations. For both escape mutants, sequence variations located in the repressor binding site of the viral genomic RNA, which decrease affinity for the repressor protein, conferred resistance to translational repression. The results clearly suggest the feasibility of the proposed strategy for the evaluation of antiviral approaches in terms of their potential to allow resistant mutants to appear. In addition, the strategy proved to be a valuable tool for observing virus-specific molecular targets under the impact of antiviral drugs

27. **Prevalence of *Escherichia coli* O157:H7 prophage-like sequences among German *Salmonella enterica* serotype *Typhimurium* phage types and their use in detection of phage type DT104 by the polymerase chain reaction. Malorny, B., Schroeter, A., Bunge, C., Helmuth, R. (2002). *Veterinary Microbiology* 87:253-265.** A 1.6kb DNA fragment identified by random amplifiable polymorphic DNA differentiation (RAPD) from a *Salmonella enterica* serotype *Typhimurium* phage type DT104 isolate was used to investigate the prevalence of the region in 160 DT104 isolates, 83 other epidemiological important *S. Typhimurium* phage types and 20 strains selected from 17 other *Salmonella* serotypes. PCR screening tests using two different primer-sets derived from the RAPD fragment's nucleotide sequence showed that 76% of the 160 DT104 isolates investigated, including subtypes DT104A, DT104B, DT104B low, DT104H and DT104L, reacted positively. High sensitivity was shown for DT104 strains expressing at least the penta-resistance pattern ACSSuT (97% of 104 strains tested). DT104 susceptible strains showed only a sensitivity of 35% (17 strains tested). In contrast, 83% of the 83 strains from the other *S. Typhimurium* phage types reacted negatively. Strains from five out of the 17 other serotypes showed a positive signal with one primer-set. The other primer-set exhibited only a positive reaction with one *S. Dublin* isolate. The analysis of a 2415bp extended sequence revealed homologies to genes encoded by *Escherichia coli* O157:H7 prophages, suggesting that the described region contains genes of a prophage specific for DT104 and related phage types

28. **Use of lacticin 481 to facilitate delivery of the bacteriophage resistance plasmid, pCBG104 to cheese starters. Mills, S., Coffey, A., O'Sullivan, L., Stokes, D., Hill, C., Fitzgerald, G. F., Ross, R. P. (2002). *Journal of Applied Microbiology* 92:238-246.** AIMS: Use of lacticin 481 to facilitate the conjugal transfer of the bacteriophage resistance plasmid pCBG104 to various starter cultures. METHODS AND RESULTS: A raw milk isolate of *Lactococcus* was found to harbour determinants for lacticin 481 production and immunity and phage resistance on a plasmid designated pCBG104. The lacticin 481 was successfully used to mobilize the phage resistance determinant to a variety of cheese starters enabling the formation of highly phage resistant starters. In addition, it facilitated the stacking of a number of phage resistance genes, namely a type I restriction modification system, a phage abortive infection system and a phage adsorption blocking system in a single Lactococcus strain without the use of recombinant techniques. The transconjugants were all shown to produce lacticin 481 and to contain the entire 481 operon. Subsequently one transconjugant was selected and successfully used for large-scale cheddar cheese manufacture. CONCLUSIONS: Lacticin 481 could be used as a food-grade selectable marker to facilitate the introduction of advantageous traits to starter cultures for industrial food fermentations. SIGNIFICANCE AND IMAPCT OF THE STUDY: Food-grade selectable markers greatly facilitate the introduction of various advantageous traits to starter cultures for industrial food fermentation. Indeed self-cloning which is becoming increasingly important for strain improvement has a requirement for the identification and demonstration of the utility of tools such as lacticin 481

29. **Optimisation of ISO 10705-1 on enumeration of F-specific bacteriophages. Mooijman, K. A., Bahar, M., Muniesa, M., Havelaar, A. H. (2002). *Journal of Virological Methods* 103:129-136.** During the European project 'Bacteriophages in bathing waters' (January 1996-June 1999), research was carried out to optimise the method for detection and enumeration of F-specific (RNA) phages in water. It was evaluated whether further optimisation would be possible/needed for the procedure as described in the standard method of the International Organisation for Standardisation (ISO) 10705-1. The research focused mainly on optimisation of the different steps for culturing the host strain WG49 *Salmonella Typhimurium*. It was concluded that all steps described in ISO 10705-1 are necessary and, if followed carefully, using a culture of host strain WG49 *Salmonella Typhimurium* of good quality, reliable results could be obtained for the enumeration of F-specific RNA phages

30. **Microbial quality of wastewater: detection of hepatitis A virus by reverse transcriptase-polymerase chain reaction. Morace, G., Aulicino, F. A., Angelozzi, C., Costanzo, L., Donadio, F., Rapicetta, M. (2002). *Journal of Applied Microbiology* 92:828-836.** AIMS: The persistent circulation of hepatitis A virus (HAV) in the Mediterranean area suggests the need for monitoring its presence in the environment. A reverse transcriptase-polymerase chain reaction (RT-PCR) was used to detect the presence of HAV in several consecutive raw sewage and final effluent samples, collected over an 8-month period from an activated sludge treatment plant in southern Italy. METHODS AND RESULTS: Two distinct purification protocols, either based on antigen-capture with monoclonal antibody (AC) or RNA extraction, were compared. The possible influence of the antibody used in the AC phase was evaluated in preliminary experiments on HAV-spiked samples, using two different monoclonal antibodies. Hepatitis A virus RNA was detected in all but one sewage environmental sample examined. The contemporary presence of enteroviruses, reoviruses and phages was observed, while HAV growth in cell culture was hampered. CONCLUSIONS: The RT-PCR technique was confirmed to be a valuable tool for the rapid monitoring of HAV in sewage samples. In addition, this study demonstrated that application of different sample purification methods can result in different levels of sensitivity of the assay and that, in the antigen-capture method, the choice of antibody can have a crucial role. SIGNIFICANCE AND IMPACT OF THE STUDY: This work underlines the need for technical uniformity in environmental studies from different laboratories for a correct and useful comparison of the results

31. **Effect of denture cleaner using ozone against methicillin-resistant *Staphylococcus aureus* and E. coli T1 phage. Murakami, H., Mizuguchi, M., Hattori, M., Ito, Y., Kawai, T., Hasegawa, J. (2002). *Dental Materials Journal* 21:53-60.** We examined the bactericidal and virucidal effectiveness of a denture cleaner that uses ozone (ozone concentration, 10 ppm)

against methicillin-resistant *Staphylococcus aureus* (MRSA) and T1 phage, respectively. In the bactericidal activity test, with the ozone supply turned on, the number of bacteria was 3.1 x 10(3) CFU/mL at the beginning of the experiment, fell to 1.0 x 10(0) CFU/mL 10 min later, and was 1.0 x 10(0) CFU/mL or less afterwards. In contrast, when the ozone supply was cut off (air bubble only), the number of bacteria was 3.4 x 10(3) CFU/mL at the beginning of the experiment, and had fallen to 3.0 x 10(3) CFU/mL 60 min later (no statistically significant difference). In the virucidal activity test, the number of phages was 1.2 x 10(6) PFU/mL before ozone treatment, fell to about 1/10 of that number 10 min later, and was 6.1 x 10(0) PFU/mL 40 min later. These results indicate that the use of ozone in this denture cleaner is effective against MRSA and viruses

32. **Evolution and spread of antibiotic resistance. Normark, B. H., Normark, S. (2002). *Journal of Internal Medicine* 252:91-106.** Antibiotic resistance is a clinical and socioeconomical problem that is here to stay. Resistance can be natural or acquired. Some bacterial species, such as *Pseudomonas aeruginosa*, show a high intrinsic resistance to a number of antibiotics whereas others are normally highly antibiotic susceptible such as group A streptococci. Acquired resistance evolve via genetic alterations in the microbes own genome or by horizontal transfer of resistance genes located on various types of mobile DNA elements. Mutation frequencies to resistance can vary dramatically depending on the mechanism of resistance and whether or not the organism exhibits a mutator phenotype. Resistance usually has a biological cost for the microorganism, but compensatory mutations accumulate rapidly that abolish this fitness cost, explaining why many types of resistances may never disappear in a bacterial population. Resistance frequently occurs stepwise making it important to identify organisms with low level resistance that otherwise may constitute the genetic platform for development of higher resistance levels. Self-replicating plasmids, prophages, transposons, integrons and resistance islands all represent DNA elements that frequently carry resistance genes into sensitive organisms. These elements add DNA to the microbe and utilize site-specific recombinases/integrases for their integration into the genome. However, resistance may also be created by homologous recombination events creating mosaic genes where each piece of the gene may come from a different microbe. The selection with antibiotics have informed us much about the various genetic mechanisms that are responsible for microbial evolution

33. **Complete nucleotide sequence and likely recombinatorial origin of bacteriophage T3. Pajunen, M. I., Elizondo, M. R., Skurnik, M., Kieleczawa, J., Molineux, I. J. (2002). *Journal of Molecular Biology* 319:1115-1132.** We report the complete genome sequence (38,208 bp) of bacteriophage T3 and provide a bioinformatic comparative analysis with other completely sequenced members of the T7 group of phages. This comparison suggests that T3 has evolved from a recombinant between a T7-like coliphage and a yersiniophage. To assess this, recombination between T7 and the *Yersinia enterocolitica* serotype O:3 phage phiYeO3-12 was accomplished in vivo; coliphage progeny from this cross were selected that had many biological properties of T3. This represents the first experimentally observed recombination between lytic phages whose normal hosts are different bacterial genera

34. **Bacteriophage resistance of a deltathyA mutant of *Lactococcus lactis* blocked in DNA replication. Pedersen, M. B., Jensen, P. R., Janzen, T., Nilsson, D. (2002). *Applied and Environmental Microbiology* 68:3010-3023.** The thyA gene, which encodes thymidylate synthase (TS), of *Lactococcus lactis* CHCC373 was sequenced, including the upstream and downstream regions. We then deleted part of thyA by gene replacement. The resulting strain, MBP71 deltathyA, was devoid of TS activity, and in media without thymidine, such as milk, there was no detectable dTTP pool in the cells. Hence, DNA replication was abolished, and acidification by MBP71 was completely unaffected by the presence of nine different phages tested at a multiplicity of infection (MOI) of 0.1. Nonreplicating MBP71 must be inoculated at a higher level than CHCC373 to achieve a certain pH within a specified time. For a pH of 5.2 to be reached in 6 h, the inoculation level of MBP71 must be 17-fold higher than for CHCC373. However, by adding a limiting amount of thymidine this could be lowered to just 5-fold the normal amount, while acidification was unaffected with MBP71 up to an MOI of 0.01. It was found that nonreplicating MBP71 produced largely the same products as CHCC373, though the acetaldehyde production of the former was higher

35. **Les bactériophages, nouvelle perspective dans le traitement des maladies infectieuses? Resch, G., Meyer, J. (2002). *Rev. Mens. Suisse Odontostomatol.* 112:643-645.** De nombreuses bactéries ont été identifiées, et cela depuis des décennies, comme étant des agents responsables de nombreuses maladies infectieuses de l'homme. Ainsi, il a été mis en évidence que certaines bactéries buccales jouent un rôle primordial dans l'étiologie de la carie et des pathologies du parodontium. Ces bactéries peuvent être, à leur tour, infectées par des virus appelés bactériophages. Ces bactériophages, qui sont des parasites obligatoires, sont capables d'altérer profondément les caractéristiques de leur hôte. Nous verrons, dans la suite, quelques aspects de la biologie de ces virus et de leur importance.

36. **The Phage Proteomic Tree: a genome-based taxonomy for phage. Rohwer, F., Edwards, R. (2002). *Journal of Bacteriology* 184:4529-4535.** There are approximately 10^{31} phage in the biosphere, making them the most abundant biological entities on the planet. Despite their great numbers and ubiquitous presence, very little is known about phage biodiversity, biogeography, or phylogeny. Information is limited, in part, because the current ICTV taxonomical system is based on culturing phage and measuring physical parameters of the free virion. No sequence-based taxonomic systems have previously been established for phage. We present here the "Phage Proteomic Tree," which is based on the overall similarity of 105 completely sequenced phage genomes. The Phage Proteomic Tree places phage relative to both their near neighbors and all other phage included in the analysis. This method groups phage into taxa that predicts several aspects of phage biology and highlights genetic markers that can be used for monitoring phage biodiversity. We propose that the Phage Proteomic Tree be used as the basis of a genome-based taxonomical system for phage

37. **Distribution of genotypes of F-specific RNA bacteriophages in human and non-human sources of faecal pollution in South Africa and Spain. Schaper, M., Jofre, J., Uys, M., Grabow, W. O. K. (2002). *Journal of Applied Microbiology* 92:657-667.** AIMS: To assess whether the distribution of genotypes of F-specific RNA bacteriophages reflects faecal pollution of human and animal origin in water environments. METHODS AND RESULTS: Stool samples, animal feedlot waste slurries and a wide variety of faecally polluted waters were studied in South Africa and Spain. Genotyping was performed by plaque and spot hybridization with genotype-specific probes. Only genotypes II and III were detected in human stool. Animal faeces contained predominantly, but not exclusively, genotypes I and IV. Raw hospital and municipal sewage contained mostly genotypes II and III, whereas genotypes I and II prevailed in settled sewage, secondary treated sewage and non-point diffuse effluents from developing communities. Abattoir wastewaters contained mostly genotypes I and IV. No differences were observed between the distribution of genotypes in Spain and South Africa. CONCLUSIONS: Although the association of genotypes II and III with human excreta and I and IV with animal excreta was statistically significant, the results suggest that the association cannot be used for absolute distinction between faecal pollution of human and animal origin. SIGNIFICANCE AND IMPACT OF THE STUDY: This study contributes greatly to understanding the usefulness of genotypes of F-specific RNA bacteriophages in source tracking of faecal wastes

38. **Genetics of the phage growth limitation (Pgl) system of *Streptomyces coelicolor* A3(2). Sumby, P., Smith, M. C. M. (2002). *Molecular Microbiology* 44:489-500.** The phage growth limitation (Pgl) system, encoded by *Streptomyces coelicolor* A3(2), confers protection against the temperate bacteriophage phiC31 and its homoimmune relatives. The Pgl phenotype is characterized by the ability of Pgl+ hosts to support a phage burst on initial infection but subsequent cycles are severely attenuated. Previously, two adjacent genes pglY and pglZ were shown to be required for Pgl. It had been shown by Southern blotting that *Streptomyces lividans*, a close relative of *S. coelicolor* and naturally Pgl-, does not contain homologues of pglYZ and that introduction of pglYZ into *S. lividans* is not sufficient to confer a Pgl+ phenotype. Moreover, the mechanism of the Pgl+<--> Pgl- phase variation associated with this phenotype is also not understood. Here we describe two novel genes, pglW and pglX, that were shown to be part of this system by complementation of Pgl- mutants and by insertional mutagenesis. pglW encodes a 169 kDa protein that includes putative motifs for both serine/threonine protein kinase activity and DNA binding. pglX encodes a 136 kDa protein with putative adenine-specific DNA methyltransferase activity. pglW and pglX have overlapping stop-start codons suggesting transcriptional and translational coupling. S1 mapping of transcripts initiating up-stream of pglW indicated that, like pglYZ, pglWX is expressed in uninfected cultures. A homologue of pglX with 76% amino acid identity was identified in *S. coelicolor*, and insertional mutagenesis indicated that this gene was not required for the Pgl+ phenotype. Southern blots indicated that *S. lividans* does not contain homologues of pglW or pglX. A plasmid encoding pglWXYZ was able to confer the Pgl+ phenotype to *S. lividans* implying that these four genes constitute the whole system

39. **50 million years of genomic stasis in endosymbiotic bacteria. Tamas, I., Klasson, L., Canback, B., Naslund, A. K., Eriksson, A. S., Wernegreen, J. J., Sandstrom, J. P., Moran, N. A., Andersson, S. G. E. (2002). *Science* 296:2376-2379.** Comparison of two fully sequenced genomes of *Buchnera aphidicola*, the obligate endosymbionts of aphids, reveals the most extreme genome stability to date: no chromosome rearrangements or gene acquisitions have occurred in the past 50 to 70 million years, despite substantial sequence evolution and the inactivation and loss of individual genes. In contrast, the genomes of their closest free-living relatives, *Escherichia coli* and *Salmonella* spp., are more than 2000-fold more labile in content and gene order. The genomic stasis of *B. aphidicola*, likely attributable to the loss of phages, repeated sequences, and recA, indicates that *B. aphidicola* is no longer a source of ecological innovation for its hosts

40. **Effectiveness of the lactococcal abortive infection systems AbiA, AbiE, AbiF and AbiG against P335 type phages. Tangney, M., Fitzgerald, G. F. (2002). *FEMS Microbiology Letters* 210:67-72.** Four lactococcal abortive infection mechanisms were introduced into strains which were sensitive hosts for P335 type phages and plaque assay experiments performed to assess their effect on five lactococcal bacteriophages from this family. Results indicate that AbiA inhibits all five P335 phages tested, while AbiG affects phiP335 itself and phiQ30 but not the other P335 species phages. AbiA was shown to retard phage Q30 DNA replication as previously reported for other phages. It was also demonstrated that AbiG, previously shown to act at a point after DNA replication in the cases of c2 type and 936 type phages, acts at the level of, or prior to phage Q30 DNA replication. AbiE and AbiF had no effect on the P335 type phages examined

41. **One of two copies of the gene for the activatable shiga toxin type 2d in *Escherichia coli* O91:H21 strain B2F1 is associated with an inducible bacteriophage. Teel, Louise D., Melton-Celsa, Angela R., Schmitt, Clare K., O'Brien, Alison D. (2002). *Infection and Immunity* 70:4282-4291.** Shiga toxin (Stx) types 1 and 2 are encoded within intact or defective temperate bacteriophages in Stx-producing *Escherichia coli* (STEC), and expression of these toxins is linked to bacteriophage induction. Among Stx2 variants, only stx(2e) from one human STEC isolate has been reported to be carried within a toxin-converting phage. In this study, we examined the O91:H21 STEC isolate B2F1, which carries two functional alleles for the potent activatable Stx2 variant toxin, Stx2d, for the presence of Stx2d-converting bacteriophages. We first constructed mutants of B2F1 that produced one or the other Stx2d toxin and found that the mutant that produced only Stx2d1 made less toxin than the Stx2d2-producing mutant. Consistent with that result, the Stx2d1-producing mutant was attenuated in a streptomycin-treated mouse model of STEC infection. When the mutants were treated with mitomycin C to promote bacteriophage induction, Vero cell cytotoxicity was elevated only in extracts of the Stx2d1-producing mutant. Additionally, when mice were treated with ciprofloxacin, an antibiotic that induces the O157:H7 Stx2-converting phage, the animals were more susceptible to the Stx2d1-producing mutant. Moreover, an stx(2d1)-containing lysogen was isolated from plaques on strain DH5alpha that had been exposed to lysates of the mutant that produced Stx2d1 only, and supernatants from that lysogen transformed with a plasmid encoding RecA were cytotoxic when the lysogen was induced with mitomycin C. Finally, electron-microscopic examination of extracts from the Stx2d1-producing mutant showed hexagonal particles that resemble the prototypic Stx2-converting phage 933W. Together these observations provide strong evidence that expression of Stx2d1 is bacteriophage associated. We conclude that despite the sequence similarity of the stx(2d1)- and stx(2d2)-flanking regions in B2F1, Stx2d1 expression is repressed within the context of its toxin-converting phage while Stx2d2 expression is independent of phage induction

42. **[Study of *Erwinia carotovora* phage resistance with the use of temperate bacteriophage ZF40]. Tovkach, F. I. (2002). *Mikrobiologija (Microbiologiia)* 71:82-88.** The causes of the unique phage resistance of the pectinolytic phytopathogenic strains of *Erwinia carotovora* were studied with the use of temperate bacteriophage ZF40. It was shown that, in these bacteria, the bacteriophage-cell interaction can be substantially blocked at the adsorption level. An adequate indicator for studying the temperate bacteriophages of erwinias was developed on the basis of mutants resistant to macromolecular bacteriocins. Various restriction-modification systems, which influence cell resistance to bacteriophages, were revealed for the first time in *E. carotovora*. The phage resistance was shown to be determined by the wide occurrence of homoimmune temperate viruses in pectinolytic erwinias

43. **[Temperate bacteriophage ZF40 of *Erwinia carotovora*: phage particle structure and DNA restriction analysis]. Tovkach, F. I. (2002). *Mikrobiologija (Microbiologiia)* 71:75-81.** Structural organization of the temperate bacteriophage ZF40 of *Erwinia carotovora* was studied. Phage ZF40 proved to be a typical member of the Myoviridae family (morphotype A1). Phage particles consist of an isometric head 58.3 nm in diameter and a contractile 86.3-nm-long tail with a complex basal plate and short tail fibers (31.5 nm). Phage tail sheath, a truncated cone in shape, is characterized by specific packaging of structural subunits. The ZF40 phage genome is 45.8 kb in size, as determined by restriction analysis, and contains DNA cohesive ends. The ZF40 phage of *Erwinia carotovora* is assumed to be a new species of bacteriophages specific for enterobacteria

44. **[Comparative study of properties of temperate erwiniophages 49 and 59]. Tovkach, F. I., Shevchenko, T. V., Gorb, T. E., Mukvich, N. S., Romaniuk, L. V. (2002). *Mikrobiolohichnyi Zhurnal* 64:65-81.** Molecular-biological properties of two relative temperate erwiniophages 49 and 59 have been comparatively studied. The both phages are highly specific with respect to sensitive bacteria and lyse only inconsiderable quantity of amylovora-like strains of *Erwinia horticola*. It has been established that erwiniophages are distinguished by the basic parameters of a single reproduction cycle in the cells of common host *E. horticola* 450. Considerable differences between phages have been also found in the areas of genomes responsible for the

establishment and maintenance of lysogenic state in the cells of the bacterium-host. Study of structure polypeptides has confirmed the identity of capsids and tails of phages 49 and 59. It has been shown that phage 49 has another, as compared to phage 59, basal plate, which availability destabilises the phage tail and leads to virion destruction under various physical effects. Virion DNA of phages 49 and 59 are of the same size--47.9 kbp, but differ as to GC-content. Using the restriction analysis it has been shown that genome of phage 49, as well as the genome of phage 59, is permuted, but its permutation is of discrete character. The fact of recombination interaction between erwiniophages 49 and 59 has been established. It is supposed that phage 49 is the recombination (hybrid) derivative of phage 59 and unknown phage, or prophage, genetic module. The given recombination, probably, took place under the persistence of different phages in the general polylysogenic system of *E. horticola*.

45. **Role of bacteriophage MAV1 as a mycoplasmal virulence factor for the development of arthritis in mice and rats. Tu, A. H., Lindsey, J. R., Schoeb, T. R., Elgavish, A., Yu, H., Dybvig, K. (2002). *Journal of Infectious Diseases* 186:432-435.** The lysogenic bacteriophage MAV1 has been shown to be a virulence factor for the development of arthritis in rats infected with *Mycoplasma arthritidis*. In the present study, arthritis was evaluated by histopathologic examination to demonstrate that MAV1 is a virulence factor not only in the rat but also in the mouse. Specifically, the MAV1 lysogen 158L3-1 was more virulent than the nonlysogen strain 158 in DBA/2NCr, C3H/HeNCr, C3H/HeJ, and C3Smn.CB17-Prkdc(scid)/J mice, as well as in LEW rats.

46. **Bacteriophage control of bacterial virulence. Wagner, P. L., Waldor, M. K. (2002). *Infection and Immunity* 70:3985-3993.** [no abstract]

47. ***Burkholderia thailandensis* E125 harbors a temperate bacteriophage specific for *Burkholderia mallei*. Woods, D. E., Jeddeloh, J. A., Fritz, D. L., DeShazer, D. (2002). *Journal of Bacteriology* 184:4003-4017.** *Burkholderia thailandensis* is a nonpathogenic gram-negative bacillus that is closely related to *Burkholderia mallei* and *Burkholderia pseudomallei*. We found that *B. thailandensis* E125 spontaneously produced a bacteriophage, termed phiE125, which formed turbid plaques in top agar containing *B. mallei* ATCC 23344. We examined the host range of phiE125 and found that it formed plaques on *B. mallei* but not on any other bacterial species tested, including *B. thailandensis* and *B. pseudomallei*. Examination of the bacteriophage by transmission electron microscopy revealed an isometric head and a long noncontractile tail. *B. mallei* NCTC 120 and *B. mallei* DB110795 were resistant to infection with phiE125 and did not produce lipopolysaccharide (LPS) O antigen due to IS407A insertions in wbiE and wbiG, respectively. wbiE was provided in trans on a broad-host-range plasmid to *B. mallei* NCTC 120, and it restored LPS O-antigen production and susceptibility to phiE125. The 53,373-bp phiE125 genome contained 70 genes, an IS3 family insertion sequence (ISBt3), and an attachment site (attP) encompassing the 3' end of a proline tRNA (UGG) gene. While the overall genetic organization of the phiE125 genome was similar to lambda-like bacteriophages and prophages, it also possessed a novel cluster of putative replication and lysogeny genes. The phiE125 genome encoded an adenine and a cytosine methyltransferase, and purified bacteriophage DNA contained both N6-methyladenine and N4-methylcytosine. The results presented here demonstrate that phiE125 is a new member of the lambda supergroup of Siphoviridae that may be useful as a diagnostic tool for *B. mallei*

48. **Genomic analysis of *Clostridium perfringens* bacteriophage phi3626, which integrates into guaA and possibly affects sporulation. Zimmer, M., Scherer, S., Loessner, M. J. (2002). *Journal of Bacteriology* 184:4359-4368.** Two temperate viruses, phi3626 and phi8533, have been isolated from lysogenic *Clostridium perfringens* strains. Phage phi3626 was chosen for detailed analysis and was inspected by electron microscopy, protein profiling, and host range determination. For the first time, the nucleotide sequence of a bacteriophage infecting *Clostridium* species was determined. The virus belongs to the Siphoviridae family of the tailed phages, the order Caudovirales. Its genome consists of a linear double-stranded DNA molecule of 33,507 nucleotides, with invariable 3'-protruding cohesive ends of nine residues. Fifty open reading frames were identified, which are organized in three major life cycle-specific gene clusters. The genes required for lytic development show an opposite orientation and arrangement compared to the lysogeny control region. A function could be assigned to 19 gene products, based upon bioinformatic analyses, N-terminal amino acid sequencing, or experimental evidence. These include DNA-packaging proteins, structural components, a dual lysis system, a putative lysogeny switch, and proteins that are involved in replication, recombination, and modification of phage DNA. The presence of genes encoding a putative sigma factor related to sporulation-dependent sigma factors and a putative sporulation-dependent transcription regulator suggests a possible interaction of phi3626 with onset of sporulation in *C. perfringens*. We found that the phi3626 attachment site attP lies in a noncoding region immediately downstream of int. Integration of the viral genome occurs into the bacterial attachment site attB, which is located within the 3' end of a guaA homologue. This essential housekeeping gene is functionally independent of the integration status, due to reconstitution of its terminal codons by phage sequence

49. **Microbial indicator removal in onsite constructed wetlands for wastewater treatment in the southeastern U.S. Barrett, E. C., Sobsey, M. D., House, C. H., White, K. D. (2001). *Water Science and Technology* 44:177-182.** Seven onsite constructed wetlands for wastewater treatment in the coastal plains of Alabama and North Carolina were studied from September 1997 to July 1998. Each site was examined for its ability to remove a range of fecal contamination indicators from settled wastewater. Indicator organisms include total and fecal coliforms, enterococci, *Clostridium perfringens*, and somatic and male-specific (F+) coliphages. Four identical domestic wastewater treatment sites in Alabama were evaluated. In these sites the Log_{10} geometric mean reductions ranged between 0.5 and 2.6 for total and fecal coliforms, 0.1 and 1.5 for enterococci, 1.2 to 2.7 for *C. perfringens*, -0.3 and 1.2 for somatic coliphages, and -0.2 and 2.2 for F+ coliphages. Three unique designs were examined in North Carolina. Log_{10} geometric mean reductions ranged between 0.8 to 4.2 for total and fecal coliforms, 0.3 to 2.9 for enterococci, 1.6 to 2.9 for C. perfringens, -0.2 and 2.8 for somatic coliphages, and -0.1 and 1.5 for F+ coliphages. Somatic and F+ coliphage detection was highly variable from month to month

50. **First evidence for a restriction-modification system in *Leptospira* sp. Brenot, A., Werts, C., Ottone, C., Sertour, N., Charon, N. W., Postic, D., Baranton, G., Saint Girons, I (2001). *FEMS Microbiology Letters* 201:139-143.** The LE1 leptophage exhibited a host range restricted to the saprophytic *Leptospira biflexa* [Saint Girons et al., Res. Microbiol. 141 (1990) 1131-1133] and mainly to the Patoc 1 strain (hereafter called PFRA) kept in the Paris, France collection. Results of titration of LE1 lysates indicated the presence of a host-controlled modification and restriction system within PUSA (Patoc 1 strain maintained in the Morgantown, WV, USA collection) that was absent in PFRA. Because genomic DNA of PITAL (Patoc 1 strain maintained in Trieste, Italy) appeared smeared in pulsed field gel electrophoresis (PFGE), this strain is likely to contain nucleases that are activated upon DNA isolation. Moreover, comparative NotI digestions of PUSA and PFRA DNAs, as visualized by PFGE, indicated that PUSA belonged to a different serovar than PFRA. Finally, 16S ribosomal sequence analysis indicated that PUSA belonged to the saprophytic *Leptospira meyeri* species, while PITAL and PFRA appertained to *L. biflexa*.

The evolutionary significance and the importance of the restriction and modification enzymes or non-specific nucleases within strains for genetic experiments are discussed

51. **Isolation and characterization of two types of actinophage infecting *Streptomyces scabies*. el Sayed, S. A., el Didamony, G., Mansour, K. (2001). *Folia Microbiologica* 46:519-526.** Two types of actinophages, phi S and phi L, were isolated from soil samples by using *Streptomyces scabies*, a potato scab pathogen, as indicator strain. The phages were partially characterized according to their physicochemical properties, plaques and particles morphology, and their host range; this varied from narrow (for phi S) to wide (for phi L). The adsorption rate constants of the phi S and phi L were 3.44 and 3.18 pL/min, and their burst sizes were 1.61 and 3.75 virions per mL, respectively. One-step growth indicated that phi S and phi L have a latent period of 1/2 h followed by a rise period of 1/2 h. The temperate character of these phages was tested in other isolates of *Streptomyces*. Four of the phages (phi SS3, phi SS12, phi SS13 and phi SS17) were identified as temperate phages, since they were able to lysogenize SS3, SS12, SS13 and SS17. phi SS3, phi SS12 and phi SS13 were homoimmune, and they were heteroimmune with respect to phi SS17. The restriction barriers of lysogenic isolates (SS12, SS13 and SS17) interfered with the blockage of plaque formation by phages (phi SS12, phi SS13 or phi SS17) propagated on them, about 75% of lysogenic isolates had restriction systems. The exposure of the lysogenic isolates (SS12, SS13 and SS17) to UV-irradiation prevented the possible restriction barriers of these isolates so that these barriers could be overcome

52. **Removal of *Salmonella* and microbial indicators in constructed wetlands treating swine wastewater. Hill, V. R., Sobsey, M. D. (2001). *Water Science and Technology* 44:215-222.** Reductions of *Salmonella* bacteria and enteric microbial indicator organisms were measured in swine wastewater treated by a field-scale surface flow (SF) constructed wetland at a commercial hog nursery in North Carolina and in laboratory-scale SF and subsurface flow (SSF) constructed wetland reactors. Overall reductions of *Salmonella*, fecal coliforms and *E. coli* were 96, 98 and 99%, respectively, in the two-cell field-scale wetland. Somatic and F-specific coliphage viral indicators were reduced by 99 and 98%, respectively. Reductions of *Salmonella*, fecal coliforms and *E. coli* were similar in the first cell of the field system and in the laboratory-scale SF wetland operated at a TKN loading of 25 kg ha(-1) d(-1) and 30 degrees C (approximately 70, 90 and 90%, respectively). In the SSF wetland reactor, Salmonella and fecal coliform reductions were 80 and 98%, respectively, at a 40 kg TKN ha(-1) d(-1) loading and 99.8 and 99.99%, respectively, at a 10 kg TKN ha(-1) d(-1) loading. These results show that SF constructed wetlands can be effective for reducing enteric pathogens in swine wastewater and that greater removals can be achieved using SSF designs and lower TKN loading rates

53. **Removal of bacterial indicators and pathogens from dairy wastewater by a multi-component treatment system. Karpiscak, M. M., Sanchez, L. R., Freitas, R. J., Gerba, C. P. (2001). *Water Science and Technology* 44:183-190.** Microbial removal by a multi-component treatment system for dairy and municipal wastewater is being studied in Arizona, USA. The system consists of paired solids separators, anaerobic lagoons, aerobic ponds and constructed wetlands cells. The organisms under study include: total coliform, fecal coliform, enterovirus, *Listeria monocytogenes*, *Clostridium perfringens*, coliphage, *Giardia lamblia* and *Cryptosporidium parvum*. Organism removal rates from dairy wastewater varied from 13.2 per cent for fecal coliform to 94.9 per cent for coliphage. It appears that the much higher turbidity of the dairy wastewater, nearly 1,300 NTU, decreased the treatment systems' ability to remove some microbial indicators and pathogens. Information from this study can be used to determine the adequacy of multi-component treatment systems for the control of wastewater-borne pathogens, both in municipal treatment systems as well as in confined animal feeding operations (CAFO). This information also can assist municipalities and the CAFO industry in the implementation of rational and efficient treatment strategies for appropriate reuse of wastewaters

54. **Viral and chemical tracer movement through contrasting soils. McLeod, M., Aislabie, J., Smith, J., Fraser, R., Roberts, A., Taylor, M. (2001). *J. Environ. Qual.* 30:2134-2140.** Land treatment of animal or human waste can result in chemical and microbial contamination of shallow ground water and/or water-ways. We investigated the fate of a host-specific *Salmonella* bacteriophage and a nonreactive chemical (Br-) tracer when applied to large intact lysimeter soil cores (500 mm diam. by 700 mm high). The soils included a poorly drained Gley Soil and well-drained Pumice, Allophanic, and Recent Soils. A depth of 30 mm of water containing the bacteriophage and Br- was applied to the soil at a rate of 5 mm h(-1) followed by up to about 1.8 pore volumes of simulated rainfall. Resulting leachates, collected continuously over at least one pore volume were analyzed for the bacteriophage and bromide (Br-) tracers. Bromide moved uniformly through the Pumice and Allophanic Soils with peak concentrations at about 1 pore volume, while the bacteriophage was detected only at trace levels or not at all. In contrast, both Br- and bacteriophage tracers moved rapidly through Gley and Recent Soils, appearing early in the leachate and then tailing off. Such flow patterns in the Gley and Recent Soils are indicative of bypass flow. Coarse soil structure in the Gley Soil, and finger-flow due to water repellency in the sandy Recent Soil are considered responsible for the observed bypass flow in these two soils. Allophanic and Pumice Soils have finer, more porous soil structure leading to a predominance of matrix flow over bypass flow. This study suggests vertical movement of viruses varies significantly with soil type

55. **A field study of virus removal in septic tank drainfields. Nicosia, L. A., Rose, J. B., Stark, L., Stewart, M. T. (2001). *J. Environ. Qual.* 30:1933-1939.** Two field studies were conducted at a research station in Tampa, Florida to assess the removal of bacteriophage PRD1 from wastewater in septic tank drainfields. Infiltration cells were seeded with PRD1 and bromide and the effects of effluent hydraulic loading rate and rainfall on virus removal were monitored. Septic tank effluent samples were collected after passage through 0.6 m of unsaturated fine sand and PRD1 was detected over an average of 67 d. Bacteriophage PRD1 breakthrough was detected at approximately the same time as bromide in all three cells except for the low-load cell (Study 1), where bromide was never detected. Log10 removals of PRD1 were 1.43 and 1.91 for the high-load cells (hydraulic loading rate = 0.063 m/d) and 2.21 for the low-load cell (hydraulic loading rate = 0.032 m/d). Virus attenuation is attributed to dispersion, dilution, and inactivation. Significant increases in PRD1 elution with rainfall were observed in the first 10 d of the study. Approximately 125 mm of rainfall caused a 1.2 log10 increase of PRD1 detected at the 0.6-m depth. Current Florida onsite wastewater disposal standards, which specify a 0.6-m distance from the drainfield to the water table, may not provide sufficient removal of viruses, particularly during the wet season

56. **Detection of homologous recombination among bacteriophage P2 relatives. Nilsson, A. S., Haggard-Ljungquist, E. (2001). *Molecular Phylogenetics and Evolution* 21:259-269.** Sequencing of five late genes from 18 isolates of P2-like bacteriophages showed that these are at least 96% identical to the genes of phage P2. A maximum-parsimony phylogenetic analysis of these genes showed excess homoplasy of a magnitude three to six times higher than that expected. Examination of the distribution of the number of homoplasies at parsimoniously informative sites and incompatibility matrices of such sites revealed a pattern typical for extensive recombination. It has been shown that phage P2 probably incorporated some functionally complete genes or gene modules by recombination with other phages or with different hosts, but homologous recombination within genes has previously not been shown. In this paper we demonstrate that homologous recombination between P2-like

bacteriophages occurs randomly at multiple breakpoints in five late genes. The rate of recombination is high but, since some phages were sampled decades apart and in different parts of the world, this has to be viewed on an evolutionary time scale. The applicability of different methods used for detection of recombination breakpoints and estimation of rates of recombination in bacteriophages is discussed

57. **Reduction of enteric microorganisms at the Upper Occoquan Sewage Authority Water Reclamation Plant. Rose, J. B., Huffman, D. E., Riley, K., Farrah, S. R., Lukasik, J. O., Hamann, C. L. (2001). *Water Environment Research* 73:711-720.** The Upper Occoquan Sewage Authority (UOSA) Water Reclamation Plant, Centreville, Virginia, is a state-of-the-art wastewater treatment plant that was created to treat area wastewater and provide protection for the Occoquan Reservoir. This study investigated UOSA's unit processes as barriers to pathogenic as well as altemative and traditional-indicator microorganisms. Samples were collected once a month for 1 year from eight sites within UOSA's advanced wastewater reclamation plant. The eight sites were monitored for indicator bacteria total and fecal coliforms, enterococci, *Clostridium*, coliphage (the virus that infects *Escherichia coli*), human enteroviruses, and enteric protozoa. Overall, the plant was able to achieve a 5- to 7-log10 reduction of bacteria, 5-log10 reduction of enteroviruses, 4-log10 reduction for *Clostridium*, and 4.6-log10 reduction of protozoa. Total coliforms, enterococci, Clostridium, coliphage, Cryptosporidium, and Giardia were all detected in four or fewer samples of the final effluent. No enteroviruses or fecal coliforms were detected in the final effluent. The microbiological quality of reclaimed water and the reservoir water were compared. In every case, the treated wastewater was of a better quality than the ambient water in the reservoir, thus indicating that the reclaimed water will not adversely affect the water quality for downstream users

58. **[Characterization of *Vibrio cholerae* eltor isolates according to their epidemic potential using new diagnostic cholera bacteriophages eltor ctx+ and ctx- and by the polymerase chain reaction]. Smirnova, N. I., Cheldyshova, N. B., Kostromitina, E. A., Kulichenko, A. N., Kutyrev, V. V. (2001). *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* 11-16.** The epidemic potential of 113 *V. cholerae* eltor strains of different origin was determined with new diagnostic cholera bacteriophages eltor ctx+ and ctx-, as well as the test for hemolytic activity. Of these strains 50 were epidemically safe and 51 were epidemically dangerous, while the epidemic potential of 12 other strains could not be detected. Determination of genes ctxA, tcpA and toxR in the strains under study by means of the polymerase chain reaction (PCR) revealed that epidemically dangerous strains carried the whole set of the above genes in 92.2% of cases. 98.0% of epidemically safe cultures were lacking either gene ctxA, or genes ctxA and tcpA, or genes ctxA, tcpA and toxR, which confirmed their incapacity to cause cholera. The results of the differentiation of the cultures with new diagnostic cholera phages coincided with the results of PCR in 90% of cases. The most complete and reliable evaluation of the epidemic potential of individual vibrio isolates may be obtained using the two compared methods. The amplification test system gives more information when isolates with unclear epidemic potential are analyzed

59. **Occurrence and die-off of indicator organisms in the sediment in two constructed wetlands. Stenstrom, T. A., Carlander, A. (2001). *Water Science and Technology* 44:223-230.** The interest in constructed wetlands for municipal wastewater and stormwater treatment has recently increased but data for the reduction efficiency of indicator organisms are often restricted to the water phase. In a full-scale wastewater wetland in Sweden fecal coliforms and enterococci were reduced by 97-99.9% and coliphages by approximately 70%. The factors affecting the reduction are however less well understood. In two full-scale wetlands, for stormwater and wastewater treatment, an assessment has been done of the particle associated fraction of indicator organisms. No significant differences in the particle-associated numbers were seen between the inlet and the outlet of the wetlands, but the amounts of sedimenting particles varied between the two sites. In the stormwater wetland the amount of sedimenting particles at the outlet was 3% of the amount at the inlet, while the wastewater wetland had much lower particle removal efficiency. The reduction of suspended particles seems to be the main factor for bacterial elimination from the water phase, governed by vegetation and design. In the sediment, survival of presumptive *E. coli*, fecal enterococci, *Clostridium* and coliphages were long with T50-values of 27, 27, 252 and 370 days, respectively. The organisms can however be reintroduced by resuspension. Viruses in the water phase may be of main concern for a risk assessment of receiving waters.

60. **Bacteriophage therapy. Summers, W. C. (2001). *Annual Review of Microbiology* 55:437-451.** Bacteriophages were recognized as epizootic infections of bacteria in 1917 and were almost immediately deployed for antibacterial therapy and prophylaxis. The early trials of bacteriophage therapy for infectious diseases were confounded, however, because the biological nature of bacteriophage was poorly understood. The early literature reviewed here indicates that there are good reasons to believe that phage therapy can be effective in some circumstances. The advent of antibiotics together with the "Soviet taint" acquired by phage therapy in the post-war period resulted in the absence of rigorous evaluations of phage therapy until very recently. Recent laboratory and animal studies, exploiting current understanding of phage biology, suggest that phages may be useful as antibacterial agents in certain conditions.

61. **Susceptibility of *Staphylococcus epidermidis* biofilm in CSF shunts to bacteriophage attack. Wood, H. L., Holden, S. R., Bayston, R. (2001). *European Journal of Pediatric Surgery* 11 Suppl 1:S56-S57.**

62. **Quantifying viral propagation in vitro: toward a method for characterization of complex phenotypes. Yin, J., Duca, K., Lam, V., Keren, I., Endler, E. E., Letchworth, G. J., Novella, I. S. (2001). *Biotechnology Progress* 17:1156-1165.** For a eukaryotic virus to successfully infect and propagate in cultured cells several events must occur: the virion must identify and bind to its cellular receptor, become internalized, uncoat, synthesize viral proteins, replicate its genome, assemble progeny virions, and exit the host cell. While these events are taking place, intrinsic host defenses activate in order to defeat the virus, e.g., activation of the interferon system, induction of apoptosis, and attempted elicitation of immune responses via chemokine and cytokine production. As a first step in developing an imaging methodology to facilitate direct observation of such complex host/virus dynamics, we have designed an immunofluorescence-based system that extends the traditional plaque assay, permitting simultaneous quantification of the rate of virual spread, as indicated by cell loss. We propose that our propagation and cell death profiles serve as phenotypic readouts, complementing genetic analysis of viral strains. As our virus/host system we used vesicular stomatitis virus (VSV) propagating in hamster kidney epithelial (BHK-21) and murine astrocytoma (DBT) cells lines. Viral propagation and death profiles were strikingly different in these two cell lines, displaying both very different initial titer and cell age effects. The rate of viral spread and cell death tracked reliably in both cell lines. In BHK-21 cells, the rate of viral propagation, as well as maximal spread, was relatively insensitive to initial titer and was roughly linear over several days. In contrast, viral plaque expansion in DBT cells was contained early in the infections with high titers, while low titer infections spread in a manner similar to the BHK-21 cells. The effet of cell age on infection spread was negligible in BHK-21 cells but not in DBTs. Neither of these effects was clearly observed by plaque assay.

63. **Horizontal gene transfer and the origin of species: lessons from bacteria. de la Cruz, F., Davies, J. (2000). *Trends in Microbiology* 8:128-133.** In bacteria, horizontal gene transfer (HGT) is widely recognized as the mechanism responsible for the

widespread distribution of antibiotic resistance genes, gene clusters encoding biodegradative pathways and pathogenicity determinants. We propose that HGT is also responsible for speciation and sub-speciation in bacteria, and that HGT mechanisms exist in eukaryotes

64. **Biological control of *Sclerotinia minor* using chitinolytic bacterium and actinomycetes. El-Tarabily, K. A., Soliman, M. H., Nassar, A. H., Al-Hassani, H. A., Sivasithamparam, K., McKenna, F., Hardy, G. E. StJ. (2000). *Plant Pathology (Oxford)* 49:573-583.**

65. **Temporal variability of viruses, bacteria, phytoplankton and zooplankton in the western English Channel off Plymouth. Rodriguez, F., Frenandez, E., Head, R. N., Harbour, D. S., Bratbak, G., Heldal, M., Harris, R. P. (2000). *Journal of the Marine Biological Association of the United Kingdom* 80:575-586.** The temporal distribution of autotrophic and heterotrophic components of the planktonic community was studied from samples collected weekly at station L4, located to the south of Plymouth, UK, from October 1992 to January 1994. Phytoplankton succession followed the typical pattern of temperate waters. the development of a summer Gyrodinium aureolum bloom being the most prominent feature. Bacterial numbers were significantly correlated with temperature during autumn and winter, whereas resource availability and predation, including viruses, appear to be the most important controlling factors in spring and summer. High mesozooplankton densities, mainly copepods, were observed throughout most of the study associated with a series of diatom blooms, and also during autumn when low phytoplankton biomass was measured. This data set was analysed in order to build up conceptual trophodynamic models whereby the role of biological communities on the cycling of organic matter could be inferred. The results obtained in this study provide empirical evidence supporting the existence of a succession of trophic organization patterns in a coastal temperate environment. Classical models (herbivorous or microbial webs) appeared episodically whereas transition models (multivorous web) dominated throughout most of the seasonal cycle.

66. **Monitoring of environmental UV radiation by biological dosimeters. Ronto, G., Berces, A., Grof, P., Fekete, A., Kerekgyarto, T., Gaspar, S., Stick, C. (2000). *Advances in Space Research* 26:2021-2028.** As a consequence of the stratospheric ozone layer depletion biological systems can be damaged due to increased UV-B radiation. The aim of biological dosimetry is to establish a quantitative basis for the risk assessment of the biosphere. DNA is the most important target molecule of biological systems having special sensitivity against short wavelength components of the environmental radiation. Biological dosimeters are usually simple organisms, or components of them, modeling the cellular DNA. Phage T7 and polycrystalline uracil biological dosimeters have been developed and used in our laboratory for monitoring the environmental radiation in different radiation conditions (from the polar to equatorial regions). Comparisons with Robertson-Berger (RB) meter data, as well as with model calculation data weighted by the corresponding spectral sensitivities of the dosimeters are presented. Suggestion is given how to determine the trend of the increase in the biological risk due to ozone depletion

67. **A hypothesis for DNA viruses as the origin of eukaryotic replication proteins. Villarreal, L. P., DeFillips, V. R. (2000). *Journal of Virology* 74:7079-7084.** The eukaryotic replicative DNA polymerases are similar to those of large DNA viruses of eukaryotic and bacterial T4 phages but not to those of eubacteria. We develop and examine the hypothesis that DNA virus replication proteins gave rise to those of eukaryotes during evolution. We chose the DNA polymerase from phycodnavirus (which infects microalgae) as the basis of this analysis, as it represents a virus of a primitive eukaryote. We show that it has significant similarity with replicative DNA polymerases of eukaryotes and certain of their large DNA viruses. Sequence alignment confirms this similarity and establishes the presence of highly conserved domains in the polymerase amino terminus. Subsequent reconstruction of a phylogenetic tree indicates that these algal viral DNA polymerases are near the root of the clade containing all eukaryotic DNA polymerase delta members but that this clade does not contain the polymerases of other DNA viruses. We consider arguments for the polarity of this relationship and present the hypothesis that the replication genes of DNA viruses gave rise to those of eukaryotes and not the reverse direction.

68. **Diffusion of bacteriophage through alginate gels. Ibrahim, L. J., Denyer, S. P., Hanlon, G. W. (1999). *Journal of Pharmacy and Pharmacology* 51:38.**

69. **Identification of two types of actionophage parasitic to potato common scab pathogens in Hokkaido, Japan. Ogiso, H., Akino, S., Ogoshi, A. (1999). *Soil Microorganisms* 53:37-43.**

70. **Sequence of Shiga toxin 2 phage 933W from *Escherichia coli* O157:H7: Shiga toxin as a phage late-gene product. Plunkett III, G., Rose, D. J., Durfee, T. J., Blattner, F. R. (1999). *Journal of Bacteriology* 181:1767-1778.** Lysogenic bacteriophages are major vehicles for the transfer of genetic information between bacteria, including pathogenicity and/or virulence determinants. In the enteric pathogen *Escherichia coli* O157:H7, which causes hemorrhagic colitis and hemolytic-uremic syndrome, Shiga toxins 1 and 2 (Stx1 and Stx2) are phage encoded. The sequence and analysis of the Stx2 phage 933W is presented here. We find evidence that the toxin genes are part of a late-phage transcript, suggesting that toxin production may be coupled with, if not dependent upon, phage release during lytic growth. Another phage gene, *stk*, encodes a product resembling eukaryotic serine/threonine protein kinases. Based on its position in the sequence, Stk may be produced by the prophage in the lysogenic state, and, like the YpkA protein of *Yersinia* species, it may interfere with the signal transduction pathway of the mammalian host. Three novel tRNA genes present in the phage genome may serve to increase the availability of rare tRNA species associated with efficient expression of pathogenicity determinants: both the Shiga toxin and serine/threonine kinase genes contain rare isoleucine and arginine codons. 933W also has homology to *lom*, encoding a member of a family of outer membrane proteins associated with virulence by conferring the ability to survive in macrophages, and *bor*, implicated in serum resistance.

71. **Bacteriophages as surface and ground water tracers. Rossi, P., Doerfliger, N., Kennedy, K., Müller, I., Aragno, M. (1998). *Hydrology and Earth System Science* 2:101-110.** Bacteriophages are increasingly used as tracers for quantitative analysis in both hydrology and hydrogeology. The biological particles are neither toxic nor pathogenic for other living organisms as they penetrate only a specific bacterial host. They have many advantages over classical fluorescent tracers and offer the additional possibility of multi-point injection for tracer tests. Several years of research make them suitable for quantitative transport analysis and flow boundary delineation in both surface and ground waters, including karst, fractured and porous media aquifers. ¶ This article presents the effective application of bacteriophages based on their use in differing Su-iss hydrological environments and compares their behaviour to conventional coloured dye or salt-type tracers. In surface water and karst aquifers, bacteriophages travel at about the same speed as the typically referenced fluorescent tracers (uranine, sulphurhodamine G extra). In aquifers of interstitial porosity, however, they appear to migrate more rapidly than fluorescent tracers, albeit with a significant reduction in their numbers within the porous media. This faster travel time implies that a modified rationale is needed

for defining some ground water protection area boundaries. Further developments of other bacteriophages and their documentation as tracer methods should result in an accurate and efficient tracer tool that will be a proven alternative to conventional fluorescent dyes.

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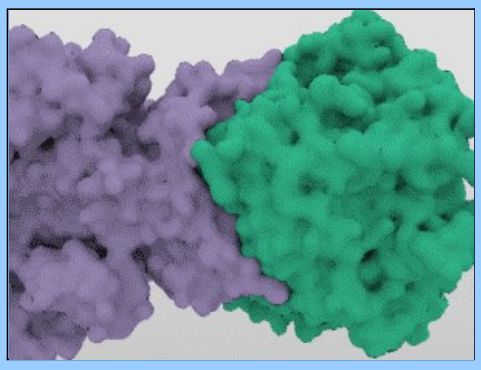

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