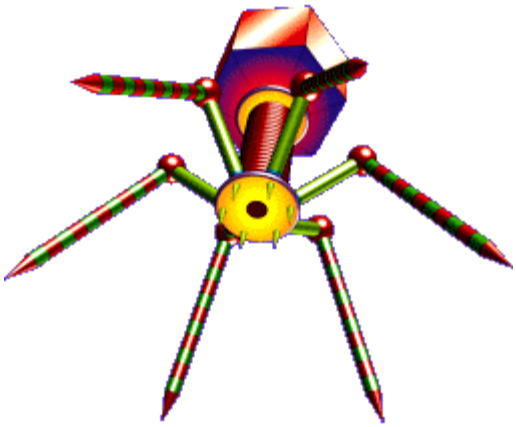

Bacteriophage Ecology Group News

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by Stephen T. Abedon (editor)

December 31, 2007 issue (#26)

Phage Meetings

Stephen T. Abedon

Bacteriophage Ecology Group News (*BEG News*) 26

You would think that phage meetings wouldn't be that difficult to keep track of. After all, there are only so many per year, and a year is a fairly long time. In this age of broadband internet connections, information can whiz back and forth at almost the speed of light. Seemingly everybody with a meeting to promote has a web site. And we're all on more list server lists than we would prefer. But the truth is that all web sites require maintenance, and with meetings that means ongoing maintenance throughout the year as we become aware of one new meeting after another. In addition, not everybody who is putting on a meeting knows about people's efforts to keep track of and advertise meetings. "If you put it on they will attend" typically seems to be a more common mantra than "if you properly advertise more will attend." Oh well.

There are at least four attempts at keeping track of phage meetings, via the web, of which I've been aware. One was the product of the Bacteriophage Ecology Group (which basically means that I conceived of the idea, executed it, maintained the beast, and, ultimately, grew tired of being in charge of the effort). The BEG Meetings page lasted from approximately the year 2000 through 2003. Remnants of the effort can still be found at www.phage.org/beg_meetings.htm. Betty Kutter took over on her Evergreen web site, and this effort continues: academic.evergreen.edu/projects/phage/generalmeeting calendar.htm (generalmeetingcalendar is one word).

The same difficulties are always present, however, and these are obtaining information and then keeping things up to date via routine maintenance. Knowledge and rigor. Strange how consistently important those two facets of science can be! A meetings page also exists on the ASM web site: www.asm.org/division/M/Meetings.html. But it too (big surprise here) is both incomplete and out of date. So clearly there has got to be a better way for the phage community to keep track of the growing number of phage-based meetings.

That way, as it always shall be, is an approach that removes from the equation a requirement that a single person exist who is willing to do all of the work and/or possess all of the necessary information. Toward that end, on October 15, 2006, I created a phage meetings page on Wikipedia: en.wikipedia.org/wiki/Phage_meetings. Wikis are not perfect. Indeed, the poor Bacteriophage Experimental Evolution page was actually deleted from Wikipedia!!!!! (see, however, en.citizendium.org/wiki/Bacteriophage_experimental_evolution, where it lives on) and the Phage

Monographs page (en.wikipedia.org/wiki/Phage_monographs) may very well be slated for extinction (yes, I've created a back up of this page in anticipation of its demise). Nevertheless, and remarkably, the existence of the phage meetings page so far has not been challenged. So, for the moment at least, we have a nice place where not only can all of us keep track of phage meetings, but all of also can also directly contribute to the upkeep of the meetings page!

Meanwhile, I'm a little burnt out on Wikipedia. Yes, the editor apparently does not like being edited (well, let's be fair, what I don't like is having my work wholesale deleted). So I'm on the look out for a wiki experience that is a bit more under my control, one where nobody can claim that scholarship is inappropriate to the mission of the site. For now, though, we have a place to keep track of our meetings, one which, let's all hope, will be around for a while: en.wikipedia.org/wiki/Phage_meetings!

W.W.C. Topley and the "Missing" Phage Reference

Paul Hyman and **Stephen T. Abedon**

Bacteriophage Ecology Group News (*BEG News*) 26

William Whiteman Carlton Topley (January 19, 1886 – January 21, 1944) was a noted British epidemiologist and bacteriologist. Educated at the City of London School, St. John's College, Cambridge and St. Thomas' Hospital, he received his MD degree and was admitted as a member to the Royal College of Physicians in 1911. He then served as the Director of the Pathological Department at Charing Cross Hospital from 1911-1922. Topley left Charing Cross Hospital to become a Professor of Bacteriology at the University of Manchester, a position he held until 1927 when he was appointed the first Professor of Bacteriology and Immunology at the London School of Hygiene and Tropical Medicine at the University of London.

Topley's research focused on experimental epidemiology particularly of microbial diseases. In 1929 he coauthored, with Graham Wilson, *The Principles of Bacteriology and Immunity* which is still published as *Topley and Wilson's Microbiology and Microbial Infections*, currently in its tenth edition. Earlier, while still at the University of Manchester, Topley and his colleagues did work on what was then referred to as the "Twort-D'Herelle phenomenon". Today we would say he was working with bacteriophages. This work produced two papers as well as a presentation to the Manchester Literary and Philosophical Society. The presentation was made on March 17, 1925, and was subsequently published later that year in the *Memoirs of the Manchester Literary and Philosophical Society* (also called *Manchester Memoirs*), a transcription of which begins on the following page. Oddly, it is not listed in the Raettig compendium of bacteriophage publications (see www.phage.org/bgnws003.htm #submissions).

This talk represents one of the earliest published reviews of the state of bacteriophage research. It was made while the nature of the bacteriophage as a type of organism was still being debated. But the basic culture techniques, isolation, and enumeration methods described are still in use today. We hope that this look back will remind our fellow phage biologists how far we have and have not traveled in our understanding bacteriophage biology.

Acknowledgements:

We would like to thank the Manchester Literary and Philosophical Society for generously allowing us to reprint Dr. Topley's article. We would also like to thank Kathy Zabak for proofreading help.

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Manchester Memoirs, Vol. lxix. (1924-1925), No. 8.[*]

VIII. "The Bacteriophage Phenomenon: Transmissible Bacterial Lysis."

Prof. W. W. C. TOPLEY, M.A., M.D., M.SC., F.R.C.P., M.R.C.S.

In choosing a subject for this paper I have tried to select some bacteriological phenomenon which is of general interest, and I think it is true that the problem I propose to discuss has this general character. It has the additional advantage that it belongs to that interesting category of questions concerning which we are in possession of a considerable mass of well-authenticated facts, but the answer to which seems the more unattainable the more we learn. Transmissible bacterial lysis, indeed, is one of those curious phenomena which obstinately refuse to fit comfortably into our general scheme. The difficulty is not so much that we do not know the facts, as that the facts themselves look all wrong. Since, as I have said, the problem is at least as interesting to the general biologist as to the bacteriologist, involving as it does the consideration of the limit which divides living from non-living things, I hope that some of those whose work has lain along biological, and especially along bio-chemical lines, will have some suggestions to offer.

Historically the facts are these. In 1915 Twort described a curious appearance in cultures of a micrococcus, which he had grown from contaminated vaccine lymph. Scattered among the confluent bacterial growth on a culture on ordinary solid nutrient agar, he found glassy areas, which, when examined microscopically, appeared to consist of a finely granular material. These vitreous areas tended to extend over the greater part of the surface growth, converting all the cocci into the granular material. If one of the vitreous areas was touched with a platinum wire, and a normal culture of the coccus was inoculated in one spot with a tiny particle of the granular material, a similar glassy area appeared, which again tended to spread throughout the surface-growth. A suspension of the granular material could be filtered through a porcelain candle, which held back all micro-organisms, but gave a filtrate, a drop of which would initiate the process on a new series of cultures. Such filtrates retained their activity over many months. They withstood heating for one hour at a temperature of 52°C., but not at 60°C. They were most active against young and actively growing organisms. They had no action on dead organisms. The lytic substance, whatever be its nature, could be transferred in an indefinitely long series from culture to culture of the bacteria on which it acted. It could not be cultivated on any known medium, apart from the living bacteria on which it acted. It acted on certain cocci nearly related to the species from which it was obtained, but it was inactive against unrelated bacteria of various kinds.

D'Herelle, in 1917, described, quite independently, a phenomenon which he observed in examining the action of filtrates of cultures, from the excreta of dysenteric patients, on the dysentery bacilli isolated from these cases. As will be seen, the observations which he records bear a striking resemblance to those reported by Twort, and almost all subsequent workers are agreed that the phenomena are essentially similar, though showing minor differences. D'Herelle himself, however, has strenuously denied this, and regards his lytic principle, which he asserts to be a living micro-organism, a parasite of the bacterium, as entirely distinct from Twort's lytic substance. This supposed ultramicroscopic parasite D'Herelle has named the Bacteriophage.

[*] Topley, W. W. C. (1925). The Bacteriophage Phenomenon: Transmissible Bacterial Lysis. Memoirs and proceedings - Manchester Literary and Philosophical Society 69(8):61-71.

Briefly summarized, D'Herelle's most important demonstrations have been as follows: Filtrates from the excreta of typhoid and dystentery [sic] convalescents contain, in a large proportion of cases, a lytic principle which has the power of dissolving or inhibiting the growth of typhoid or dysentery bacilli, and which can be transmitted in series indefinitely. This action may be shown by adding a drop of lytic filtrate to a young broth culture of a sensitive organism, which shows subsequent partial or complete clearing.

Such filtrates are usually not strictly specific in their action—that is, they are usually active against certain closely related bacteria, as well as against the actual bacterium infecting the patient from whom the filtrate was obtained.

The activity of the lytic principle is often feeble when first isolated, but it rapidly increases when transmitted in series from one culture of the sensitive organism to another, so that eventually a few drops of a filtrate diluted 10,000,000 times or more will suffice to initiate the lytic phenomenon. At the same time it frequently happens that the range of activity, as regards the microbial species acted upon, extends with the absolute increase in lytic power. So that the later filtrates of a series will produce lysis of bacterial species which before were unaffected. In some cases, if a given lysin is transmitted in series through cultures of a bacterium other than that from which it was originally obtained, it gains the power of lysing this heterologous organism in very high dilutions, while leaving unaffected, or even losing more or less completely, its power to lyse the original homologous bacterium. Thus, the lytic agent appears to show in some degree the power of adaptation.

Lysis occurs most readily in young, actively growing cultures of the sensitive bacterium. Antiseptics or temperatures, which in any way retard the growth of the bacteria without killing them, interfere with the lytic principle, though they neither destroy it nor lessen its activity, when it is transferred to fresh cultures.

It is impossible to transmit the lytic principle in dead cultures of bacteria or in bacterial filtrates. The presence of living multiplying bacteria is essential.

There is evidence that the lytic principle is particulate in nature. If the action of a lytic filtrate be studied by allowing a drop of such a filtrate, in increasing dilution, to run over the surface of an agar slope thickly inoculated with a susceptible bacterium, the lytic action, in suitable dilutions, is evidenced by the appearance of isolated circular clear areas, with sharply circumscribed borders. These clear circular areas the plaques vierges of D'Herelle and the subsequent French workers, the löcher of the German investigators, are regarded by D'Herelle as colonies of the bacteriophage which have dissolved the surrounding bacteria. He has recorded experiments, in which the use of progressive dilutions of a lytic filtrate has allowed these colonies to be counted, in much the same way as we count bacteria in a fluid culture. A filtrate giving 200 lytic areas at a given dilution will give 20 such areas when the dilution is increased tenfold, and so on. He has also demonstrated that prolonged centrifugalisation increases the concentration of the lytic principle in the lower layers of the fluid, so that the count of the lytic areas is increased.

In the course of pathological work with which this paper is concerned, I have had occasion to observe many of the phenomena referred to above. To obtain a clear picture of the fundamental facts, we may consider the technique employed in isolating and testing a lytic filtrate. It is not necessary to have recourse to a man or animal suffering from any particular infection, for, whatever may be our view about the nature of the lytic principle, there is now no doubt that a substance, or substances, capable of initiating lysis in many species of susceptible bacteria are commonly present in the human or animal intestinal canal.

We start, then, by taking a specimen of intestinal content and transferring it to an adequate quantity, say 250c.c., of ordinary nutrient broth. We incubate this for 24 hours or more at 37°C. We act here on the assumption that, if the sample of intestinal contents selected contains a lytic principle, it will contain bacteria on which that lysin acts. If this assumption be correct there will occur, during the period of incubation, multiplication of sensitive bacteria in the presence of the lysin, and this will lead to an increase in the concentration of the lysin itself. At the end of 24 hours, or after some convenient period, we filter the mixed bacterial culture through a porcelain candle of suitable porosity, a Chamberland L2 is the type usually employed. This will give a bacteria-free filtrate which may or may not contain a lysin, active against the bacterium which we have selected for study. In choosing our bacterium, if our desire be simply to study some problem in connection with bacteriophage action in general, we shall select some species which is known to be sensitive to lysins frequently found in normal animal excreta. The B. dysneteriæ of Shiga is a good example. We shall now inoculate a flask of broth or of peptone-water with the bacterium selected, and incubate it for a few hours to allow of a moderate degree of bacterial growth. We shall then add a few c.c. of our filtrate and return the flask to the incubator. Lytic action may be obvious within a few hours, as shown by a partial or complete clearing of the culture, followed in many cases by a subsequent increase in turbidity due to secondary bacterial growth. If, however, there is no such obvious evidence of lysis, we shall take a loopful of the culture and inoculate a tube or plate of solid medium, so as to allow of copious surface growth. We shall examine these cultures after a further 24 hours' incubation, and may find that the growths show the presence of typical plaques vierges, or of the curious malformed, bitten or nibbled colonies, which are scarcely less characteristic of bacteriophage action. If, at this stage, we find that a lytic agent is present, we shall proceed to increase its activity by a series of transmissions, or passages, in cultures of the sensitive organism. For this purpose we shall filter our first lytic culture, and add some drops of the filtrate to a second young and actively growing culture of the sensitive organism. This process we shall repeat again and again, finally obtaining a filtrate which may cause complete clearing of young broth cultures of our sensitive bacterium in a few hours. If we wish to obtain a quantitative measurement of our final product, we proceed by the method of dilution, testing the action of constant amounts of progressive dilutions of our filtrate on the surface growth of cultures of our susceptible bacterium. If the lytic areas are well marked, we may actually enumerate them, and make a count of the number of lytic particles in our original filtrate. If the lytic areas are ill-defined we may simply test progressive dilutions until we find the limit at which evidence of bacteriophage action ceases. It has become usual to proceed by progressive tenfold dilutions, and to express the activity of a filtrate by means of a lysin exponent, which is simply the index expressing the power of 10 which corresponds to the highest dilution which yields evidence of lytic action—this is, the lysin exponent is simply the logarithm of the highest active dilution. It is by no means unusual to obtain lysin exponents of nine or more—that is to say, filtrates diluted at least 1,000,000,000 times may still be active.

The original observations of Twort and of D'Herelle have been followed by a vast amount of research work during the past eight years, represented by a terrifying mass literature. It is quite impossible to do more than indicate briefly the more important of the additional facts which have come to light, and the rival theories which have been based upon them. It may be said at once that D'Herelle's conception of the lytic principle as a living ultra-microscopic organism has not gone unchallenged. It has, indeed, met with a rather limited acceptance, and at one period had few supporters. It is still true to say that the general trend of opinion is against this explanation, but the difficulties presented by other interpretations have produced a slight reaction in favour of D'Herelle's view.

The opposing theories are three in number. The lytic principle has been regarded as a ferment, which produces some change in the bacteria in consequence of which they not only undergo lysis,

but themselves produce the same ferment, or some similar one, which in its turn acts on successive bacterial generations. In support of this conception experiments are recorded which show that the lytic principle has, in fact, many of the properties of a ferment. Its heat resistance, which is higher than Twort believed, and is certainly not less than 70°C., corresponds well with the know resistance of any ferments. The lytic principle can be precipitated with actone [sic] or with an ether-alcohol mixture, and can be recovered almost intact. It can be absorbed by colloidal metals. It can be removed from a filtrate by absorption with aluminum-hydroxide, and recovered by solution in acetic acid. The degree to which it is retained in the pores of a candle varies with the hydrogen-ion concentration of the filtered fluid. If the hydrogen-ion concentration be increased more of the lytic principle is retained during filtration; if it be diminished more passes through. The lysin can be absorbed with Kuselgur, and recovered by solution in weak ammonia.

Another conception, that of Bordet and his school, is somewhat more vague. It is the theory of heredity transmissible autolysis. It is enunciated by Bordet himself as fellows: “Under some disturbing influence, the nature of which we remain quite free to discuss, a nutritive vitiation of the bacterium is primarily induced testified to by the appearance of the lytic agent. After this the interference of the external agent is no longer necessary. Henceforth, the reproduction of the principle requires nothing more than the presence of living microbes, which, having absorbed a sufficient quantity of it, liberate new amounts of the same again at a certain stage of their evolution.”

Bordet bases his argument largely on the proved necessity for the participation of actively multiplying bacteria in the process of increasing the activity of the lytic principle. It is not enough that the bacteria should be alive, they must be multiplying in order that a lytic substance should be set free. Bordet’s hypothesis can certainly be reconciled with many of the known facts, and deserves the careful consideration which should be accorded to all his views, but the essential nature of the transmissible autolysis remains to be explained.

A third view is that the lytic principle, the bacteriophage itself, represents some stage in the life-history of the parasite, or some alternative if abnormal mode of reproduction, so that once the appropriate stimulus is provided bacterial multiplication is given a new direction, in a proportion at least of the bacteria attacked.

One point which has been fully established, especially perhaps by Arkwright, is that the lytic principle is a potent cause of bacterial variation. Under its influence abnormal forms appear. Many of these are resistant to the action of the lysin, and attempts to propagate it in cultures of such variant strains result in failure. Some variants give rise to large mucoid colonies, quite unlike those of the original strain from which they were derived. These sometimes remain mucoid indefinitely in subsequent generations, sometimes the lytic principle must be frequently reapplied in order to maintain the propagation of this type. Sometimes the variants are non-motile, when the original bacterium was motile. Some times they are unaffected by certain antisera which agglutinated the original strain from which they came.

The supporters of these latter hypotheses are alike in believing that, after the original stimulus, the lytic principle is elaborated by the bacteria themselves, and support has been sought for this view by attempts to show that the lysin may be found in old cultures of pure bacterial strains, which have been cultivated in laboratories for many years, and which have not therefore been subject to contamination with the supposed ultra-microscopic bacteriophage, which D’Herelle believed to be a normal inhabitant of the intestinal tract of most animal species. In spite of repeated attempts, it may be said with some confidence that it is only very occasionally that the lysin can be demonstrated in old cultures, which have been long isolated from their natural surroundings, and hence from all

chance of contamination. Since we know that the lytic principle may be transmitted through many successive sub-cultures, without its presence being obvious, unless special efforts are made to demonstrate it, the supporters of the theory of the ultra-microscopic parasite may fairly contend that such occasional successes are to be expected.

It is, perhaps, well to emphasise that, although the lysin has many of the properties of a ferment, it does not behave in the same way as the ferments most familiar to the biochemist. There is no evidence that the bacteria are digested, that there is any protein cleavage in the chemical sense. The phenomenon is that of cytolysis, the disintegration of the bacterial cell, as such, and the setting free of its protoplasmic contents. It is interesting in this connection to note that the presence of colloids in the fluid medium such as gelatine for example, prevents the occurrence of the lytic phenomenon.

It is perhaps natural to enquire whether a morphological study might not assist in elucidating the problem. Unfortunately, here, as in the study of undoubted filterable viruses, we are at the limit of microscopical observation. We can detect the changes in the bacteria themselves. Under the action of a lytic filtrate they increase in size, often to an enormous extent, and may assume bizarre shapes. They tend to become clumped together in masses. Their protoplasm, at first clear, becomes opalescent and then definitely granular. Many of these granules are quite clearly observable with dark-ground illuminations, but their exact morphology cannot be made out. A broth culture which has undergone lysis may show nothing but these granules. A preparation made from a colony on solid media, showing lytic change, will frequently show, when observed by dark-ground illuminations, normal or distorted bacteria and bacterial ghosts, lying among masses of this granular material.

It is, I think, true that the morphological study of this phenomenon has been somewhat neglected, in comparison with the attention which has been directed to its study by other means, and we may hope that better methods of microscopy, and especially microphotography, may yield information of real value. It is, however, probable that we shall have to wait for technical improvements. The limits of resolution imposed by the wave-lengths of the light used for illumination form, at the moment, an insuperable barrier to the adequate study of the real form of particles of this order of size.

I do not want to deal at any length with the pathological or medical aspects of the problem, but as they may be regarded as yielding some relevant data concerning its essential nature, they may be touched on briefly. It is clear that, if the bacteriophage be in reality a parasite of a parasite, a being which attacks and kills bacteria, it is a conceivable hypothesis that the administration of a lytic filtrate, active against bacterium A, to animal B, in whose tissues bacterium A is multiplying with disastrous results, will terminate the conflict in B's favour. On this point D'Herelle himself has no doubts. The discovery of the bacteriophage has, for him, revolutionized the study of immunity, and rendered our present-day conceptions antiquated and useless. He believes that infective diseases can be cured by the administration of an appropriate bacteriophage culture, and that the whole story of epidemiology is simply a history of the conflict between bacterial parasites and their still smaller foes, with animals or man as interested but relatively quiescent spectators. He believes that it is just as possible to catch an attack of immunity against a disease, as to catch the disease itself—a conception, by the way, that has many supporters, quite apart from the factor of bacteriophage lysis. He considers that it should be possible to stop an epidemic of typhoid, for instance, by adding a suitable bacteriophage to the public water supply of the affected town. It would be unwise to predict the final verdict as to the importance of bacteriophage lysis in the treatment or prevention of disease, but it is fair to say that skeptics exist. In my own Department, since we are particularly interested in the experimental study of epidemic disease, we have submitted this question to direct experiment. It is sufficient to say that,

up to the present, our results have not led us to adopt an optimistic attitude. From the point of view of preventing or cutting short an experimental bacterial infection, the bacteriophage would appear to be a singularly inert substance.

We may consider one point more. D'Herelle has adopted quite definitely, the theory of the big flea and the little flea, and adds "and so *ad infinitum*" to his creed without a qualm. He states quite clearly that we have no right to set a lower limit to the side [sic] of living organisms. This is, of course, absurd. It is quite easy to set a lower limit to the size of living organisms, if we mean by that term living cells. We know that there are living organisms with a diameter of about 0.5 μ. We can assert with complete confidence that there are no living organisms with a diameter of less than that of, let us say, an average-sided [sic] protein molecule. The point is, where within this range is the limit set? It is very probable that the size of the smallest living thing may be below that of the particles in a lytic filtrate, but I own to a feeling that we must be approaching the limit at which the inclusion of the necessary apparatus for nutrition and reproduction within a single cell will lead to uncomfortable crowding.

Briefly to recapitulate the arguments which have been advanced.

The lytic principle is an ultra-microscopic parasite, because it is particulate in nature, has the power of reproduction through an endless series of sub-cultures in symbiosis with a sensitive bacterium, and possesses a certain power of adaptation. It is not a living organism, because it can only increase in amount when the sensitive bacterium is actually dividing, a limitation which is not in accordance with most known facts of infection, because it can be precipitated by such agents as acetone, or aluminum hydroxide, and be recovered in an active form by solution in such substances as acetic acid or ammonia, and because its heat-resistance and persistent activity on prolonged storage suggest a chemical substance rather than a living organism. All the latter characteristics are, however, quite compatible with the active substance being a ferment. Moreover, its particulate nature is no evidence against this view, since there is not *a priori* reason why ferments should not be particulate, and every reason to believe, from analogy, that a ferment might readily be absorbed on to any particulate substance which was present in the filtrate. It is, however, almost impossible to believe that the substance is a ferment, since we should have to accept the view that it could only act on organisms which are actively dividing, having no effect on dead bacteria, or on living bacteria which were not undergoing multiplication. Moreover, a ferment cannot reproduce itself, so that we should have to believe that the organisms themselves produced more of the ferment, when they were undergoing destruction by it. A mechanism which reacts to a hurtful stimulus by promptly producing more of the harmful substance, and so leads straight to race suicide, does not seem likely to have led to prolonged survival, yet the bacteriophage and sensitive bacteria are widely distributed in nature. We can only conclude on a note of interrogation: What is the bacteriophage?

The Development of Plaques and the Mechanism of Phage Action in Solidified Agar

as translated by **Siobain Duffy** and **Stephen T. Abedon**

Bacteriophage Ecology Group News (*BEG News*) 26

Mayr-Harting, A. (1958). Die Entwicklung von Phagenloechern und der mechanismus der Phagenwirkung in festen Naehrboeden. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 1. Abt., Originale* (*Zbl. f. Bakt. Paras. Infek. u. Hyg.*) 171:380-392.

From the Department of Bacteriology of Bristol University
(Chairman: Prof. Dr. K. E. Cooper)

The Development of Plaques and the Mechanism of Phage Action in Solidified Agar

Anna Mayr-Harting

With 7 Figures in the Text
Received on 13 December 1957

D' HERELLE occasionally reports, above all in his book “Le Bactériophage et son Comportement” (1926), that growing phage plaques on agar do not appear to change [in size] after they are visible to the eye. The plaques do not get bigger nor do the bacteria overrun them. This statement has never been challenged outside of a work by K. v. ANGERER (1924), which I sadly became aware after the following results were already completed.

The isolation of a phage, which forms plaques that can potentially reach 12 mm in diameter allowed for research of plaque development. Already the first experiment clearly showed that plaques grow long after they become visible. The present study was undertaken in order to determine the general properties of this growth and the conditions that affect it.

Agars and Strains

The usual medium of this institute was used throughout: Ox-heart infusion; Bacto-Peptone 1%; NaCl 0.6%; Oxoid-Agar 2%; pH 7.6.

The strain KS, which was used for amplification of the phage and in all of the experiments, was a typical bacterial coli from human feces.

The phage strain came from a mixture of a large number of phages, that we use often for instruction purposes. This contained: 1. a mixture of coliphage from the Laboratory of Bacteriophage in Paris, that Dr. N. A. Boulgakov had kindly left, 2. “Enterofagos”, a mixture of phages for intestinal flora, from the company MedicoBiological Laboratories Ltd, London, 3. some phages from Bristol waste water. Despite frequent investigations of this phage mixture we had never noticed any large-plaque phage components. Also neither the strain KS itself nor any other strains of *E. coli* were recovered from any part of the phage mixture.

The plaques show a concentric structure. The inner circle is completely sterile; a lightly clouded zone surrounds it; then follows a narrow, nearly sterile ring, and finally again a somewhat overgrown [region].

The last [ring] shows a strange phenomenon that will be discussed shortly. During the first 24-30 hours it is not usually seen. In isolated plaques it only rudimentarily occurs. Where however plaques flow together, the ring occurs to a greater extent (Fig. 1). Merging plaques therefore were excluded from all measurements.

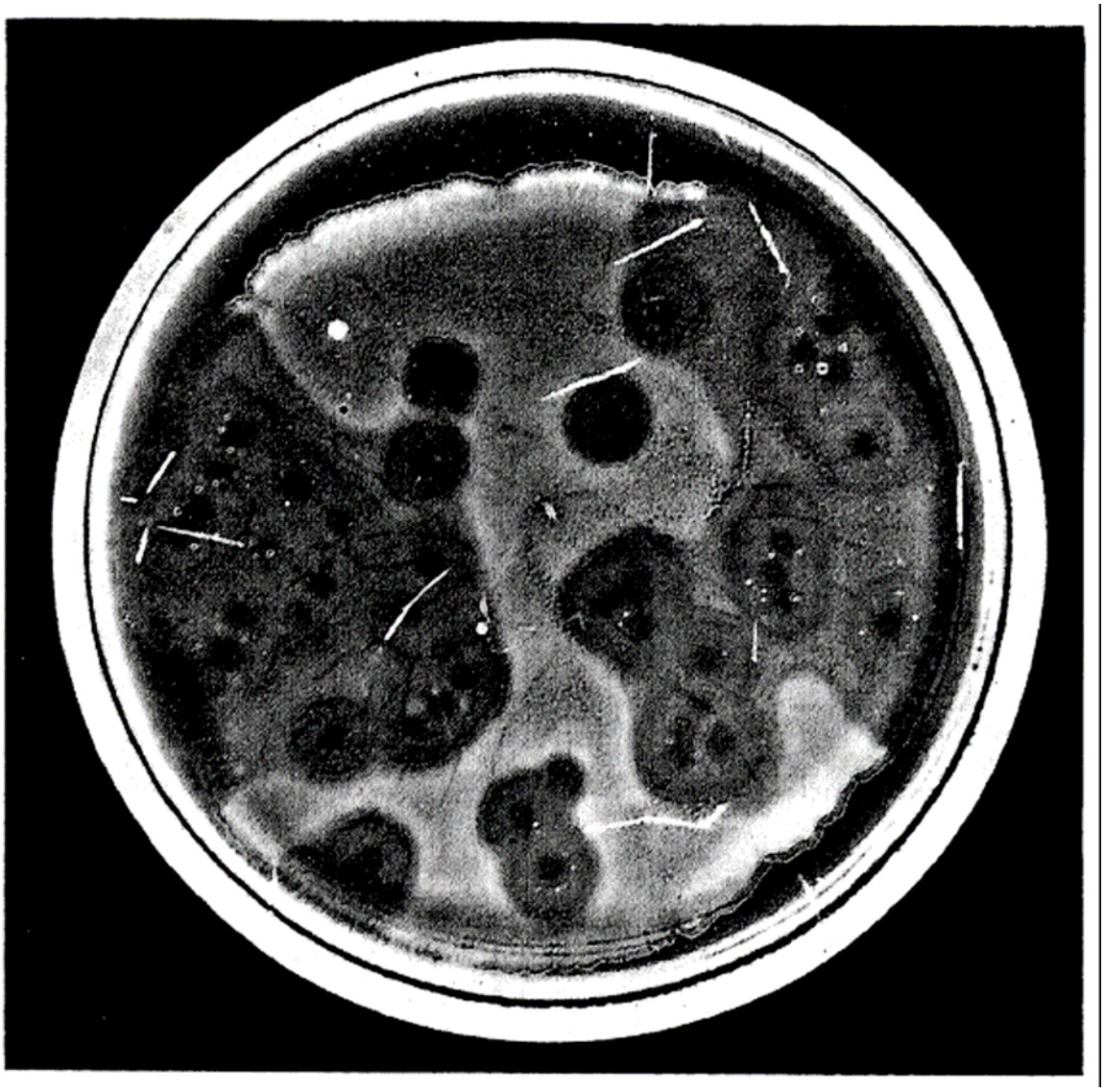

Fig 1. Direct photocopy of one of the plates with *E. coli* and phages. Synergistic effect of merging plaques.

If one lays a ring of the phage lysate on the bacteria-inoculated agar, that location appears perfectly sterile after incubation. A zone of partial lysis was already visible far into the bacterial lawns after 24 hours. This effect, as well as the effect of the merging plaques, extends over such distances that it was necessary to test if these outer zones were really components of a plaque or whether it was due to a lysin produced by the phages, in the sense of SERTIC (1929).

Three strips of a bacterial lawn, of about a centimeter wide, were spread on agar plates; the remaining sterile gap between them was about 3 mm. Phage lysate was dripped on four points on the middle stripe. Although after 24 hours the cloudy zones of the plaques in the middle of the stripes were wider than the room between the stripes, there was no overgrowth [of the plaques] into the other stripes, and, after 48 hours, [the plaques overgrew] only in two places in the stripes, where, apparently, the bacteria from two stripes were confluent. It can therefore be assumed, that the partially cleared zones are a direct result of the phages, not of a soluble lysin (Fig. 2).

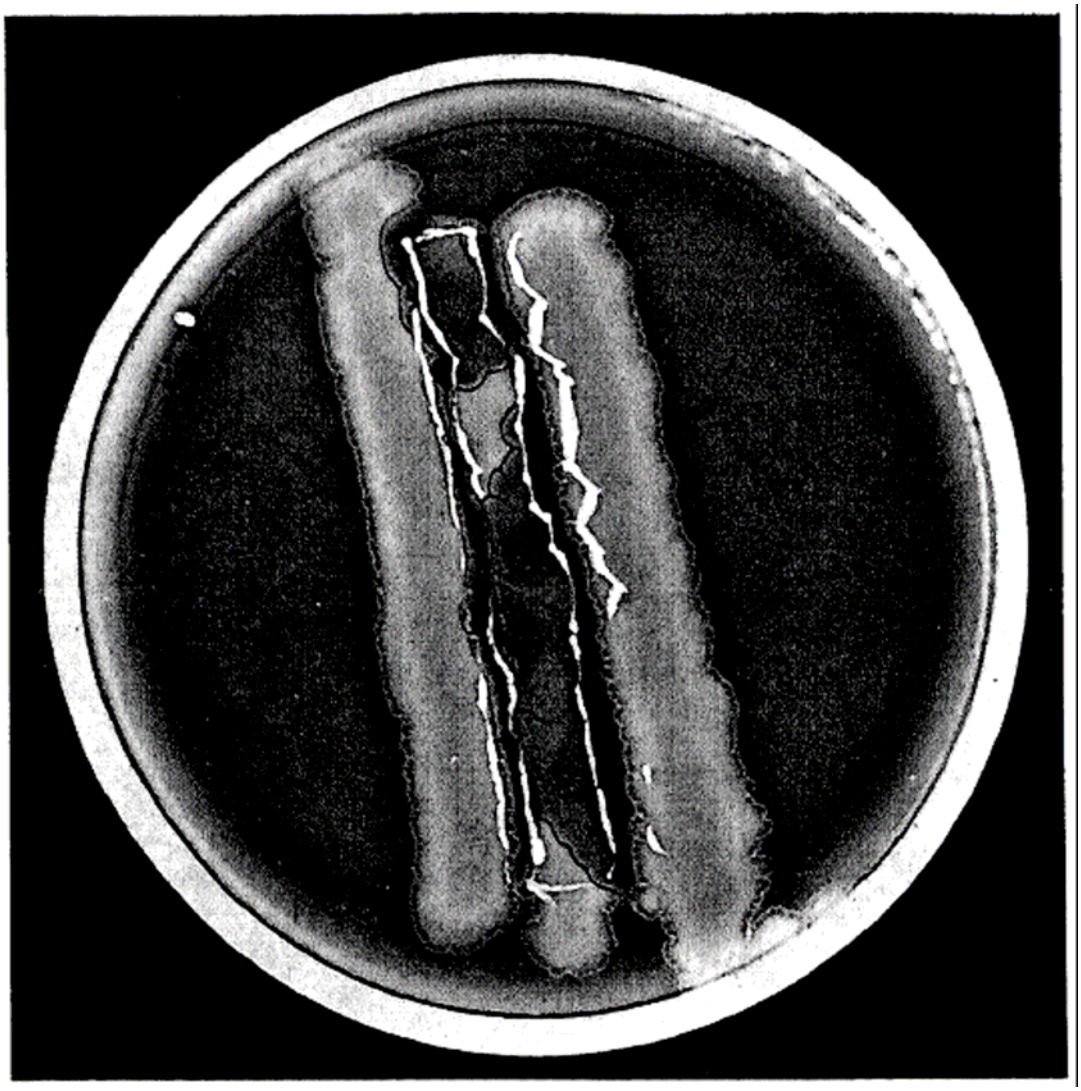

Fig. 2. Direct photocopy of one [plate] with three stripes of *E. coli* culture. Phage was dropped onto the middle striple at four points. Confluence of the phage between stripes only occured where the bacterial stripes grew into each other. At these [points], where the phages turned towards the sides of the inoculated stripes, [there] was a notable pearlescence of the edges, which was not very remarkable by eye, but showed up clearly in white in the photograph.

Methods

The method employed was basically that of M. ADAMS (1952). Onto a 20ml agar Petri plate (9 cm diameter) was poured a 4ml top layer, which contained phages and bacteria. For the latter [top agar], 2 ml liquefied agar was mixed in a 50°C constant water bath with 1 ml of the bacterial culture or a resuspension and 1 ml of the appropriate phage dilution in broth. After solidifying, which occurred

almost instantly, the plates were dried open for 15 minutes in the incubator, then closed and further incubated.

The diameters of the plaques were measured with a caliper, through the glass, on the underside of the plate. The Nonius-scale of this instrument permits the reading [of measurements] of tenths of millimeters. The thickness of the glass and agar can distort the measurement by 0.1-0.2 mm.

Results

Fig. 3 shows the growth curves for three different plaques and three colonies of *E. coli*. The plaques were already visible after two to three hours. Their diameters grew linearly for 15-20 hours more. However, during this time the diameters of bacterial colonies grew exponentially. After transferring the data onto a logarithmic scale, the growth of the colony diameter for the first 10 hours had a constant slope and then the rate of increase became slower; however, some individual colonies grew with a constant slope all day.

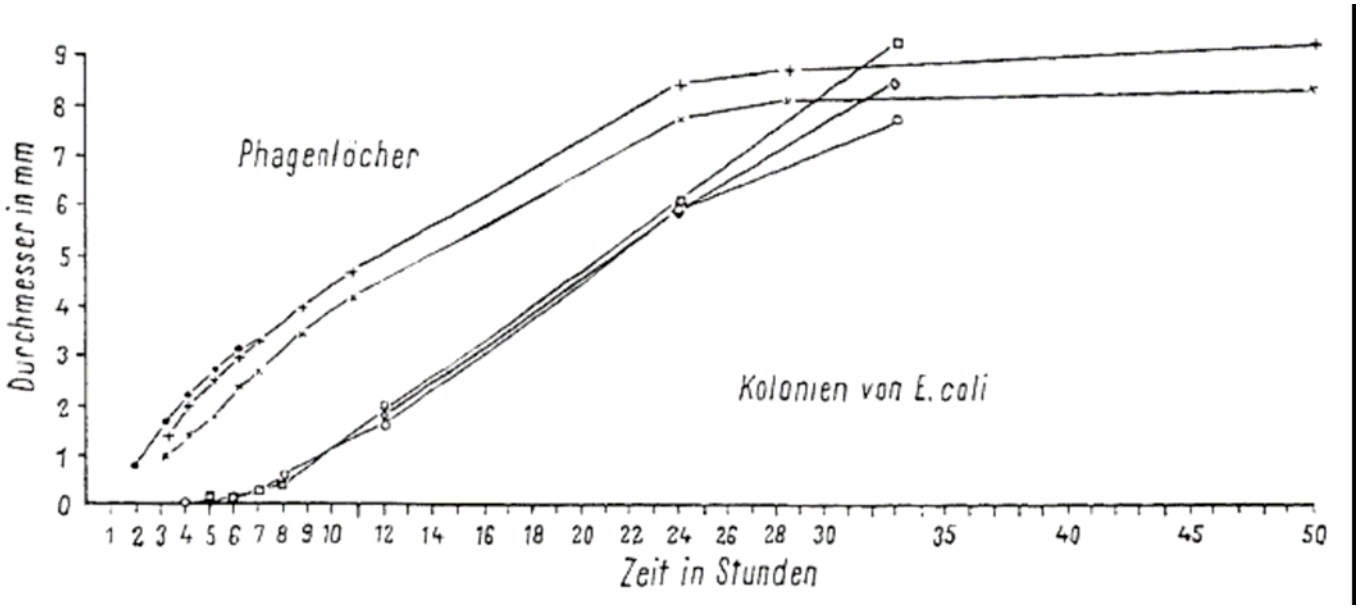

Fig. 3. Growth curves for the diameter of phages and *E. coli* colonies. The curve for the colonies was collected in two ways. For the first eight hours the colonies were observed microscopically and after eight hours [the growth of] three colonies that were the same size whether they were observed microscopically or macroscopically were followed on a plate. (translation from figure: *x* = time in hours, *y* axis = diameter in mm; plaques, colonies of *E. coli*)

In order to determine the environmental conditions that affect the growth and end size of plaques, quantitative research should be undertaken that does not focus on the growth of [an] individual plaque but [instead on] the average diameter of all of the non-overlapping plaques on the plate.

The environmental factors that were of importance were the thickness of the agar layer and the density of the bacterial inocula, and also, to a smaller degree, the concentration of the agar.

The age of the bacterial culture was completely irrelevant. Cultures from 6, 12, 24, and 48 hours gave plaques of the same size provided that the cultures were all diluted so that they all had

the same number of living cells. Also, cold shock of the culture and cold shock followed by incubation had no influence on plaque growth.

The effect of placing poured phage plates in the refrigerator for 3 hours prior to incubation had the result of plaque growth lagging three hours behind the control culture. They eventually reached the same size.

Incubation at 22°C gave totally irregular results that cannot be explained at this time.

An experiment was conducted in salt-poor agar. The under layer was salt free. The over layer contained 1/40 or 1/80 of the normal 0.6%, respecitively. On the first plate the plaques were normal and in the latter the plaques were a bit smaller than in the controls.

D' HERELLE stated that the number of the plaques on a plate influenced the range of the sizes of the plaques in the same way as the number of bacterial colonies and their distance from each other influences the size of bacterial colonies. This cannot be said at all in the experiments presented here. Individual grown plaques have the same size without the ability to see the [other plaques in their] neighborhood. Completely in contrast to colonies of *E. coli*, which seldom overlap when they have reached a given size, but instead have a sterile space between them such that they remain independent, neighboring plaques grow over each other, which is an impressive synergistic phenomenon.

The agar concentration of the media, in combination with the variation in the incubation tested in the study, had a clear, but not very large effect. In Fig. 4 we showed that plaques became larger with lower concentrations of agar (in the top agar) but in one case [the plaques] were smaller on average [in lower-concentration agar] than in higher-concentration agar. In any case, the concentration of [agar] in the bottom agar layer had a clearer effect than that of the top agar. The plaques on the plate that had a bottom agar concentration of only 1% agar could only be seen up to 6 hours, and then the inoculum — the bacteria and phage — dissolved into an indefinite form.

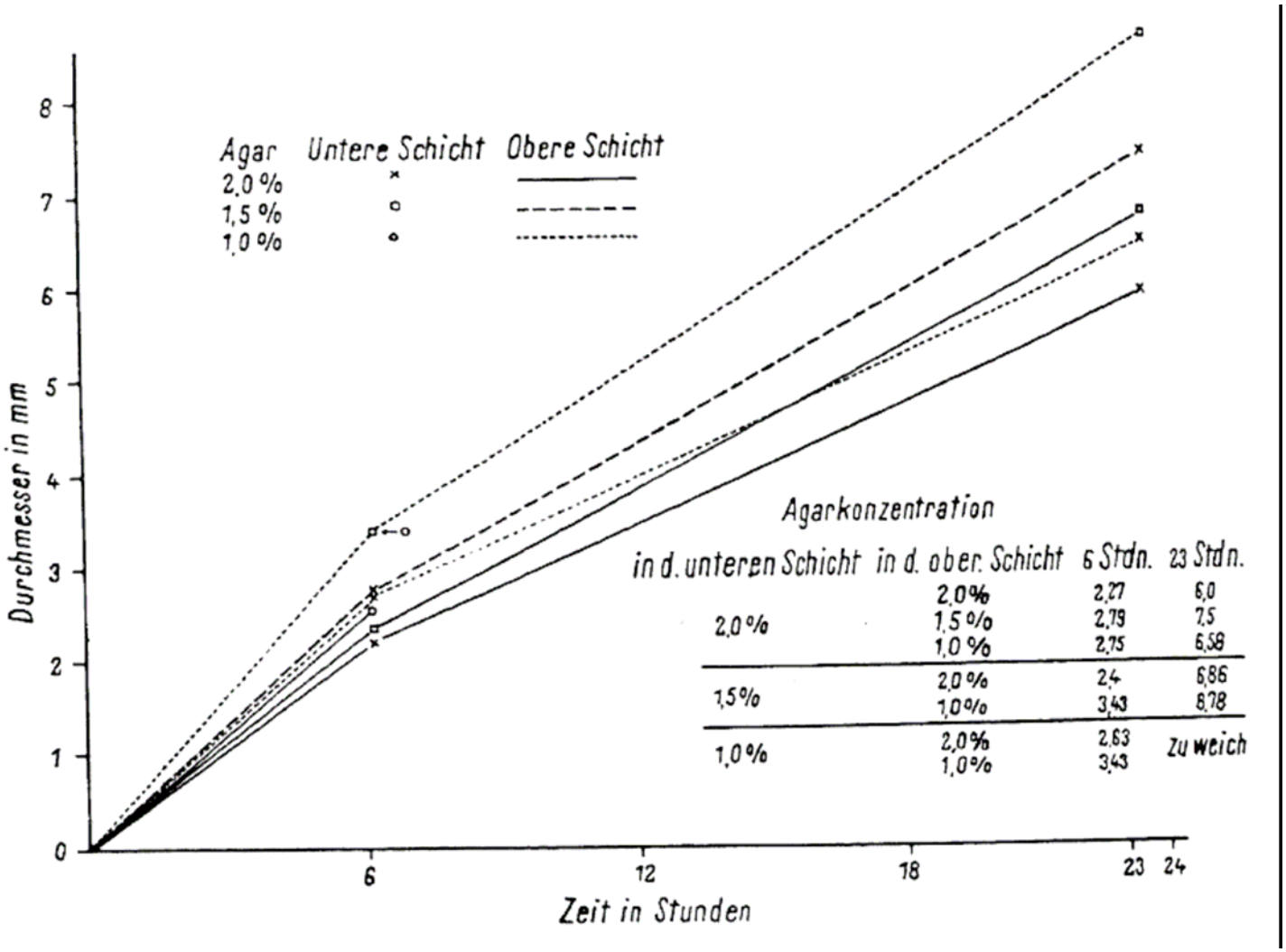

Fig. 4. Plaque size at different agar concentrations. (*x* axis = time in hours, *y* = diameter in millimeters. Bottom layer agar concentration as symbols, top layer agar concentration as lines. Table: Agar concentration in the bottom layer (first column) and in the top layer (second column); diameter of the plaques after 6 hours (third column) and 23 hours (fourth column). “Zu weich” – too soft)

The thickness of the lower agar layer during the first 6 hours affected plaque growth insignificantly. On the thinner layers, however, plaque growth ended sooner, while the thicker layers permitted longer enlargement of the plaque diameter (Fig. 5). The difference between the average diameters of the plaques on 10 ml and on 30 ml agar amounted to, after 45 hours, 5 mm and more.

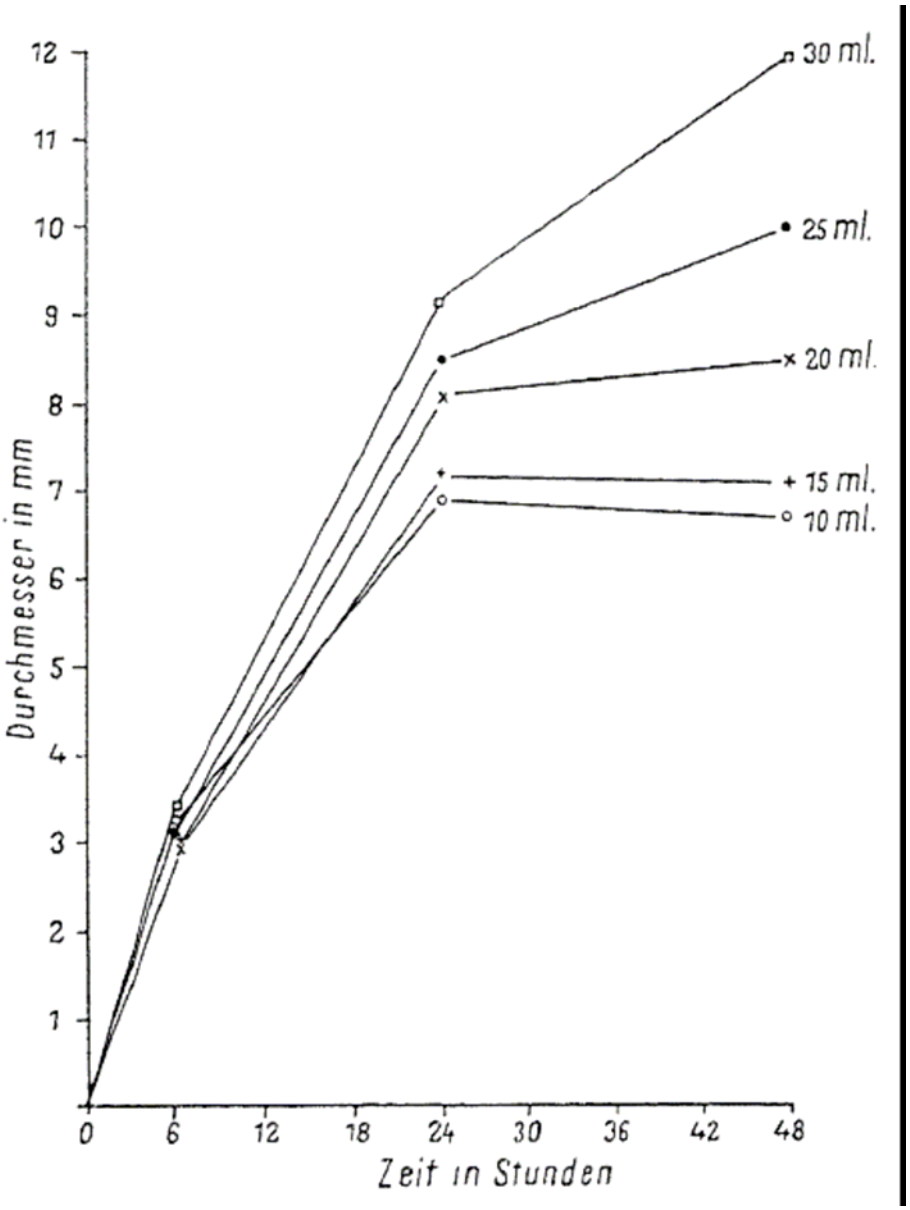

Fig. 5. Growth of plaques with differently thick bottom agar layers. (*x* axis = time in hours , *y* = diameter in millimeters)

The effect of the bacterial density is recorded in Fig. 6. The concentrated inoculum was a dense resuspension of an agar slant [literally “diagonal agar”]. In all experiments of this type the average diameter of the colonies was the same size from the concentrated to the thousand-fold diluted inoculum. In the ten thousand-fold dilution, the bacteria colonies in the agar no longer appeared confluent to the naked eye, and the plaques were very irregular in size and shape. In the research shown the average [plaque] diameter in the ten thousand-fold diluted inoculum was still larger than in the thousand-fold diluted; in another experiment, however, with the greatest dilution there was a drop in the average plaque diameter, although the largest single diameters were still bigger than the biggest of the previous dilution. In contrast to the influence of the thickness of the agar layer, the bacterial density influenced the growth of plaques from the beginning onward.

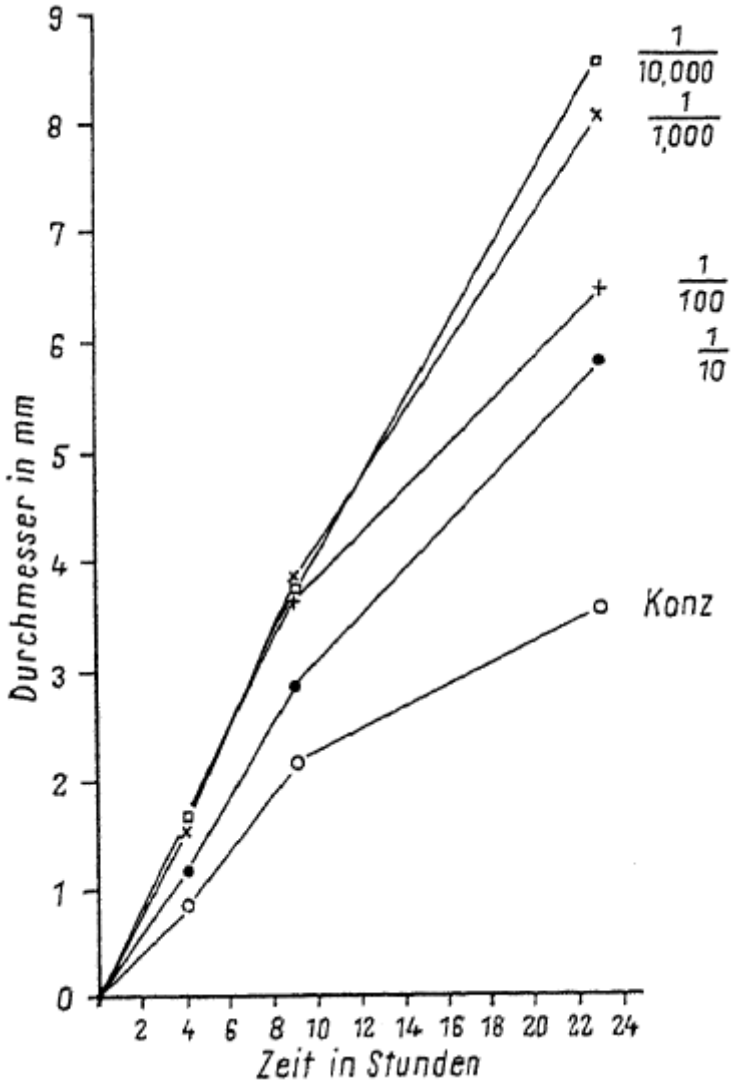

Fig. 6. Growth of plaques on plates seeded with different bacterial densities. (*x* axis = time in hours, *y* axis = diameter in mm, “Konz” -- concentrated)

Not only the size, but also the number of developing plaques is dependent on the density of the bacterial inoculum. This observation has not been well considered with regard to routine titration of phage suspensions, although von ANGERER made a similar observation. The highest count of plaques develops in a bacterial inoculum of 2000 million per 4 ml. With decreasing bacterial inoculum, the number of plaques decreases slowly but steadily (Fig. 7). Also with an increase of the bacterial inoculum, to over 2000 million/4 ml, the number of the developing plaques decreases. This drop is quantitatively very small, but this effect was seen in all of our experiments so that it is unlikely it is an experimental error.

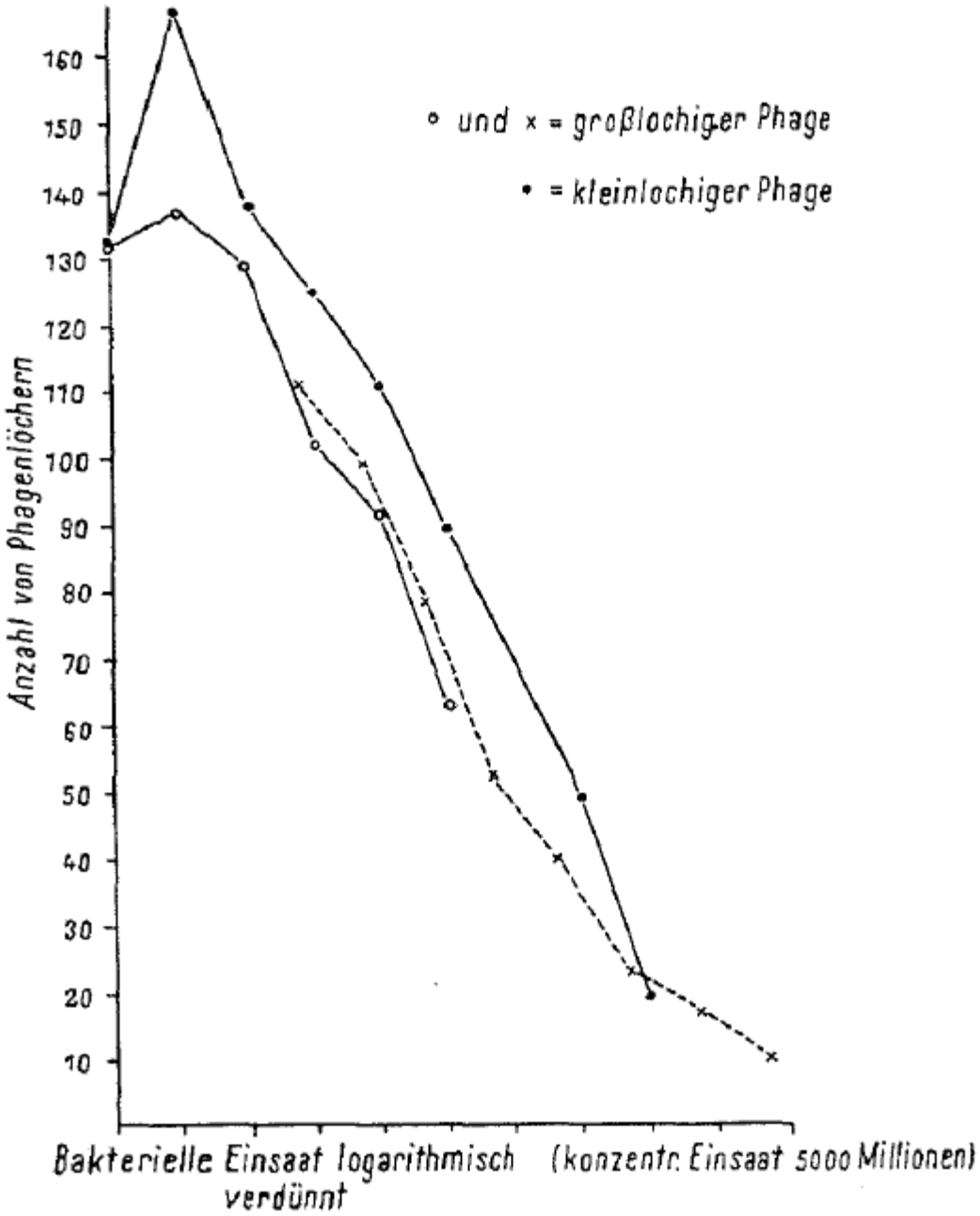

Fig. 7. Reduction of the number of plaques with deceasing density of bacterial inoculum. The absolute numbers for one of the two replicates for the large-plaquing phages were determined in order to place both replicates on the same number scale. (*x* axis = Bacterial inoculum logarithmically diluted (concentrated inoculum 5000 million), *y* axis = Number of plaques; open circles and crosses: large-plaque-forming phage, closed circles: small-plaque-forming phage)

Discussion

Two possible models for the growth of plaques present themselves: 1. the growth of bacterial colonies 2. the diffusion of dissolved substances through a gel.

1. The comparison with bacterial colonies is valid in the sense that all phage particles of a plaque are descendants of a single particle. D' HERELLE always stressed the similar behavior of

bacterial and phage colonies. The most remarkable thing is kind and/or strain-specific size of both, which persists despite the influence of environmental factors. D' HERELLE already determined that plaques are larger than bacterial colonies, ever larger with [increasing] media volumes, into which the metabolites can diffuse away.

The statement of D'HERELLE that the total number of plaques on a plate and their distance from each other determines the size of the plaques, as is in the case with bacterial colonies, could not be confirmed in the phages examined here. For the growth of bacterial colonies, the number of and distance to neighboring colonies have a quantitative effect on size (MAYR HARTING 1947). Plaques grow, each in their own world, independent of other plaques on the plate. Coli colonies, whose borders come nearer to each other in the course of growth, almost never merge, but leave a narrow sterile distance between them. Plaques merge with ease; in case of our giant phages it was even determined there was an enlargement of the plaque surface where two phage holes ran into each other.

The growth curve of the diameter of bacterial colonies proceeds completely differently than those of the diameter of plaques.

2. To compare plaque formation with diffusion of dissolved substances through a gel, it would be as if to say: physical-chemically, the phage is seen as a big colloid-piece. It will therefore diffuse through a sieve of agar, although, importantly, more slowly than small pieces of dissolved molecular material. For a plaque to be achieved this way, the phage particles, which are liberated by the decay of an infected bacterium, must only diffuse so far that they reach the next regularly spaced bacterium in the agar. These become infected and will, when the reproduction of the phage inside has completed, become new centers, from which phage will diffuse out into the agar. One must therefore imagine the growth of a plaque as waves of diffusing phage, which lead to the appearance of approximately circularly arranged new centers of diffusion.

The diffusion from an individual infection center can not be determined on account of the size of the phage particles. Possibly it follows curves, as VESTERDAL (1947) described exhaustively for the diffusion of one substance, thus by no means in arithmetic progression. However, as the individual bacteria develop into microcolonies, the distance between them is only slowly reduced at first, and the advance of phages between the individual infection-centers goes approximately as slowly, and this results in the arithmetic growth curve of the plaque diameters.

The distances between bacteria, which the phage particles travel, are considerable. The smallest bacterial inoculum, which still results in clearly delineated plaques, is about 250,000 per ml. In this case, the distance between the centers of bacterial cells is 160µ[m]. Notably, one obtains fuzzily-bounded plaques, even with smaller bacterial inocula. Therefore, the phage particles diffuse 160µ[m] away and, occasionally, farther.

The original phage infection of the bacterial germs, which represents the origin of the plaque, is also dependent on a diffusion event, which does not necessarily take place before pouring the molten mixture onto the plate. V. ANGERER, who expressly postulated the diffusion of the phage particles for the formation of plaques through the agar, tried - in incomprehensible contradiction to own hypothesis - to determine the diffusion rates of the phage, by adding agar at time intervals from 0-30 minutes to the mixture of bacteria and phages in liquid medium, and then pouring into plates. From the fact that the number of plaques in the mixture didn’t increase after the 15 minute treatment (it was no larger than it was in the mixture plated immediately after mixing), he concluded that the

absorption of phage onto the bacteria occurs instantaneously and that the v. SMOLUCHOWSKIS formula for the adsorption of phage to bacteria was not applicable.

Later works that deal with the adsorption of phage to bacteria (SCHLESINGER 1932, DELBRÜCK 1940, PUCK *et al*. 1951 and 1953) proved, that this [absorption] absolutely follows diffusion laws. All these works exclusively used liquid medium with suspended bacteria and phages in relatively high concentration. The experimental determination of absorption rates allowed the calculation of the diffusion coefficient and size of phage particles. PUTNAM (1950), however, pointed out that the convection stream and other factors always cause an error, apparently always towards fast diffusion, which increases with diminishing concentration of phage. The phage concentration in v. ANGERERS' experiments was low. The experimental error, which after PUTNAM's [observation about] low phage concentrations is more remarkable, reflects an apparent higher diffusion rate, may have therefore promoted collisions between bacteria and phages in v. ANGERERS liquid mixture. In addition, after pouring his plates the unadsorbed phages still had plenty of time to diffuse to their bacterial victims, and this [occurs] without substantial disturbance by PUTNAM's obstacles of liquid medium, which are strongly diminished if the diffusion takes place in agar.

The reduction in plaque number demonstrated by the same quantity of a phage suspension [inoculated] with a reduced bacterial inoculum permits us to estimate the distance that an individual phage particle can assuredly cover. The highest number of plaques was achieved with a bacterial inoculum of 2000 million in 4 ml. The average distance between bacteria here was 12µ[m]. This means that for a phage particle its prospects worsen after a collision with a bacterium, if at the beginning of the experiment it is located will be more than 6µm away from the next bacterium. That an individual phage particle has less chance for success as a brood of 50-200 phage particles diffused from a burst infectious center does not require a miracle-just probability.

The reduction in plaque number with an increased bacterial inoculum over 2000 million by 4 ml is probably ascribable to the bacterial population here being close to a critical amount, where the phage no longer has a visible effect. The phages, which adsorb with some delay, do not then have enough time to develop a plaque. One would have to actually expect that reduced bacterial seed would stably make the journey of the phage longer, while the critical bacterial population would be reached through the delay. That the plaque number nevertheless decreases indicates that the delay of bacterial growth does not increase the diffusion distance.

ELFORD and ANDREWES (1932) found an extensive, but not absolute correlation between the size of the phage particles in the ultra-filtration experiment and plaque diameter on agar. In general, the smaller the phage, the larger the plaque diameter. This relationship would also be expressed in a drastic reduction in the number of small-plaque-forming phages with decreased bacterial seed. An experiment was therefore undertaken in which a small-plaque-forming phage was poured into plates with a dilution series of bacterial suspensions. Against all expectations, it showed that, under identical experimental conditions and with the same bacterial strain, the number of plaques fell similarly for both [large and small plaque-forming] phages with decreasing bacterial seed (Fig. 7). This observation indicates that both phages have the same diffusion rate and, therefore, the same size particles. If this is correct, one must conclude that plaque size is not solely determined by phage size and the dependent diffusion rate of the phage particle, but that other factors play into it. It calls into question the influence of the number of phage particles liberated in a 'burst,' and the statistical probability of collision; perhaps the 'generation time' of the phage, i.e. the time that passes between the individual diffusion waves, could be more important. These points are currently being further examined.

D'HÉRELLE tried to explain the general cessation of plaque growth and the sharpness of the plaque edge as strain-specific attributes, analogous to [those traits of] bacterial colonies, which should be based on a "vaccination" of the surrounding medium. Plaques, which concern the boundary of growth, actually behave much more like the inhibition zones of antibiotics. For plaques, as for antibiotics in the [punched] hole-test, the zone of inhibition of bacterial growth gets larger as the bacterial inoculum decreases. This not comparable in any way to the end of growth of a bacterial colony. LINTON (personal communication) found that the border of the inhibition zones of all antibiotics depends on how far the inhibiting concentration of the antibiotic diffuses, before a critical population size is reached. If the inoculum, *e.g*. of Klebsiella pneumoniae, amounts to 6.78 x 10^7 living cells/ml, even the highest concentration of streptomycin is not capable of creating a visible inhibition zone. The sharp delimitation of most plaques indicates that a critical population density also exists here, which does not permit further visible enlargement of plaques. This [density] must be, however, substantially greater than that for antibiotics.

A difference in the behavior of phage plaques and antibiotic zones of inhibition is that, as the former grow, the position of the border of the latter, which is determined by bacteria and antibiotic concentrations, does not change in location once it becomes visible (COOPER and LINTON 1952).

Summary [author's translation]

The diameter of phage holes grows arithmetically, in contrast to the logarithmic growth of the diameter of bacterial colonies. The growth and the final size of the phage hole depend on the distance that the phage particles can travel before a critical bacterial population is reached. This, in turn, is determined by properties of the phage, the bacterial strain and the medium. Elford and Andrewes have shown that the most important property of the phage is its particle size, which determines its diffusion rate. The agar concentration of the medium will influence the diffusion rate to a greater or lesser degree, dependent on the particle size and other factors. Some of the present work indicates that properties of the phage other than its basic diffusion rate contribute to the size of the phage hole; one would have to consider here the "generation time" of the phage, and its burst size.

The bacterial inoculum influences the size of the phage holes mainly by its numbers. The denser the inoculum, the smaller the holes.

The density of the bacterial inoculum affects not only the size but also the number of phage holes which develop from aliquots of a phage suspension. The number of phage holes is reduced at a regular slow rate, with dilution of the bacterial inoculum.

The mechanism by which the thickness of the agar layer affects the size of the holes remains at present unexplained. It is suggested that it is an effect on bacteria, perhaps influencing their synthesizing faculties and, therefore, the burst size of the phage.

The consideration of the adsorption of phages on bacteria as a physico-chemical problem has led to a better understanding of this complex subject. In the present paper it is suggested, that this aspect might usefully be extended to the events in solid medium, where the complicating factors of fluid media - like convection currents, and active and Brownian movements of bacteria – are absent.

[additional author translations of this summary into French, Spanish and Russian are not presented]

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New BEG Members

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name		address/research interests
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	Interests:	Isolation and characterization of phages active against bacterial plant pathogen, especially lytic phages active against *Ralstonia solanacerum*, the cause of brown rot disease of potato.
Kalyan Banerjee	PI	Vice-President, MACS, Agharkar Research Institute, Agarkar Road, Off Law College Road, Pune 411 004, Maharashtra State, INDIA
	Interests:	Study of bacteriophages of *Salmonella* spp. pathogenic to humans and poultry.
Seth Bordenstein	PI	7 MBL Street, The Marine Biological Laboratory, Woods Hole, MA 02543
	Interests:	Structure, function, and evolution of bacteriophages in obligate intracellular symbionts. My main study system is the widespread and heritable bacterium *Wolbachia* that infects numerous arthropod and nematode species.
Sabah Jassim	PI	Head of Microbiology Department, Zayed Complex for Herbal Research & Traditional Medicine, P.O Box: 3542, Abu Dhabi, United Arab Emirates
	Interests:	Phage-breeding, phage-amplification, and phage-based biocontrol and bioprocessing technologies, with specific emphasis on EHEC and VTEC O157 phages.
Richard J. Obiso Jr.	PI	Director, Life Sciences Division, Luna Innovations Inc, 3157 State Street, Blacksburg, VA 24060
	Interests:	At Luna Innovations, we are committed to utilizing bacteriophages in three areas: detection, decontamination of food pathogens, and, in bacteriophage therapy. We have a number of bacteriophages that will enter the food market and we are looking forward to novel bacteriophage therapy concepts.
S. Somnath Pai		National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Lakeside Campus, Fine Arts Avenue, Kochi - 682 016, Kerala, India
	Interests:	Development of phage therapy for *Vibrio* infections in prawn larviculture and study phage ecology in hatchery and estuarine systems.

New Phage-Ecology References

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why papers covering more than just bacteriophages are included)

1. Anders,R., Chrysikopoulos,C.V. (2006). **Evaluation of the factors controlling the time-dependent inactivation rate coefficients of bacteriophage MS2 and PRD1.** *Environ. Sci. Technol.* 40:3237-3242. **Abstract:** Static and dynamic batch experiments were conducted to study the effects of temperature and the presence of sand on the inactivation of bacteriophage MS2 and PRD1. The experimental data suggested that the inactivation process can be satisfactorily represented by a pseudo-first-order expression with time-dependent rate coefficients. The time-dependent rate coefficients were used to determine pertinent thermodynamic properties required for the analysis of the molecular processes involved in the inactivation of each bacteriophage. A combination of high temperature and the presence of sand appears to produce the greatest disruption to the surrounding protein coat of MS2. However, the lower activation energies for PRD1 indicate a weaker dependence of the inactivation rate on temperature. Instead, the presence of air-liquid and air-solid interfaces appears to produce the greatest damage to specific viral components that are related to infection. These results indicate the importance of using thermodynamic parameters based on the time-dependent inactivation model to better predict the inactivation of viruses in groundwater.

2. Angly,F.E., Felts,B., Breitbart,M., Salamon,P., Edwards,R.A., Carlson,C., Chan,A.M., Haynes,M., Kelley,S., Liu,H., Mahaffy,J.M., Mueller,J.E., Nulton,J., Olson,R., Parsons,R., Rayhawk,S., Suttle,C.A., Rohwer,F. (2006). **The marine viromes of four oceanic regions.** *PLoS Biol.* 4:e368. **Abstract:** Viruses are the most common biological entities in the marine environment. There has not been a global survey of these viruses, and consequently, it is not known what types of viruses are in Earth's oceans or how they are distributed. Metagenomic analyses of 184 viral assemblages collected over a decade and representing 68 sites in four major oceanic regions showed that most of the viral sequences were not similar to those in the current databases. There was a distinct "marine-ness" quality to the viral assemblages. Global diversity was very high, presumably several hundred thousand of species, and regional richness varied on a North-South latitudinal gradient. The marine regions had different assemblages of viruses. Cyanophages and a newly discovered clade of single-stranded DNA phages dominated the Sargasso Sea sample, whereas prophage-like sequences were most common in the Arctic. However most viral species were found to be widespread. With a majority of shared species between oceanic regions, most of the differences between viral assemblages seemed to be explained by variation in the occurrence of the most common viral species and not by exclusion of different viral genomes. These results support the idea that viruses are widely dispersed and that local environmental conditions enrich for certain viral types through selective pressure.

3. Awais,R., Fukudomi,H., Miyanaga,K., Unno,H., Tanji,Y. (2006). **A recombinant bacteriophage-based assay for the discriminative detection of culturable and viable but nonculturable *Escherichia coli* O157:H7.** *Biotechnol. Prog.* 22:853-859. **Abstract:** A previously green fluorescent protein (GFP)-labeled PP01 virulent bacteriophage, specific to *Escherichia coli* O157:H7, was used to construct lysozyme-inactivated GFP-labeled PP01 phage (PP01e-/GFP). The new recombinant phage lacked lytic activity because of the inactivation of gene e, which produces the lysozyme responsible for cell lysis. Gene e was inactivated by inserting an amber stop codon. Prolonged incubation of E. coli O157:H7 cells with PP01e-/GFP did not lead to cell lysis, while the propagation of PP01e-/GFP in host cells increased the intensity of green fluorescence. Retention of cell morphology and increase in fluorescence enabled the direct visualization and enumeration of *E. coli* O157:H7 cells within an hour. The PP01e-/GFP system, when combined with nutrient uptake analysis, further allowed the discriminative detection of culturable, viable but nonculturable (VBNC), and dead cells in the stress-induced aquatic environment. Stress-induced cells, which retained culturability, allowed phage propagation and produced bright green florescence. Nonculturable cells (VBNC and dead) allowed only phage adsorption but no proliferation and remained low fluorescent. The low-fluorescent nonculturable cells were further differentiated into VBNC and dead cells on the basis of nutrient uptake analysis. The low-fluorescent cells, which grew in size by nutrient incorporation during prolonged incubation in nutrient medium, were defined as metabolically active and in the VBNC state. The elongated VBNC cells were then easily recognizable from dead cells. The proposed assay enabled the detection and quantification of VBNC cells. Additionally, it revealed the proportion of culturable to VBNC cells within the population, as opposed to conventional techniques, which demonstrate VBNC cells as a differential value of the total viable count and the culturable cell count.

4. Bae,T., Baba,T., Hiramatsu,K., Schneewind,O. (2006). **Prophages of *Staphylococcus aureus* Newman and their contribution to virulence.** *Mol. Microbiol.* 62:1035-1047. **Abstract:** Four prophages (phiNM1-4) were identified in the genome of *Staphylococcus aureus* Newman, a human clinical isolate. phiNM1, phiNM2 and

phiNM4, members of the siphoviridae family, insert at different sites (poiA, downstream of isdB and geh) in the staphylococcal chromosome. phiNM3, a beta-haemolysin (hlb) converting phage, encodes modulators of innate immune responses (sea, sak, chp and scn) in addition to other virulence genes. Replication of phiNM1, phiNM2 and phiNM4 occurs in culture and during animal infection, whereas phiNM3 prophage replication was not observed. Prophages were excised from the chromosome and *S. aureus* variants lacking phiNM3 or phiNM1, phiNM2 and phiNM4 displayed organ specific virulence defects in a murine model of abscess formation. *S. aureus* Newman lacking all four prophages was unable to cause disease, thereby revealing essential contributions of prophages to the pathogenesis of staphylococcal infections.

5. Baker,A.C.**,** Goddard,V.J.**,** Davy,J.**,** Schroeder,D.C.**,** Adams,D.G.**,** Wilson,W.H. (2006). **Identification of a diagnostic marker to detect freshwater cyanophages of filamentous cyanobacteria.** *Appl. Environ. Microbiol.* 72:5713-5719. **Abstract:** Cyanophages are viruses that infect the cyanobacteria, globally important photosynthetic microorganisms. Cyanophages are considered significant components of microbial communities, playing major roles in influencing host community diversity and primary productivity, terminating cyanobacterial water blooms, and influencing biogeochemical cycles. Cyanophages are ubiquitous in both marine and freshwater systems; however, the majority of molecular research has been biased toward the study of marine cyanophages. In this study, a diagnostic probe was developed to detect freshwater cyanophages in natural waters. Oligonucleotide PCR-based primers were designed to specifically amplify the major capsid protein gene from previously characterized freshwater cyanomyoviruses that are infectious to the filamentous, nitrogen-fixing cyanobacterial genera Anabaena and Nostoc. The primers were also successful in yielding PCR products from mixed virus communities concentrated from water samples collected from freshwater lakes in the United Kingdom. The probes are thought to provide a useful tool for the investigation of cyanophage diversity in freshwater environments.

6. Ballester,N.A.**,** Fontaine,J.H.**,** Margolin,A.B. (2005). **Occurrence and correlations between coliphages and anthropogenic viruses in the Massachusetts Bay using enrichment and ICC-nPCR.** *J. Water Health* 3:59-68. **Abstract:** We evaluated a two-step enrichment procedure to detect coliphages and an integrated cell culture-nested polymerase chain reaction (ICC-nPCR) to detect human astrovirus, enteroviruses, rotavirus and adenovirus type 40 and 41 in marine water samples collected by the Massachusetts Water Resource Authority (MWRA). MWRA has been monitoring its receiving waters for coliphages, anthropogenic viruses and indicator bacteria in order to evaluate the impact of Boston's Deer Island Sewage Treatment Plant discharge. Coliphages and enteric viruses were originally assayed using single agar overlay and most probable number cell culture (MPN) methods, respectively. Reanalysis of these samples for enteric viruses by ICC-nPCR demonstrated that 46% were positive for at least one virus compared with 23% with the MPN method. Use of the enrichment method showed a 47% increase in the detection of male specific and somatic coliphages compared with the single agar overlay method. Correlations between the presence of coliphages, enteric viruses and indicator bacteria were based on proximity to the treatment plant discharge, seasonal variations and site levels. The presence of enteric viruses was significantly correlated to coliphages but not to indicator bacteria. Preliminary comparative results demonstrate that effective and efficient monitoring of anthropogenic contamination can be achieved using these more sensitive and specific techniques.

7. Bearden,C.M.**,** Agarwal,A.**,** Book,B.K.**,** Vieira,C.A.**,** Sidner,R.A.**,** Ochs,H.D.**,** Young,M.**,** Pescovitz,M.D. (2005). **Rituximab inhibits the in vivo primary and secondary antibody response to a neoantigen, bacteriophage phiX174.** *Am. J. Transpl.* 5:50-57. **Abstract:** The response to primary immunization in patients treated with Rituximab (RIT) is not clear. We studied the in vivo antibody response of chronic renal failure (CRF) patients to the neoantigen bacteriophage phiX174 given alone or after ablation with RIT. Eighteen CRF subjects received two immunizations with phiX174 separated by 6 weeks. Nine subjects received a single dose of RIT. The intensity and immunoglobulin isotype of the antibody response (K(*v*)) were measured post-infusion. In addition, three subjects previously immunized and treated with RIT underwent a third and fourth immunization with phiX174 and a tetanus control 2 years later. RIT significantly decreased peak K(*v*) responses when compared to both historic non-CRF controls and to CRF subjects. CRF itself decreased peak K(*v*) responses compared to non-CRF controls. Percent-ratio of anti-phage IgM to IgG was significantly decreased in RIT treated subjects. One of three subjects treated with RIT was found to have developed a partial B cell tolerance to phiX174 administration 2 years later. RIT decreases antibody production and isotype switching to neoantigens and might be useful to prevent antibody response to therapeutic drugs and to newly transplanted organs.

8. Beilstein,F.**,** Dreiseikelmann,B. (2006). **Bacteriophages of freshwater *Brevundimonas vesicularis* isolates.** *Res. Microbiol.* 157:213-219. **Abstract:** Nine strains of *Brevundimonas vesicularis* were isolated from surface water of three ponds in Bielefeld, Germany. With those strains as indicators seven bacteriophages with different host ranges were isolated. Molecular characterization showed that all phages contained linear double-stranded DNA with a similar genome size of about 37 kb. Restriction analysis and hybridization of phage DNAs revealed that three of these phages are closely related to each other. These phages had morphologies typical of the family

Siphoviridae. Their genomes contained cohesive ends. Four phages were classified into the family of Podoviridae. Restriction analysis of the DNAs of these phages did not reveal any similarities. The DNA of these phages were terminally redundant. All phages were unable to transduce plasmids or marker genes.

9. Bradford,S.A.**,** Tadassa,Y.F.**,** Jin,Y. (2006). **Transport of coliphage in the presence and absence of manure suspension.** *J. Environ. Qual.* 35:1692-1701. **Abstract:** Mechanisms of coliphage transport and fate in the presence and absence of manure suspension were studied in saturated column experiments. In the presence of manure suspension, little inactivation of indigenous somatic coliphage occurred and the transport was controlled by deposition. The deposition followed a power law distribution with depth, and the magnitude increased with decreasing sand size. Comparison of the cumulative size distribution of manure components in the suspension initially and after passage through sand, suggested that particles retained by mechanical filtration and/or straining decreased the effective pore size and potentially induced straining of the somatic coliphage. A 2-site kinetic deposition model was used to estimate the magnitudes of attachment and straining in the presence of manure suspension, and provided a good description of the data. Modeling results indicated that straining accounted for 16 to 42% of the deposited somatic coliphage, and that both straining and attachment increased with decreasing sand size due to smaller pores and higher surface area, respectively. In the absence of manure suspension, ϕX174 (a representative somatic coliphage) and MS2 (a male-specific RNA coliphage) transport was controlled by inactivation induced by the solid phase. This conclusion was based on comparison of coliphage transport behavior at 5 and 20 degrees C, mass balance information, and numerical modeling. Comparison of somatic coliphage transport data in the presence and absence of manure suspension revealed much higher effluent concentrations in the presence of manure. This difference was attributed to lower inactivation and higher detachment rates. The observed coliphage transport behavior suggests that survival of viruses may be extended in the presence of manure suspensions, and that transport studies conducted in the absence of manure suspension may not accurately characterize the transport potential of viruses in manure-contaminated environments.

10. Brigati,J.R.**,** Petrenko,V.A. (2005). **Thermostability of landscape phage probes.** *Analyt. Bioanalyt. Chem.* 382:1346-1350. **Abstract:** Immunoassays have traditionally relied on antibodies as diagnostic probes. Their use outside of a laboratory, however, may be problematic because antibodies are often unstable in severe environmental conditions. Environmental monitoring requires thermostable probes, such as landscape phage, that carry thousands of foreign peptides on their surfaces, are superior to antibodies, and can operate in non-controlled conditions. While parent wild-type phage are known to be extremely stable in various media at high temperatures, no work has been done to demonstrate the stability of landscape phage probes. We examined the thermostability of a landscape phage probe and a monoclonal antibody specific for β-galactosidase in parallel in an enzyme-linked immunosorbent assay (ELISA) format. They were both stable for greater than six months at room temperature, but at higher temperatures the antibody degraded more rapidly than the phage probe. Phage retained detectable binding ability for more than six weeks at 63 degrees C, and three days at 76 degrees C. The activation energy of phage degradation was determined to be 1.34 x 10(5) J/mol. These results confirm that phage probes are highly thermostable and can function even after exposure to high temperatures during shipping, storage and operation.

11. Brockhurst,M.A.**,** Buckling,A.**,** Rainey,P.B. (2006). **Spatial heterogeneity and the stability of host-parasite coexistence.** *J. Evol. Biol.* 19:374-379. **Abstract:** Spatially heterogeneous environments can theoretically promote more stable coexistence of hosts and parasites by reducing the risk of parasite attack either through providing permanent spatial refuges or through providing ephemeral refuges by reducing dispersal. In experimental populations of *Pseudomonas aeruginosa* and the bacteriophage PP7, spatial heterogeneity promoted stable coexistence of host and parasite, while coexistence was significantly less stable in the homogeneous environment. Phage populations were found to be persisting on subpopulations of sensitive bacteria. Transferring populations to fresh microcosms every 24 h prevented the development of permanent spatial refuges. However, the lower dispersal rates in the heterogeneous environment were found to reduce parasite transmission thereby creating ephemeral refuges from phage attack. These results suggest that spatial heterogeneity can stabilize an otherwise unstable host-parasite interaction even in the absence of permanent spatial refuges.

12. Brown,C.M.**,** Lawrence,J.E.**,** Campbell,D.A. (2006). **Are phytoplankton population density maxima predictable through analysis of host and viral genomic DNA content?** *J. Mar. Bio. Assoc. UK* 86:491-498. **Abstract:** Phytoplankton:virus interactions are important factors in aquatic nutrient cycling and community succession. The number of viral progeny resulting from an infection of a cell critically influences the propagation of infection and concomitantly the dynamics of phytoplankton populations. Host nucleotide content may be the resource limiting viral particle assembly.We present evidence for a strong linear correlation between measured viral burst sizes and viral burst sizes predicted from the host DNA content divided by the viral genome size, across a diversity of phytoplankton:viral pairs. An analysis of genome sizes therefore supports predictions of taxon-specifc phytoplankton population density thresholds beyond which viral proliferation can trim populations or terminate

phytoplankton blooms. We present corollaries showing that host:virus interactions may place evolutionary pressure towards genome reduction of both phytoplankton hosts and their viruses.

13. Brown,S.P., Le Chat,L., De Paepe,M., Taddei,F. (2006). **Ecology of microbial invasions: amplification allows virus carriers to invade more rapidly when rare.** *Curr. Biol.* 16:2048-2052. **Abstract:** Locally adapted residents present a formidable barrier to invasion . One solution for invaders is to kill residents . Here, we explore the comparative ecological dynamics of two distinct microbial mechanisms of killing competitors, via the release of chemicals (e.g., bacteriocins) and via the release of parasites (e.g., temperate phage). We compared the short-term population dynamics of susceptible *E. coli* K12 and isogenic carriers of phage varphi80 in experimental cultures to that anticipated by mathematical models using independently derived experimental parameters. Whereas phages are a direct burden to their carriers because of probabilistic host lysis, by killing competitor bacteria they can indirectly benefit bacterial kin made immune by carrying isogenic phage. This is similar to previously described bacteriocin-mediated effects. However, unlike chemical killing, viable phage trigger an epidemic among susceptible competitors, which become factories producing more phage. Amplification makes phage carriers able to invade well-mixed susceptibles even faster when rare, whereas chemical killers can only win in a well-mixed environment when sufficiently abundant. We demonstrate that for plausible parameters, the release of chemical toxins is superior as a resident strategy to repel invasions, whereas the release of temperate phage is superior as a strategy of invasion.

14. Bull,J.J., Regoes,R.R. (2006). **Pharmacodynamics of non-replicating viruses, bacteriocins and lysins.** *Proc. R. Soc. Lond. B Biol. Sci.* 273:2703-2712. **Abstract:** The pharmacodynamics of antibiotics and many other chemotherapeutic agents is often governed by a 'multi-hit' kinetics, which requires the binding of several molecules of the therapeutic agent for the killing of their targets. In contrast, the pharmacodynamics of novel alternative therapeutic agents, such as phages and bacteriocins against bacterial infections or viruses engineered to target tumour cells, is governed by a 'single-hit' kinetics according to which the agent will kill once it is bound to its target. In addition to requiring only a single molecule for killing, these agents bind irreversibly to their targets. Here, we explore the pharmacodynamics of such 'irreversible, single-hit inhibitors' using mathematical models. We focus on agents that do not replicate, i.e. in the case of phage therapy, we deal only with non-lytic phages and in the case of cancer treatment, we restrict our analysis to replication of incompetent viruses. We study the impact of adsorption on dead cells, heterogeneity in adsorption rates and spatial compartmentalization.

15. Capra,M.L., Del,L.Q., Ackermann,H.W., Moineau,S., Reinheimer,J.A. (2006). **Characterization of a new virulent phage (MLC-A) of *Lactobacillus paracasei*.** *J. Dairy Sci.* 89:2414-2423. **Abstract:** A new virulent bacteriophage (MLC-A) was recently isolated in Argentina from a probiotic dairy product containing a strain of *Lactobacillus paracasei*. Observation of the lysate with an electron microscope revealed bacteriophage particles with an icosahedral capsid of 57 +/- 2 nm; with a collar and a noncontractile tail of 156 +/- 3 nm terminating with a baseplate to which a tail fiber was attached. Therefore, phage MLC-A belongs to the Siphoviridae family. This phage was able to survive the pasteurization process and was resistant to alcohols and sodium hypochlorite (400 mg/kg). Only peracetic acid could inactivate high-titer suspensions of phages in a short time. The maximum rates of phage adsorption to its host cells were obtained at 30 degrees C with a pH between 5 and 7, and in the presence of calcium or magnesium ions. The host range of phage MLC-A encompassed *L. paracasei* and *Lactobacillus casei* strains, but it was not able to infect *Lactobacillus rhamnosus* or *Lactobacillus gasseri* strains. One-step growth kinetics of its lytic development revealed latent and burst periods of 30 and 135 min, respectively, with a burst size of about 69 +/- 4 plaque-forming units per infected cell. Phage MLC-A had a distinctive restriction profile when compared with the 2 well-studied *Lactobacillus* phages, PL-1 and J-1. The genome size of the MLC-A phage was estimated to be approximately 37 kb. This study presents the description of the first phage specific for *L. paracasei* isolated in Argentina. The isolation of phage MLC-A indicates that, beside lactic acid bacteria starters, probiotic cultures can also be sensitive to virulent phages in industrial processes.

16. Carey-Smith,G.V., Billington,C., Cornelius,A.J., Hudson,J.A., Heinemann,J.A. (2006). **Isolation and characterization of bacteriophages infecting *Salmonella* spp.** *FEMS Microbiol. Lett.* 258:182-186. **Abstract:** Bacteriophages infecting *Salmonella* spp. were isolated from sewage using soft agar overlays containing three *Salmonella* serovars and assessed with regard to their potential to control food-borne salmonellae. Two distinct phages, as defined by plaque morphology, structure and host range, were obtained from a single sample of screened sewage. Phage FGCSSa1 had the broadest host range infecting six of eight *Salmonella* isolates and neither of two *Escherichia coli* isolates. Under optimal growth conditions for *S. Enteritidis* PT160, phage infection resulted in a burst size of 139 PFU but was apparently inactive at a temperature typical of stored foods (5 degrees C), even at multiplicity of infection values in excess of 10 000. While neither isolate had characteristics that would make them candidates for biocontrol of *Salmonella* spp. in foods, phage FGCSSa1 behaved unusually when grown on two *Salmonella* serotypes at 37 degrees C in that the addition of phages appeared to retard growth of the host, presumably by the lysis of a fraction of the host cell population.

17. Casas,V., Miyake,J., Balsley,H., Roark,J., Telles,S., Leeds,S., Zurita,I., Breitbart,M., Bartlett,D., Azam,F., Rohwer,F. (2006). **Widespread occurrence of phage-encoded exotoxin genes in terrestrial and aquatic environments in Southern California.** *FEMS Microbiol. Lett.* 261:141-149. **Abstract:** Many human diseases are caused by pathogens that produce exotoxins. The genes that encode these exotoxins are frequently encoded by mobile DNA elements such as plasmids or phage. Mobile DNA elements can move exotoxin genes among microbial hosts, converting avirulent bacteria into pathogens. Phage and bacteria from water, soil, and sediment environments represent a potential reservoir of phage- and plasmid-encoded exotoxin genes. The genes encoding exotoxins that are the causes of cholera, diphtheria, enterohemorrhagic diarrhea, and *Staphylococcus aureus* food poisoning were found in soil, sediment, and water samples by standard PCR assays from locations where the human diseases are uncommon or nonexistent. On average, at least one of the target exotoxin genes was detected in approximately 15% of the more than 300 environmental samples tested. The results of standard PCR assays were confirmed by quantitative PCR (QPCR) and Southern dot blot analyses. Agreement between the results of the standard PCR and QPCR ranged from 63% to 84%; and the agreement between standard PCR and Southern dot blots ranged from 50% to 66%. Both the cholera and shiga exotoxin genes were also found in the free phage DNA fraction. The results indicate that phage-encoded exotoxin genes are widespread and mobile in terrestrial and aquatic environments.

18. Chao,L., Rang,C.U., Wong,L.E. (2002). **Distribution of spontaneous mutants and inferences about the replication mode of the RNA bacteriophage φ6.** *J. Virol.* 76:3276-3281. **Abstract:** When a parent virus replicates inside its host, it must first use its own genome as the template for replication. However, once progeny genomes are produced, the progeny can in turn act as templates. Depending on whether the progeny genomes become templates, the distribution of mutants produced by an infection varies greatly. While information on the distribution is important for many population genetic models, it is also useful for inferring the replication mode of a virus. We have analyzed the distribution of mutants emerging from single bursts in the RNA bacteriophage φ6 and find that the distribution closely matches a Poisson distribution. The match suggests that replication in this bacteriophage is effectively by a stamping machine model in which the parental genome is the main template used for replication. However, because the distribution deviates slightly from a Poisson distribution, the stamping machine is not perfect and some progeny genomes must replicate. By fitting our data to a replication model in which the progeny genomes become replicative at a given rate or probability per round of replication, we estimated the rate to be very low and on the on the order of 10(-4). We discuss whether different replication modes may confer an adaptive advantage to viruses.

19. Characklis,G.W., Dilts,M.J., Simmons,O.D., Likirdopulos,C.A., Krometis,L.A., Sobsey,M.D. (2005). **Microbial partitioning to settleable particles in stormwater.** *Water Res.* 39:1773-1782. **Abstract:** The degree to which microbes in the water column associate with settleable particles has important implications for microbial transport in receiving waters, as well as for microbial removal via sedimentation (i.e. detention basins). The partitioning behavior of several bacterial, protozoan and viral indicator organisms is explored in three urban streams under both storm and dry weather conditions. The fraction of organisms associated with settleable particles in stormwater is estimated through use of a centrifugation technique which is calibrated using suspensions of standard particles (e.g., glass, latex). The fraction of organisms associated with settleable particles varies by type of microbe, and the partitioning behavior of each organism generally changes between dry weather and storm conditions. Bacterial indicator organisms (fecal coliforms, *Escherichia coli*, enterococci) exhibited relatively consistent behavior, with an average of 20-35% of organisms associated with these particles in background samples and 30-55% in storm samples. *Clostridium perfringens* spores exhibited the highest average level of particle association, with storm values varying from 50% to 70%. Results related to total coliphage partitioning were more variable, with 20-60% associated with particles during storms. These estimates should be valuable in surface water quality modeling efforts, many of which currently assume that all microbes exist as free (unattached) organisms.

20. Chauvatcharin,N., Ahantarig,A., Baimai,V., Kittayapong,P. (2006). **Bacteriophage WO-B and *Wolbachia* in natural mosquito hosts: infection incidence, transmission mode and relative density.** *Molecular Ecology* 15:2451-2461. **Abstract:** Bacteriophages of *Wolbachia* bacteria have been proposed as a potential transformation tool for genetically modifying mosquito vectors. In this study, we report the presence of the WO-B class of *Wolbachia*-associated phages among natural populations of several mosquito hosts. Eighty-eight percent (22/25) of *Wolbachia*-infected mosquito species surveyed were found to contain WO-B phages. WO-B phage orf7 sequence analysis suggested that a single strain of WO-B phage was found in most singly (23/24) or doubly (1/1) *Wolbachia*-infected mosquitoes. However, the single *Wolbachia* strain infecting *Aedes perplexus* was found to harbour at least two different WO-B phages. Phylogenetic analysis suggested that horizontal transmission of WO-B phages has occurred on an evolutionary scale between the *Wolbachia* residing in mosquitoes. On an ecological scale, a low trend of co-transmission occurred among specific WO-B phages within *Wolbachia* of each mosquito species.

Assessment of the density of WO-B phage by real-time quantitative polymerase chain reaction (RTQ-PCR) revealed an average relative density of 7.76 x 10(5)+/- 1.61 x 10(5) orf7 copies per individual mosquito for a single *Wolbachia* strain infecting mosquitoes, but a threefold higher density in the doubly *Wolbachia*-infected *Aedes albopictus*. However, the average combined density of WO-B phage(s) did not correlate with that of their *Wolbachia* hosts, which varied in different mosquito species. We also confirmed the presence of WO-B-like virus particles in the laboratory colony of *Ae. albopictus* (KLPP) morphologically, by transmission electron microscopy (TEM). The viral-like particles were detected after purification and filtration of *Ae. albopictus* ovary extract, suggesting that at least one WO-B-like phage is active (temperate) within the *Wolbachia* of this mosquito vector. Nevertheless, the idea of utilizing these bacteriophages as transformation vectors still needs more investigation and is likely to be unfeasible.

21. Chen,F.**,** Wang,K. **,** Stewart,J.**,** Belas,R. (2006). **Induction of multiple prophages from a marine bacterium: a genomic approach.** *Appl. Environ. Microbiol.* 72:4995-5001. **Abstract:** Approximately 70% of sequenced bacterial genomes contain prophage-like structures, yet little effort has been made to use this information to determine the functions of these elements. The recent genomic sequencing of the marine bacterium *Silicibacter* sp. strain TM1040 revealed five prophage-like elements in its genome. The genomes of these prophages (named prophages 1 to 5) are approximately 74, 30, 39, 36, and 15 kb long, respectively. To understand the function of these prophages, cultures of TM1040 were treated with mitomycin C to induce the production of viral particles. A significant increase in viral counts and a decrease in bacterial counts when treated with mitomycin C suggested that prophages were induced from TM1040. Transmission electron microscopy revealed one dominant type of siphovirus, while pulsed-field gel electrophoresis demonstrated two major DNA bands, equivalent to 35 and 75 kb, in the lysate. PCR amplification with primer sets specific to each prophage detected the presence of prophages 1, 3, and 4 in the viral lysate, suggesting that these prophages are inducible, but not necessarily to the same level, while prophages 2 and 5 are likely defective or non-mitomycin C-inducible phages. The combination of traditional phage assays and modern microbial genomics provides a quick and efficient way to investigate the functions and inducibility of prophages, particularly for a host harboring multiple prophages with similar sizes and morphological features.

22. Chetochine,A.S.**,** Brusseau,M.L.**,** Gerba,C.P.**,** Pepper,I.L. (2006). **Leaching of phage from Class B biosolids and potential transport through soil.** *Appl. Environ. Microbiol.* 72:665-671. **Abstract:** The objective of this study was to investigate leaching and transport of viruses, specifically those of an indigenous coliphage host specific to *Escherichia coli* ATTC 15597 (i.e., MS-2), from a biosolid-soil matrix. Serial extractions of 2% and 7% (solids) class B biosolid matrices were performed to determine the number of phage present in the biosolids and to evaluate their general leaching potential. Significant concentrations of coliphage were removed from the biosolids for each sequential extraction, indicating that many phage remained associated with the solid phase. The fact that phage was associated with or attached to solid particles appeared to influence the potential for release and subsequent transport of phage under saturated-flow conditions, which was examined in a series of column experiments. The results indicated that less than 8% of the indigenous coliphage initially present in the biosolids leached out of the biosolid-soil matrix. A fraction of this was subsequently transported through the sandy porous medium with minimal retention. The minimal retention observed for the indigenous phage, once released from the biosolids, was consistent with the results of control experiments conducted to examine MS-2 transport through the porous medium.

23. Clark,J.R.**,** March,J.B. (2006). **Bacteriophages and biotechnology: vaccines, gene therapy and antibacterials.** *Trends Biotechnol.* 24:212-218. **Abstract:** In recent years it has been recognized that bacteriophages have several potential applications in the modern biotechnology industry: they have been proposed as delivery vehicles for protein and DNA vaccines; as gene therapy delivery vehicles; as alternatives to antibiotics; for the detection of pathogenic bacteria; and as tools for screening libraries of proteins, peptides or antibodies. This diversity, and the ease of their manipulation and production, means that they have potential uses in research, therapeutics and manufacturing in both the biotechnology and medical fields. It is hoped that the wide range of scientists, clinicians and biotechnologists currently researching or putting phages to practical use are able to pool their knowledge and expertise and thereby accelerate progress towards further development in this exciting field of biotechnology.

24. Claverie,J.-M.**,** Ogata,H.**,** Audic,S.**,** Abergel,C.**,** Suhre,K.**,** Fournier,P.-E. (2006). **Mimivirus and the emerging concept of "giant" virus.** *Virus Res.* 17:133-144. **Abstract:** The recently discovered *Acanthamoeba polyphaga* Mimivirus is the largest known DNA virus. Its particle size (750 nm), genome length (1.2 million bp) and large gene repertoire (911 protein coding genes) blur the established boundaries between viruses and parasitic cellular organisms. In addition, the analysis of its genome sequence identified many types of genes never before encountered in a virus, including aminoacyl-tRNA synthetases and other central components of the translation machinery previously thought to be the signature of cellular organisms. In this article, we examine how the finding of such a giant virus might durably influence the way we look at microbial biodiversity, and lead us to revise the

classification of microbial domains and life forms. We propose to introduce the word "girus" to recognize the intermediate status of these giant DNA viruses, the genome complexity of which makes them closer to small parasitic prokaryotes than to regular viruses.

25. Clokie,M., Millard,A.D., Mehta,J.Y., Mann,N.H. (2006). **Virus isolation studies suggest short-term variations in abundance in natural cyanophage populations of the Indian Ocean.** *J. Mar. Bio. Assoc. UK* 86:499-505. **Abstract:** Cyanophage abundance has been shown to fluctuate over long timescales and with depth, but little is known about how it varies over short timescales. Previous short-term studies have relied on counting total virus numbers and therefore the phages which infect cyanobacteria cannot be distinguished from the total count. ¶ In this study, an isolation-based approach was used to determine cyanophage abundance from water samples collected over a depth profile for a 24h period from the Indian Ocean. Samples were used to infect *Synechococcus* sp. WH7803 and the number of plaque forming units (pfu) at each time point and depth were counted. At 10m phage numbers were similar for most time-points, but there was a distinct peak in abundance at 0100 hours. Phage numbers were lower at 25m and 50m and did not show such strong temporal variation. No phages were found below this depth. Therefore, we conclude that only the abundance of phages in surface waters showed a clear temporal pattern over a short timescale. Fifty phages from a range of depths and time points were isolated and purified. The molecular diversity of these phages was estimated using a section of the phage-encoded psbD gene and the results from a phylogenetic analysis do not suggest that phages from the deeper waters form a distinct subgroup.

26. Clokie,M.R.J., Mann,N.H. (2006). **Marine cyanophages and light.** *Environ. Microbiol.* 8:2074-2082. **Abstract:** In contrast to the phages of heterotrophic hosts, light can play a key role in all aspects of the life cycle of phages infecting ecologically important marine unicellular cyanobacteria of the genera *Synechococcus* and *Prochlorococcus*. Phage adsorption, replication, modulation of the host cell metabolism, and survival in the environment following lysis, all exhibit light-dependent components. The analysis of cyanophage genomes has revealed the acquisition of key photosynthetic genes during the course of evolution, such as those encoding central components of the light harvesting apparatus. These discoveries are beginning to reveal novel features of the interactions between parasite and host that shape the biology of both.

27. Comeau,A.M., Chan,A.M., Suttle,C.A. (2006). **Genetic richness of vibriophages isolated in a coastal environment.** *Environ. Microbiol.* 8:1164-1176. **Abstract:** The purpose of this study was to characterize *Vibrio parahaemolyticus* viruses (VpVs) isolated from different environments within and adjacent to the Strait of Georgia, and to examine the relative influences of distance and environment on host-range and genetic richness. Nearly all seawater enrichment cultures (29/31) generated isolates, implying that VpVs were widespread in the virioplankton, yet at low abundances (< 1 l(-1)). Viruses were not detected in sediments (n = 99). Fourteen of the 16 viruses characterized were siphoviruses, with genome sizes ranging from approximately 45-106 kb, and half were capable of infecting other *Vibrio* species. The VpVs infected bacteria isolated from oysters and sediments fairly well (55% and 46% of the host-virus combinations, respectively), but were unable to infect many of the bacteria isolated from the water column (< 13% of 112 combinations). When compared with VpVs from oysters, it was clear that the major determinant of phenotypic (host-range) and genetic richness (by the DP-RAPD assay) was not geography, but the source environment from which the VpVs originated. Therefore, the VpV population within the Strait of Georgia is a highly diverse mixture of phenotypes and genotypes.

28. Cornick,N.A., Helgerson,A.F., Mai,V., Ritchie,J.M., Acheson,D.W.K. (2006). **In vivo transduction of an Stx-encoding phage in ruminants.** *Appl. Environ. Microbiol.* 72:5086-5088. **Abstract:** We assessed the ability of a kanamycin-marked Stx phage to move into a commensal, ovine *Escherichia coli* strain in the ruminant gastrointestinal tract. Transduction was detected in 19/24 sheep tested, resulting in the recovery of 47 transductants. Subtherapeutic doses of the quinolone antibiotic enrofloxacin did not increase the rate of transduction.

29. Coward,C., Grant,A.J., Swift,C., Philp,J., Towler,R., Heydarian,M., Frost,J.A., Maskell,D.J. (2006). **Phase-variable surface structures are required for infection of *Campylobacter jejuni* by bacteriophages.** *Appl. Environ. Microbiol.* 72:4638-4647. **Abstract:** This study characterizes the interaction between *Campylobacter jejuni* and the 16 phages used in the United Kingdom typing scheme by screening spontaneous mutants of the phage-type strains and transposon mutants of the sequenced strain NCTC 11168. We show that the 16 typing phages fall into four groups based on their patterns of activity against spontaneous mutants. Screens of transposon and defined mutants indicate that the phage-bacterium interaction for one of these groups appears to involve the capsular polysaccharide (CPS), while two of the other three groups consist of flagellatropic phages. The expression of CPS and flagella is potentially phase variable in *C. jejuni*, and the implications of these findings for typing and intervention strategies are discussed.

30. Dabrowska,K., Switala-Jelen,K., Opolski,A., Weber-Dabrowska,B., Gorski,A. (2005). **Bacteriophage penetration in vertebrates.** *J. Appl. Microbiol.* 98:7-13. **Abstract:** Bacteriophages are viruses that infect bacteria. They are the most numerous life forms on earth. As antibiotic resistance is becoming an increasingly worldwide challenge, bacteriophages as potential antimicrobial agents are being more intensively explored. Some very important questions involve their ability to penetrate higher organisms, as this determines potential phage activity in antibacterial treatment. Higher organisms are widely exposed to bacteriophages, which penetrate them quite freely. Bacteriophage activity can be influenced by specific antibodies which, together with the nonspecific immune system, can contribute to their rapid clearance from the organism. Bacteriophages can also interact directly with mammalian cells and even play a role in the development of some nonbacterial diseases, although they are not able to multiply in these cells. All aspects of the interaction between phages and higher organism are of interest and importance for further medical and biochemical applications.

31. Davies,C.M., Logan,M.R., Rothwell,V.J., Krogh,M., Ferguson,C.M., Charles,K., Deere,D.A., Ashbolt,N.J. (2006). **Soil inactivation of DNA viruses in septic seepage.** *J. Appl. Microbiol.* 100:365-374. **Abstract:** AIMS: To generate field-relevant inactivation data for incorporation into models to predict the likelihood of viral contamination of surface waters by septic seepage. METHODS AND RESULTS: Inactivation rates were determined for PRD1 bacteriophage and Adenovirus 2 in two catchment soils under a range of temperature, moisture and biotic status regimes. Inactivation rates presented for both viruses were significantly different at different temperatures and in different soil types (alpha = 0.05). Soil moisture generally did not significantly affect virus inactivation rate. Biotic status significantly affected inactivation rates of PRD1 in the loam soil but not the clay-loam soil. Adenovirus 2 was inactivated more rapidly in the loam soil than PRD1 bacteriophage. CONCLUSIONS: Virus inactivation rates incorporated into models should be appropriate for the climate/catchment in question with particular regard to soil type and temperature. Given that PRD1 is similar in size to adenoviruses, yet more conservative with regard to inactivation in soil, it may be a useful surrogate in studies of Adenovirus fate and transport. SIGNIFICANCE AND IMPACT OF THE STUDY: A better understanding of the factors that govern virus fate and transport in catchments would facilitate the design of barrier measures to prevent viral contamination of surface waters by septic seepage.

32. Davis,J.A., Farrah,S.R., Wilkie,A.C. (2006). **Adsorption of viruses to soil: impact of anaerobic treatment.** *Water Sci. Technol.* 54:161-167. **Abstract:** The adsorption of viruses in untreated flushed dairy manure wastewater (FDMW), anaerobically digested flushed dairy manure wastewater (ADFDMW) and groundwater to sandy soil was investigated. Batch adsorption studies showed differential adsorption of viruses in groundwater to soil. Less than 75% of PRD1 and MS2 added to groundwater adsorbed after 1 h, but greater than 95% of ΦX174 and poliovirus 1 adsorbed to the soil. Adsorption differences in groundwater were related to the isoelectric points of the viruses. Suspending phages in untreated and treated wastewater reduced adsorption compared with groundwater. For MS2, more phages were adsorbed using ADFDMW than with FDMW. Adsorption of poliovirus 1 was not affected by FDMW and ADFDMW. Small column studies (6 x 2.5 cm) produced a similar trend in that adsorption was observed with groundwater and both FDMW and ADFDMW reduced virus adsorption. Groundwater, FDMW or ADFDMW did not affect the adsorption of poliovirus 1 in column studies. The major difference between FDMW and ADFDMW was in mobilisation of adsorbed viruses. The application of FDMW to soil columns with adsorbed viruses caused significantly more viruses to be mobilised than did the application of rainwater or ADFDMW. These results showed that treating FDMW by anaerobic digestion increased the adsorption of viruses to soil and decreased detachment of adsorbed viruses. As the potential for new zoonotic pathogens becomes known, the treatment of animal wastes may become mandatory. The assessment and management of viruses in manure for addressing possible risk to animal and human health is of interest.

33. Dawson,D.J., Paish,A., Staffell,L.M., Seymour,I.J., Appleton,H. (2005). **Survival of viruses on fresh produce, using MS2 as a surrogate for norovirus.** *J. Appl. Microbiol.* 98:203-209. **Abstract:** AIMS: To study the survival and removal of viruses from fresh fruit and vegetables using the bacteriophage MS2 as a potential surrogate for noroviruses. METHOD AND RESULTS: Survival of MS2 in buffer and on fresh produce was studied at 4, 8 and 22 degrees C. At 4 and 8 degrees C a reduction of <1 log10 was observed after 50 days in buffer; however a reduction in excess of 1 log10 occurred within 9 days at 22 degrees C. Similar results were obtained with fresh produce with virus survival times exceeding the shelf life of the produce. In washing experiments, using a chlorine wash (100 ppm), in all but one case <1.5 log10 MS2 bacteriophage was removed from fruit and vegetables. The mean across all produce types was 0.89 log10. With potable water, reduction was lower (0.3 log mean across all produce types). CONCLUSIONS: MS2 survived for prolonged periods, both in buffer and on fresh produce, at temperatures relevant to chilled foods. It was not removed effectively by chlorine washing. SIGNIFICANCE AND IMPACT OF THE STUDY: Bacteriophage MS2 has been evaluated as a potential surrogate for noroviruses on fresh produce. Experimental results together with current knowledge of norovirus resistance and survival indicate that MS2 could be used as an effective surrogate in future evaluations.

34. de Siqueira,R.S.**,** Dodd,C.E.R.**,** Rees,C.E.D. (2006). **Evaluation of the natural virucidal activity of teas for use in the phage amplification assay.** *Int. J. Food Microbiol.* 111:259-262. **Abstract:** Many natural products have intrinsic antimicrobial activity. In this study we have examined infusions from nine types of loose-leaf tea for their ability to inactivate bacteriophage, for use as an alternative to plant extract in a phage-based *Salmonella* detection assay. The results demonstrated that tea infusions, either freshly prepared or stored at 4 degrees C had virucidal action against two phages, Felix 01 and P22. Crucially, for use in the detection assay, there was no antibacterial effect of the virucide on the target bacteria. Therefore, tea was a good candidate to replace pomegranate as the virucidal agent in the phage amplification assay.

35. Dennehy,J.J.**,** Friedenberg,N.A.**,** Holt,R.D.**,** Turner,P.E. (2006). **Viral ecology and the maintenance of novel host use.** *Am. Nat.* 167:429-439. **Abstract:** Viruses can occasionally emerge by infecting new host species. However, the early phases of emergence can hinge upon ecological sustainability of the virus population, which is a product of both within-host population growth and between-host transmission. Insufficient growth or transmission can force virus extinction before the latter phases of emergence, where genetic adaptations that improve host use may occur. We examined the early phase of emergence by studying the population dynamics of RNA phages in replicated laboratory environments containing native and novel host bacteria. To predict the breadth of transmission rates allowing viral persistence on each species, we developed a simple model based on in vitro data for phage growth rate over a range of initial population densities on both hosts. Validation of these predictions using serial passage experiments revealed a range of transmission rates for which the native host was a source and the novel host was a sink. In this critical range of transmission rates, periodic exposure to the native host was sufficient for the maintenance of the viral population on the novel host. We argue that this effect should facilitate adaptation by the virus to utilize the novel host--often crucial in subsequent phases of emergence.

36. Desselberger,U. (2005). **Report on an ICTV-sponsored symposium on Virus Evolution.** *Arch. Virol.* 150:629-635. **Abstract:** A symposium on Virus Evolution, sponsored by the International Committee on Taxonomy of Viruses (ICTV), was held at the 23rd Annual Meeting of the American Society for Virology (ASV) in Montreal, Canada on July 10, 2004. It was organized by Ann Palmenberg (University of Madison-Wisconsin) and Andrew Ball (President, ICTV; University of Alabama at Birmingham) and was supported by Academic Press/Elsevier, Bristol Myers Squibb, The University of Alabama at Birmingham School of Medicine, US National Biodefense Analysis and Countermeasures Center,Wyeth Lederle Vaccines, and the ASV.

37. Duda,R.L.**,** Hendrix,R.W.**,** Huang,W.M.**,** Conway,J.F. (2006). **Shared architecture of bacteriophage SPO1 and herpesvirus capsids.** *Curr. Biol.* 16:R11-R13. **Abstract:** [first paragraph] Viruses have probably existed for as long as cells. Recent structural studies of viral capsids have revealed similarities that span the domains of life and point to distant evolutionary connections between viruses that pre-date the division of their host organisms into domains [1-3]. Comparisons of adenovirus and phage PRD1 demonstrate this emerging theme: these viruses share a unique T=25 capsid geometry with unusual 'trimeric hexons' and a common core fold for the major capsid proteins [4]. We describe a novel structural link between herpesviruses and the bacteriophage SPO1 revealed by cryo-electron microscopy (cryoEM) data showing that the SPO1 capsid has icosahedral geometry with triangulation number T=16, a value previously associated uniquely with herpesviruses, as well as an asymmetric capsid surface molecule reminiscent of the 'triplex' molecule of HSV-1. We propose that the similarities go deeper, to a common capsid protein core fold of the phage HK97 class [5]. The shared architecture suggests a common ancestor for herpesviruses and phage SPO1 and supports a distinct lineage for herpesviruses and the tailed phages.

38. Duplessis,M.**,** Levesque,C.M.**,** Moineau,S. (2006). **Characterization of *Streptococcus thermophilus* host range phage mutants.** *Appl. Environ. Microbiol.* 72:3036-3041. **Abstract:** To investigate phage-host interactions in *Streptococcus thermophilus*, a phage-resistant derivative (SMQ-301R) was obtained by challenging a Tn917 library of phage-sensitive strain *S. thermophilus* SMQ-301 with virulent phage DT1. Mutants of phages DT1 and MD2 capable of infecting SMQ-301 and SMQ-301R were isolated at a frequency of 10(-6). Four host range phage mutants were analyzed further and compared to the two wild-type phages. Altogether, three genes (orf15, orf17, and orf18) contained point mutations leading to amino acid substitutions and were responsible for the expanded host range. These three proteins were also identified in both phages by N-terminal sequencing and/or matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. The results suggest that at least three phage structural proteins may be involved in phage-host interactions in *S. thermophilus*.

39. Effantin,G.**,** Boulanger,P.**,** Neumann,E.**,** Letellier,L.**,** Conway,J.F. (2006). **Bacteriophage T5 structure reveals similarities with HK97 and T4 suggesting evolutionary relationships.** *J. Mol. Biol.* 361:993-1002. **Abstract:** Evolutionary relationships between viruses may be obscure by protein sequence but unmasked by structure. Analysis of bacteriophage T5 by cryo-electron microscopy and protein sequence analysis reveals analogies with HK97 and T4 that suggest a mosaic of such connections. The T5 capsid is consistent with the HK97 capsid protein fold but has a different geometry, incorporating three additional hexamers on each icosahedral facet. Similarly to

HK97, the T5 major capsid protein has an N-terminal extension, or Delta-domain that is missing in the mature capsid, and by analogy with HK97, may function as an assembly or scaffold domain. This Delta-domain is predicted to be largely coiled-coil, as for that of HK97, but is approximately 70% longer correlating with the larger capsid. Thus, capsid architecture appears likely to be specified by the Delta-domain. Unlike HK97, the T5 capsid binds a decoration protein in the center of each hexamer similarly to the "hoc" protein of phage T4, suggesting a common role for these molecules. The tail-tube has unusual trimeric symmetry that may aid in the unique two-stage DNA-ejection process, and joins the tail-tip at a disk where tail fibers attach. This intriguing mix of characteristics embodied by phage T5 offers insights into virus assembly, subunit function, and the evolutionary connections between related viruses.

40. Filee,J., Comeau,A.M., Suttle,C.A., Krisch,H.M. (2006). **[T4-type bacteriophages: ubiquitous components of the "dark matter" of the biosphere].** *Med. Sci.* 22:111-112.

41. Filee,J., Forterre,P. (2005). **Viral proteins functioning in organelles: a cryptic origin?** *Trends Microbiol.* 13:510-513. **Abstract:** Although mitochondria derive from alpha-proteobacteria, many proteins acting in this organelle did not originate from bacteria. In particular, phylogenetic evidence indicates that RNA polymerase, DNA polymerase and DNA primase--with homologues encoded by T3/T7-like bacteriophages--have replaced the ancestral proteins of bacterial origin. To date, there was no clear explanation for this puzzling observation. Bacterial genomics has now revealed the presence of cryptic prophages that are related to T3/T7 in several genomes of proteobacteria. We propose that such a prophage was present in the ancestral alpha-proteobacterium at the origin of mitochondria and that RNA polymerase, DNA polymerase and DNA primase encoded by this prophage replaced the original bacterial enzymes to function in mitochondria. Another T3/T7 viral-like RNA polymerase is functional in the chloroplast, indicating that a strong selection pressure has favored replacement of some cellular proteins by viral proteins in organelle evolution.

42. Filippini,M., Buesing,N., Bettarel,Y., Sime-Ngando,T., Gessner,M.O. (2006). **Infection paradox: high abundance but low impact of freshwater benthic viruses.** *Appl. Environ. Microbiol.* 72:4893-4898. **Abstract:** The discovery of an abundant and diverse virus community in oceans and lakes has profoundly reshaped ideas about global carbon and nutrient fluxes, food web dynamics, and maintenance of microbial biodiversity. These roles are exerted through massive viral impact on the population dynamics of heterotrophic bacterioplankton and primary producers. We took advantage of a shallow wetland system with contrasting microhabitats in close proximity to demonstrate that in marked contrast to pelagic systems, viral infection, determined directly by transmission electron microscopy, and consequently mortality of prokaryotes were surprisingly low in benthic habitats in all seasons. This was true even though free viruses were abundant throughout the year and bacterial infection and mortality rates were high in surrounding water. The habitats in which we found this pattern include sediment, decomposing plant litter, and biofilms on aquatic vegetation. Overall, we detected viruses in only 4 of a total of approximately 15,000 bacterial cells inspected in these three habitats; for comparison, nearly 300 of approximately 5,000 cells suspended in the water column were infected. The strikingly low incidence of impact of phages in the benthos may have important implications, since a major portion of microbial biodiversity and global carbon and nutrient turnover are associated with surfaces. Therefore, if failure to infect benthic bacteria is a widespread phenomenon, then the global role of viruses in controlling microbial diversity, food web dynamics, and biogeochemical cycles would be greatly diminished compared to predictions based on data from planktonic environments.

43. Fischetti,V.A., Nelson,D., Schuch,R. (2006). **Reinventing phage therapy: are the parts greater than the sum?** *Nat. Biotech.* 24:1508-1511. **Abstract:** Although whole phage continue to generate interest as an alternative to antibiotics, focus is shifting to the use of purified phage components as antibacterial agents.

44. Foppen,J.W.A., Okletey,S., Schijven,J.F. (2006). **Effect of goethite coating and humic acid on the transport of bacteriophage PRD1 in columns of saturated sand.** *J. Contam. Hydrol.* 85:287-301. **Abstract:** The transport of bacteriophage PRD1, a model virus, was studied in columns containing sediment mixtures of quartz sand with goethite-coated sand and using various solutions consisting of monovalent and divalent salts and humic acid (HA). Without HA and in the absence of sand, the inactivation rate of PRD1 was found to be as low as 0.014 day(-1) (at 5+/-3 degrees C), but in the presence of HA it was much lower (0.0009 day(-1)), indicating that HA helps PRD1 to survive. When the fraction of goethite in the sediment was increased, the removal of PRD1 also increased. However, in the presence of HA, C/C0 values of PRD1 increased by as much as 5 log units, thereby almost completely eliminating the effect of addition of goethite. The sticking efficiency was not linearly dependent on the amount of goethite added to the quartz sand; this is apparently due to surface charge heterogeneity of PRD1. Our results imply that, in the presence of dissolved organic matter (DOM), viruses can be transported for long distances thanks to two effects: attachment is poor because DOM has occupied favourable sites for attachment and

inactivation of virus may have decreased. This conclusion justifies making conservative assumptions about the attachment of viruses when calculating protection zones for groundwater wells.

45. Fraser,J.S.**,** Yu,Z.**,** Maxwell,K.L.**,** Davidson,A.R. (2006). **Ig-like domains on bacteriophages: a tale of promiscuity and deceit.** *J. Mol. Biol.* 359:496-507. **Abstract:** The immunoglobulin (Ig) fold is one of the most important structures in biology, playing essential roles in the vertebrate immune response, cell adhesion, and many other processes. Through bioinformatic analysis, we have discovered that Ig-like domains are often found in the constituent proteins of tailed double-stranded (ds) DNA bacteriophage particles, and are likely displayed on the surface of these viruses. These phage Ig-like domains fall into three distinct sequence families, which are similar to the classic immunoglobulin domain (I-Set), the fibronectin type 3 repeat (FN3), and the bacterial Ig-like domain (Big2). The phage Ig-like domains are very promiscuous. They are attached to more than ten different functional classes of proteins, and found in all three morphogenetic classes of tailed dsDNA phages. In addition, they reside in phages that infect a diverse set of gram negative and gram positive bacteria. These domains are deceptive because many are added to larger proteins through programmed ribosomal frameshifting, so that they are not always detected by standard protein sequence searching procedures. In addition, the presence of unrecognized Ig-like domains in a variety of phage proteins with different functions has led to gene misannotation. Our results demonstrate that horizontal gene transfer involving Ig-like domain encoding DNA has occurred commonly between diverse classes of both lytic and temperate phages, which otherwise display very limited sequence similarities to one another. We suggest that phage may have been an important vector in the spread of Ig-like domains through diverse species of bacteria. While the function of the phage Ig-like domains is unknown, several lines of evidence suggest that they may play an accessory role in phage infection by weakly interacting with carbohydrates on the bacterial cell surface.

46. Gamage,S.D.**,** Patton,A.K.**,** Strasser,J.E.**,** Chalk,C.L.**,** Weiss,A.A. (2006). **Commensal bacteria influence *Escherichia coli* O157:H7 persistence and Shiga toxin production in the mouse intestine.** *Infect. Immun.* 74:1977-1983. **Abstract:** The presence of commensal flora reduced colonization of *Escherichia coli* O157:H7 and production of Shiga toxin (Stx) in the murine intestine. Stx production was not detected in mice colonized with *E. coli* that were resistant to the Shiga toxin phage, but it was detected in mice colonized with phage-susceptible *E. coli*.

47. Goerke,C.**,** Koller,J.**,** Wolz,C. (2006). **Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in *Staphylococcus aureus*.** *Antimicrob. Agents Chemother.* 50:171-177. **Abstract:** In *Staphylococcus aureus* strains of human origin, phages which integrate into the chromosomal gene coding for β-hemolysin (*hlb*) are widely distributed. Most of them encode accessory virulence determinants such as staphylokinase (*sak*) or enterotoxins. Here, we analyzed the effects of ciprofloxacin and trimethoprim on phage induction and expression of phage-encoded virulence factors by using isolates from patients with cystic fibrosis for which the induction of *hlb*-converting phages was demonstrated in vivo (C. Goerke, S. Matias y Papenberg, S. Dasbach, K. Dietz, R. Ziebach, B. C. Kahl, and C. Wolz, J. Infect. Dis. 189:724-734, 2004) as well as a ϕ13 lysogen of phage-cured strain 8325-4. Treatment of lysogens with subinhibitory concentrations of either antibiotic resulted in (i) delysogenization of strains resembling the isolates picked up after chronic lung infection and (ii) replication of phages in the bacterial host in a dose-dependent manner. Ciprofloxacin treatment resulted in enhanced *recA* transcription, indicating involvement of the SOS response in phage mobilization. Induction of ϕ13 was linked to elevated expression of the phage-encoded virulence gene *sak*, chiefly due to the activation of latent phage promoters. In summary, we could show the induction of *hlb*-converting phages and a subsequent virulence modulation of the host bacterium by ciprofloxacin and trimethoprim.

48. Gons,H.J.**,** Hoogveld,H.L.**,** Simis,S.G.H.**,** Tijdens,M. (2006). **Dynamic modelling of viral impact on cyanobacterial populations in shallow lakes: implications of burst size.** *J. Mar. Bio. Assoc. UK* 86:537-542. **Abstract:** Laboratory experiments with whole water-columns from shallow, eutrophic lakes repeatedly showed collapse of the predominant filamentous cyanobacteria. The collapse could be due to viral activity, from the evidence of electron microscopy of infected cyanobacterial cells and observed dynamics of virus-like particles. Burst-size effects on single-host single-virus dynamics was modelled for nutrient-replete growth of the cyanobacteria and fixed viral decay rate in the water column. The model combined previously published equations for nutrient-replete cyanobacterial growth and virus–host relationship. According to the model results, burst sizes greater than 200 to 400 virions per cell would result in host extinction, whereas lower numbers would allow coexistence, and even stable population densities of host and virus. High-nutrient status of the host cells might accommodate a large burst size. The ecological implication could be that burst-size increase accompanying a transition from phosphorus to light-limited cyanobacterial growth might destabilize the virus–host interaction and result in the population collapse observed in the experiments.

49. Griffiths,W.D.**,** Bennett,A.**,** Speight,S.**,** Parks,S. (2005). **Determining the performance of a commercial air purification system for reducing airborne contamination using model micro-organisms: a new test methodology.** *J. Hosp. Infect.* 61:242-247. **Abstract:** The performance of a duct-mounted air disinfection system, designed to reduce airborne pathogens in the hospital environment, was determined using a new testing methodology. The methodology places the equipment in a test duct, a microbial aerosol is generated and then sampled simultaneously before and after the test system. This allows a percentage efficiency value to be calculated. The air disinfection system is a novel chemical-coated filter and ultraviolet (UV) radiation air purification system, operating at a flow rate of 500 m(3)/h, against aerosols of MS2 phage and *Mycobacterium vaccae* (surrogates of viral and mycobactericidal pathogens). A three UV lamp system was effective against airborne phages, removing an average of 97.34% of the aerosolized challenge. With the UV component switched off, the average efficiency dropped to 61.46%. This demonstrates that the chemical-coated filter component plays a more significant role than the UV radiation in destroying phages. When six UV lamps were used, the system was able to remove mycobacteria with an efficiency exceeding 99.99%. This test methodology can be used to assess manufacturers' claims of efficacy of equipment against airborne micro-organisms in the hospital environment.

50. Guan,D.**,** Kniel,K.**,** Calci,K.R.**,** Hicks,D.T.**,** Pivarnik,L.F.**,** Hoover,D.G. (2006). **Response of four types of coliphages to high hydrostatic pressure.** *Food Microbiol.* 23:546-551. **Abstract:** Pressure inactivation of four types of coliphages, φiX 174 (ssDNA virus), MS2 (ssRNA virus), λ imm434 (dsDNA virus) and T4 (dsDNA virus), was studied to evaluate their potential as human enteric viral surrogates for use in validation of commercial pressure processing treatments. Phage var φX 174 demonstrated an unexpected high resistance to pressure with no more than 1-log(10) reduction observed following exposures to 350-600 MPa. There was no greater than 1-log(10) reduction below 500 MPa for MS2 in modified phosphate-buffered saline, but a 3.3-log(10) reduction was observed for MS2 pressurized at 600 MPa. Coliphages λ imm434 and T4 were relatively sensitive to pressure in demonstrating inactivation at 350 MPa. At 21 degrees C, λ imm434 was inactivated in modified phosphate-buffered saline or Dulbecco's Modified Eagle's Medium plus 5% fetal bovine sera by at least 7.5-log(10) when exposed to 400 MPa for 5 min. Treatment at 450 MPa for 5 min was necessary to obtain a log(10) reduction of 6-7 for T4.

51. Guan,J.**,** Chan,M. **,** Allain,B.**,** Mandeville,R.**,** Brooks,B.W. (2006). **Detection of multiple antibiotic–resistant *Salmonella enterica* Serovar Typhimurium DT104 by phage replication–competitive enzyme-linked immunosorbent assay.** *J. Food Prot.* 739-742. **Abstract:** A phage replication-competitive enzyme-linked immunosorbent assay (PR-cELISA) was developed for the detection of multiple antibiotic-resistant *Salmonella* Typhimurium DT104. In the PR-cELISA procedure, a phage, BP1, was inoculated into a log-phase bacterial culture at a ratio of 1:100. After a 3-h incubation of the mixture, BP1 replication was measured by cELISA based on the competitive binding between BP1 and biotinylated BP1 to *Salmonella* Typhimurium smooth lipopolysaccharide. Among the 84 *Salmonella* strains and 9 non-*Salmonella* strains that were tested by PR-cELISA, BP1 detected 39 of 40 *Salmonella Typhimurium strains*, 2 of 10 *Salmonella* non-Typhimurium somatic group B strains, and 5 of 18 *Salmonella* somatic group D1 strains. With the addition of chloramphenicol to the culture medium, PR-cELISA detected all 27 multiple antibiotic-resistant *Salmonella* Typhimurium DT104 and none of the other *Salmonella* strains or non-*Salmonella* strains tested. The results demonstrated that PR-cELISA has potential applications for the detection of multiple antibiotic-resistant *Salmonella* Typhimurium DT104.

52. Guglielmotti,D.M.**,** Reinheimer,J.A.**,** Binetti,A.G.**,** Giraffa,G.**,** Carminati,D.**,** Quiberoni,A. (2006). **Characterization of spontaneous phage-resistant derivatives of *Lactobacillus delbrueckii* commercial strains.** *Int. J. Food Microbiol.* 111:126-133. **Abstract:** A total of 44 spontaneous phage-resistant mutants were isolated from three commercial *Lactobacillus delbrueckii* strains by secondary culture and agar plate methods. Phenotypic characteristics related to their phage-resistance capacities, i.e. plaquing efficiency, phage-resistance stability, lysogeny and adsorption rates were determined. The morphological, biochemical (sugar fermentation patterns) and technological (acidifying and proteolytic activities and acidification kinetics) properties of mutants were also studied. Amplification and restriction analysis of the 16S rRNA gene (PCR-ARDRA) was applied to confirm strain identity at the subspecies level. Random amplification of polymorphic DNA (RAPD-PCR) was used to determine genetic diversity among the isolates and their respective parent strains. The secondary culture method was the most useful for obtaining phage-resistant mutants. Phage resistance stability was a variable property among the isolates, but a high level of resistance was exhibited as quantified by the efficiency of plaquing. Furthermore, a total absence of spontaneous lysogeny was demonstrated. Adsorption rates were heterogeneously distributed among the three groups of mutants. All mutants isolated from two sensitive strains were similar to them with respect to technological properties. Two groups of mutants with distinctive technological properties were isolated from the other sensitive strain. PCR-ARDRA revealed that two out of three sensitive strains identified commercially as *Lb. delbrueckii* subsp. *bulgaricus* were actually *Lb. delbrueckii* subsp. *lactis*. Some of the phage-resistant mutants that were obtained might be used in culture rotation programs without regulatory restrictions when commercial strains become sensitive to phages present in industrial environments.

53. Hagens,S., Habel,A., Blasi,U. (2006). **Augmentation of the antimicrobial efficacy of antibiotics by filamentous phage.** *Microb. Drug Resist.* 12:164-168. **Abstract:** A significant increase in sensitivity to several antibiotics was observed in vitro after infection of the two *Pseudomonas aeruginosa* strains O1 and K with the filamentous phage Pf3 and Pf1, respectively. Moreover, upon infection with phage Pf1 a *P. aeruginosa* K strain harboring a plasmid-borne gentamicin resistance gene could be resensitized to the antibiotic. We further show that BALB/c mice were rescued from lethal infections with *P. aeruginosa* K by concomitant treatment with phage Pf1 and low concentrations of gentamicin, neither of which was able to cure the infection when administered alone.

54. Han,J., Jin,Y., Willson,C.S. (2006). **Virus retention and transport in chemically heterogeneous porous media under saturated and unsaturated flow conditions.** *Environ. Sci. Technol.* 40:1547-1555. **Abstract:** Retention and transport of colloids and microorganisms are complex processes, especially in the vadose zone due to the more complicated water flow regime and additional interfacial reactions involved. In this study, we examined the retention and transport behavior of two bacteriophages, MS-2 and ϕX174, in homogeneous and chemically heterogeneous media under variably saturated conditions. Column experiments with glass beads (treated to have either hydrophilic or hydrophobic surface properties) were conducted using a phosphate-buffered saline solution at different pore water ionic strengths ranging from 0.025 to 0.163 M. In columns packed with 100% hydrophilic glass beads, retention of the viruses increased with decreasing water content and increasing ionic strength, a result similar to those reported in the literature. However, greater retention of both MS-2 and ϕX174 was observed in saturated columns than in unsaturated columns packed with a 1:1 mixture of hydrophilic and hydrophobic glass beads, especially at high ionic strengths. This result contradicts the common belief that viruses (and colloids in general) are subject to greater removal in unsaturated media. Our study suggests that while the mechanisms controlling colloid interfacial interactions (i.e., attachment on solid-water and air-water interfaces and film straining) on the pore scale are relevant, nonuniform wetting conditions due to heterogeneous grain surface hydrophobicity can strongly influence water flow and phase interconnection. Under these conditions, hydrodynamic effects on the mesopore scale will dominate pore-scale interfacial reactions in controlling the extent of colloid retention and movement in unsaturated media.

55. Haramoto,E., Katayama,H., Oguma,K., Yamashita,H., Tajima,A., Nakajima,H., Ohgaki,S. (2006). **Seasonal profiles of human noroviruses and indicator bacteria in a wastewater treatment plant in Tokyo, Japan.** *Water Sci. Technol.* 54:301-308. **Abstract:** The seasonal profiles of microorganisms in raw sewage, secondary-treated sewage, and final effluent at a wastewater treatment plant in Tokyo, Japan, were quantitatively determined each month for one year, from July 2003 to June 2004. Human noroviruses, which were determined by real-time PCR, in raw sewage varied from 0.17-260 copies/mL for genotype 1 and from 2.4-1900 copies/mL for genotype 2, showing much higher values in winter, the epidemic season. The concentration of total coliforms, *Escherichia coli*, or F-specific phages in raw sewage was almost constant throughout the year. Human noroviruses of genotype 2 were removed most effectively (3.69 log10 on average) at the wastewater treatment plant, followed by *E. coli* (3.37 log10), total coliforms (3.05 loglo), F-specific phages (2.81 log10), and human noroviruses of genotype 1 (2.27 log10). The removal ratio of human noroviruses was almost constant, independent of the initial concentration of the viruses in raw sewage, which led to the increasing concentration of human noroviruses in final effluent in winter. None of the tested bacteria was judged to be a reliable indicator of human noroviruses in final effluent.

56. Hatfull,G.F., Pedulla,M.L., Jacobs-Sera,D., Cichon,P.M., Foley,A., Ford,M.E., Gonda,R.M., Houtz,J.M., Hryckowian,A.J., Kelchner,V.A., Namburi,S., Pajcini,K.V., Popovich,M.G., Schleicher,D.T., Simanek,B.Z., Smith,A.L., Zdanowicz,G.M., Kumar,V., Peebles,C.L., Jacobs,W.R.J., Lawrence,J.G., Hendrix,R.W. (2006). **Exploring the mycobacteriophage metaproteome: phage genomics as an educational platform.** *PLoS Genetics* 2:e92. **Abstract:** Bacteriophages are the most abundant forms of life in the biosphere and carry genomes characterized by high genetic diversity and mosaic architectures. The complete sequences of 30 mycobacteriophage genomes show them collectively to encode 101 tRNAs, three tmRNAs, and 3,357 proteins belonging to 1,536 "phamilies" of related sequences, and a statistical analysis predicts that these represent approximately 50% of the total number of phamilies in the mycobacteriophage population. These phamilies contain 2.19 proteins on average; more than half (774) of them contain just a single protein sequence. Only six phamilies have representatives in more than half of the 30 genomes, and only three-encoding tape-measure proteins, lysins, and minor tail proteins-are present in all 30 phages, although these phamilies are themselves highly modular, such that no single amino acid sequence element is present in all 30 mycobacteriophage genomes. Of the 1,536 phamilies, only 230 (15%) have amino acid sequence similarity to previously reported proteins, reflecting the enormous genetic diversity of the entire phage population. The abundance and diversity of phages, the simplicity of phage isolation, and the relatively small size of phage genomes support bacteriophage isolation and comparative genomic analysis as a highly suitable platform for discovery-based education.

57. Hausler,T. (2006). **Bug killers.** *Nat. Med.* 12:600-601. **Abstract:** Viruses that can kill bacteria were once wildly popular. Will the rising problem of antibiotic resistance bring them back? Thomas Häusler reports.

58. Hendrix,R.W. (2005). **Bacteriophage evolution and the role of phages in host evolution.** pp. 55-65 In Waldor,M.K., Friedman,D.I., and Adhya,S.L. (eds.), *Phages: Their Role in Bacterial Pathogenesis and Biotechnology*. ASM Press, Washington DC. **Abstract:** [first paragraph] The tailed double-stranded DNA bacteriophages have been evolving for perhaps 3 billion years or more, but it is only in very recent years that we have come to the beginning of a real understanding of the genetic mechanisms behind that evolution as well as an appreciation for the major role that phages have in the evolution of their bacterial hosts. Because the host range of a typical phage is narrow, estimates of the abundance of phages in the environment based on the number of plaques formed on a few bacterial strains that can be grown in the laboratory have been low by many orders of magnitude. It was only when environmental samples were examined directly by electron microscopy that it became clear not only that tailed phages are remarkably abundant in theenvironment but that they probably constitute a numerical majority of organisms on the planet. In the first such measurements (2), the concentration of particles with characteristic morphology of tailed phages in Norwegian fjord water was about 10(7) per mel. Subsequent measurements from several other environmental sources have found similarly large, and in some cases, substantially larger numbers (31). Estimates of the total global population of phages can be made from these measurements and from the sizes of the different environmental compartments, and estimates are on the order of 10(31) total viral particles. This is a truly astronomical number, in that 10(31) tailed phages, laid end to end, would extend into space to a distance of 200 million light years. In all of the environmental samples examined, there were roughly 5 to 10 phage particles for every bacterial cell, which is the basis for the claim above that phage are a majority of the organisms on Earth.

59. Hertveldt,K.**,** Lavigne,R.**,** Pleteneva,E.**,** Sernova,N.**,** Kurochkina,L.**,** Korchevskii,R.**,** Robben,J.**,** Mesyanzhinov,V.**,** Krylov,V.N.**,** Volckaert,G. (2005). **Genome comparison of *Pseudomonas aeruginosa* large phages.** *J. Mol. Biol.* 354:536-545. **Abstract:** *Pseudomonas aeruginosa* phage EL is a dsDNA phage related to the giant ϕKZ-like Myoviridae. The EL genome sequence comprises 211,215 bp and has 201 predicted open reading frames (ORFs). The EL genome does not share DNA sequence homology with other viruses and micro-organisms sequenced to date. However, one-third of the predicted EL gene products (gps) shares similarity (Blast alignments of 17-55% amino acid identity) with ϕKZ proteins. Comparative EL and ϕKZ genomics reveals that these giant phages are an example of substantially diverged genetic mosaics. Based on the position of similar EL and phiKZ predicted gene products, five genome regions can be delineated in EL, four of which are relatively conserved between EL and ϕKZ. Region IV, a 17.7 kb genome region with 28 predicted ORFs, is unique to EL. Fourteen EL ORFs have been assigned a putative function based on protein similarity. Assigned proteins are involved in DNA replication and nucleotide metabolism (NAD+-dependent DNA ligase, ribonuclease HI, helicase, thymidylate kinase), host lysis and particle structure. EL-gp146 is the first chaperonin GroEL sequence identified in a viral genome. Besides a putative transposase, EL harbours predicted mobile endonucleases related to H-N-H and LAGLIDADG homing endonucleases associated with group I intron and intein intervening sequences.

60. Hewson,I.**,** Winget,D.M.**,** Williamson,K.E.**,** Fuhrman,J.A.**,** Wommack,K.E. (2006). **Viral and bacterial assemblage covariance in oligotrophic waters of the West Florida Shelf (Gulf of Mexico).** *J. Mar. Bio. Assoc. UK* 86:591-603. **Abstract:** Viruses are hypothesized to cause enhanced diversity in bacterial communities by regulating the outcome of intertaxon competition. However, concomitant documentation of viral and bacterial assemblage composition in oligotrophic waters are rare, particularly in situ over time, and there is almost no information on the temporal variability in virioplankton assemblage composition in oligotrophic water masses. Assemblage composition of viruses (via pulsed-field gel electrophoresis, PFGE) and bacteria (via automated rRNA intergenic spacer analysis, ARISA) was compared during surface lagrangian drifter deployments in the oligotrophic Gulf of Mexico during summer 2001, 2002, and 2003. In vertical profile, viruses and bacteria both had maximum abundances in surface waters, which decreased with depth; however, the richness of their assemblages was not significantly different between depths, suggesting independence of biomass and diversity. Viral assemblages changed rapidly (0.17-0.32 Jaccard index d-1), which was similar to the rate of change in bacterial assemblages reported in surface waters. Patterns of viral and bacterial assemblage composition were significantly related ($P<0.001$, $r=0.58$ between node ranks), and both assemblages clustered primarily by year and then by depth. These cultivation-independent observations demonstrate relationships between viral and bacterial assemblages, which are dynamic in patches of open ocean water. Even at the relatively low phylogenetic resolution of the ARISA and PFGE methods, the results support the idea that viruses may influence the species composition of host assemblages.

61. Hewson,I.**,** Fuhrman,J.A. (2006). **Viral impacts upon marine bacterioplankton assemblage structure.** *J. Mar. Bio. Assoc. UK* 86:577-589. **Abstract:** This study examined the relationship between viral infection and the

richness, diversity and composition of bacterial assemblages in the water column. Viruses were enriched by ultrafiltration, added to water column incubation experiments at 15 locations in the North Atlantic, North Pacific, Gulf of Mexico and Southern California. In a separate experiment, viruses were removed from bacterioplankton by diafiltration at the San Pedro Ocean Time Series Station. Bacterial assemblage composition was observed using a high throughput and sensitive molecular fingerprinting analysis, automated rRNA intergenic spacer analysis (ARISA). Diazotrophs were used as a model functional group to represent rare organisms hypothesized to benefit from viral activity, and their richness and diversity was determined by terminal restriction fragment length polymorphism of a nitrogenase gene fragment (*nifH*). The enrichment and removal experiments demonstrated mixed impacts of viral pressure upon bacterial communities, and we observed significant effects of viruses on several microbial parameters in all but two experiments. However, there was no consistent response of viral enrichment on total bacterial and diazotroph assemblages at stations with similar environmental conditions, suggesting that untested variables, small spatial scale factors, or stochastic processes influence the outcome of viral activities. Across all experiments, the relative abundance of the more common operational taxonomic units (OTUs) in fingerprints were not significantly impacted compared to the abundance of rare OTUs. These data indicate that viruses may have significant influence upon community structure of bacterioplankton; however, effects were not consistent between sampling locations nor water masses.

62. Hijnen,W.A.M.**,** Brouwer-Hanzens,A.J.**,** Charles,K.J.**,** Medema,G.J. (2005). **Transport of MS2 phage, *Escherichia coli*, *Clostridium perfringens*, *Cryptosporidium parvum*, and *Giardia intestinalis* in a gravel and a sandy soil.** *Environ. Sci. Technol.* 39:7860-7868. **Abstract:** To define protection zones around groundwater abstraction wells and safe setback distances for artificial recharge systems in water treatment, quantitative information is needed about the removal of microorganisms during soil passage. Column experiments were conducted using natural soil and water from an infiltration site with fine sandy soil and a river bank infiltration site with gravel soil. The removal of phages, bacteria, bacterial spores, and protozoan (oo)-cysts was determined at two velocities and compared with field data from the same sites. The microbial elimination rate (MER) in both soils was generally >2 log, but MER in the gravel soil was higher than that in the fine sandy soil. This was attributed to enhanced attachment, related to higher metal-hydroxides content. From the high sticking efficiencies (>1) and the low influence of flow rate on MER it was deduced that straining played a significant role in the removal of *Escherichia coli* and *Cryptosporidium parvum* oocysts in the gravel soil. Lower removal of oocysts than the 4-5 times smaller *E. coli* and spores in the fine sand indicates that the contribution of straining is variable and needs further attention in transport models. Thus, simple extrapolation of grain size and particle size to the extent of microbial transport underground is inappropriate. Finally, the low MER of indigenous *E. coli* and *Clostridium perfringens* observed in the soil columns as well as under field conditions and the second breakthrough peak found for *Cryptosporidium* and spores in the fine sandy soil upon a change in the feedwater pH indicate a significant role of detachment and retardation to microbial transport and the difficulty of extrapolation of quantitative column test results to field conditions.

63. Hill,E. (2006). **The cyanophage molecular mixing bowl of photosynthesis genes.** *PLoS Biol.* 4:e264. **Abstract:** [first paragraph] Among the wealth of microbial organisms inhabiting marine environments, cyanobacteria (blue-green algae) are the most abundant photosynthetic cells. *Prochlorococcus* and *Synechococcus*, the two most common cyanobacteria, account for 30% of global carbon fixation (through the photosynthetic process in which sugars are manufactured from carbon dioxide and water). By drawing on natural resources, these microbes use photosystems (PS) I and II (the two reaction centers in photosynthesis) to harness energy.

64. Hillier,K. (2006). **Babies and bacteria: phage typing, bacteriologists, and the birth of infection control.** *Bull. Hist. Med.* 80:733-761. **Abstract:** During the 1950s, *Staphylococcus aureus* became a major source of hospital infections and death, particularly in neonates. This situation was further complicated by the fact that *Staphylococcus* quickly gained resistance to most antibiotics. Controlling these infections was a pressing concern for hospital workers, especially bacteriologists who tackled it through the use of a new epidemiologic tool: phage typing. This article argues that during the mid- to late 1950s a series of staphylococcal hospital and nursery epidemics united phage typers, brought international recognition to the usefulness of their technique, and, in the process, contributed to the establishment of the new field of infection control. Through the use of this new tool, phage typers established themselves as experts in infection control and, in some places, became essential members of newly formed infection-control committees. The nursery epidemics represent a particularly important test for phage typing and infection control, for this staphylococcal strain (80/81) was especially virulent and spread rapidly beyond the hospital to the wider community. The epidemiologic information provided by phage typers was vital for devising practical advice on how to control this deadly strain of *Staphylococcus* and also for transforming the role of the hospital bacteriologist from mere technician into infection-control expert.

65. Huff,W.E.**,** Huff,G.R.**,** Rath,N.C.**,** Donoghue,A.M. (2006). **Evaluation of the influence of bacteriophage titer on the treatment of colibacillosis in broiler chickens.** *Poult. Sci.* 85:1373-1377. **Abstract:** Two studies were

conducted to determine the efficacy of bacteriophage SPR02 and DAF6 at varying titers to treat colibacillosis in chickens. In Study 1, the treatments consisted of a control, i.m. injection of bacteriophage SPR02 or DAF6, *Escherichia coli* airsac challenge, and *E. coli* challenge followed by treatment at different titers with bacteriophage SPR02 or DAF6. The *E. coli*- challenged birds were injected with 6 x 10(4) cfu into the left thoracic airsac at 7 d of age. Immediately after the birds were challenged with *E. coli*, they were treated by administration of bacteriophage SPR02 or DAF6 by i.m. injection into the left thigh with 4 x 10(8), 10(6), 10(4), or 10(2) pfu. Study 2 was identical to Study 1, with the exception that the *E. coli* challenge was increased to 9 x 10(4) cfu, and the titers of SPR02 and DAF6 were slightly less at 3 x 10(8), 10(6), 10(4), and 10(2) pfu. Both studies were concluded when the birds were 3 wk of age. Mortality in the birds challenged with *E. coli* in Studies 1 and 2 was 48 and 47%, respectively. The only consistently effective bacteriophage treatment was the highest titer (10(8) pfu) of bacteriophage SPR02, which significantly reduced mortality from 48 and 47% in the birds only challenged with *E. coli* (positive control) to 7% in both studies, which was not significantly different from the unchallenged negative control treatments. These studies indicate that an effective multiplicity of infection for i.m. treatment with SPR02 was 10(4) in this experimental model of colibacillosis. Bacteriophage administered at sufficient titers can be effective therapeutic agents and provide an alternative to antibiotics in the treatment of bacterial diseases.

66. Hutchison,M.L.**,** Thomas,D.J.I.**,** Walters,L.D.**,** Avery,S.M. (2006). **Shiga toxin-producing *Escherichia coli*, faecal coliforms and coliphage in animal feeds.** *Lett. Appl. Microbiol.* 43:205-210. **Abstract:** AIMS: Animal feeds (n = 226), collected from pastures or feeding troughs on UK farms and from feed manufacturers' bulk stores, were analysed for *Escherichia coli* harbouring shiga-toxin genes (stx), faecal coliforms, coliphages and stx-harbouring bacteriophages. METHODS AND RESULTS: Samples comprised of 79 fresh grasses, 26 silages and 121 dried or heat-processed feeds (DPF). Five of the 79 (6.3%) fresh grass samples contained stx(2)-*E. coli*. stx-*E. coli* were not detected in the silages or DPF that were examined. Faecal coliforms were detected in 75/79 (94.9%) of fresh grasses, 19/26 (73.1%) of silages and 36/121 (29.8%) of processed feeds. Coliphages were detected in 63/79 (79.7%) and 18/26 (69.2%) of fresh grasses and silages, respectively. Coliphages were isolated at a significantly lower prevalence of 5% (6/121) from processed feeds. Although stx(2)-phage was isolated from the enrichment of a single grass sample, stx-phages were not detected in any of the silage or processed feeds. We did not detect stx(1)-phage in any of the samples collected. CONCLUSIONS: Pastures have the potential to act as transmission vectors for stx-harbouring *E. coli* for grazed livestock. SIGNIFICANCE AND IMPACT OF THE STUDY: This is the first study to report on the prevalence of *E. coli* harbouring stx genes, faecal coliforms, coliphages and stx-harbouring bacteriophages in a range of feedstuffs destined for consumption by UK livestock. This study provides information on the risk of feeds to the spread of stx-phages between livestock and/or the environment.

67. Irfan,S.**,** Hasan,R.**,** Kanji,A.**,** Hassan,Q.**,** Azam,I. (2006). **Evaluation of a microcolony detection method and phage assay for rapid detection of *Mycobacterium tuberculosis* in sputum samples.** *S. E. Asian J. Trop. Med. Pub. Health* 37:1187-1195. **Abstract:** Early and rapid diagnosis of tuberculosis is necessary for both treatment and control of the disease. This study evaluated two microcolony observation techniques based on liquid and solid media and a mycobacteriophage assay, to evaluate their effectiveness in the diagnosis of pulmonary TB compared with a standard culture (BACTEC 460 and LJ medium). Middlebrook7H9 (M7H9) broth based on microcolony determination detected 57/61 positives cultures (n = 200) with a sensitivity of 93.4% and a specificity of 87.1%. M7H11 agar detected 57/62 positive cultures (n = 198) with a sensitivity of 91.9% and a specificity of 89.7%. The mycobacteriophage assay detected 98/143 (68.5%) of positive samples. The time to positivity was 48 hours in the mycobacteriophage assay versus 7 days in both the M7H9 broth and M7H11 agar. The costs in comparison with the culture (BACTEC 460 and LJ) were 33% and 48% for the microcolony and mycobacteriophage methods, respectively. Microcolony methods were rapid and cost effective compared to standard cultures. The mycobacteriophage assay, despite its lower sensitivity, has a short turn around time, and may be recommended as a screening test in countries with a low prevalence of tuberculosis.

68. Jain,R.**,** Knorr,A.L.**,** Bernacki,J.**,** Srivastava,R. (2006). **Investigation of bacteriophage MS2 viral dynamics using model discrimination analysis and the implications for phage therapy.** *Biotechnol. Prog.* 22:1650-1658. **Abstract:** Lytic phages infect their bacterial hosts, use the host machinery to replicate, and finally lyse and kill their hosts, releasing progeny phages. Various mathematical models have been developed that describe these phage-host viral dynamics. The aim of this study was to determine which of these models best describes the viral dynamics of lytic RNA phage MS2 and its host *Escherichia coli* C-3000. Experimental data consisted of uninfected and infected bacterial cell densities, free phage density, and substrate concentration. Parameters of various models were either determined directly through other experimental techniques or estimated using regression analysis of the experimental data. The models were evaluated using a Bayesian-based model discrimination technique. Through model discrimination it was shown that phage-resistant cells inhibited the growth of phage population. It was also shown that the uninfected bacterial population was a quasispecies consisting of phage-sensitive and phage-resistant bacterial cells. When there was a phage attack the phage-sensitive cells died out and the phage-resistant cells were selected for and became the dominant strain of the bacterial population.

69. Jenkins,C.A.**,** Hayes,P.K. (2006). **Diversity of cyanophages infecting the heterocystous filamentous cyanobacterium *Nodularia* isolated from the brackish Baltic Sea.** *J. Mar. Bio. Assoc. UK* 86:529-536. **Abstract:** A collection of 17 cyanophage isolates able to infect the heterocystous, filamentous cyanobacterium *Nodularia spumigena* has been established from the Baltic Sea. These cyanophages have been characterized based on their morphology, cross infectivity and genetic structure. Short fragments (450bp) of the gene encoding the major capsid protein (g23) were amplified and sequenced from several isolates, and the encoded protein was found to be 99% identical across all the *N. spumigena*-specific cyanophages tested. These results suggest that the *Nodularia*-specific cyanophages are very closely related. However, these cyanophages were found to be diverse in terms of their morphology and host range. Cyanophages belonging to two families within the order Caudovirales, Myoviridae and Siphoviridae, were included in the collection of isolates. The cyanophage particles are large in comparison with cyanophages previously isolated from the marine environment, with the largest capsid measuring 127×122×888nm. Host ranges of the cyanophage isolates varied, some being able to infect up to five genotypically distinct strains of *Nodularia spumigena*, while others were very specific, infecting only one strain. We conclude that *Nodularia*-specific cyanophages form a diverse community in surface waters during summer and autumn months and that they may play a role both in the transfer of genetic information between *Nodularia* lineages and in promoting changes in the genetic structure of the host population.

70. Jiao,N.**,** Zhao,Y. **,** Luo,T.**,** Wang,X. (2006). **Natural and anthropogenic forcing on the dynamics of virioplankton in the Yangtze river estuary.** *J. Mar. Bio. Assoc. UK* 86:543-550. **Abstract:** Seasonal investigation of virus dynamics by flow cytometry was conducted in the Yangtze river estuarine area in April, August, November 2002 and February 2003, and a supplemental investigation in the inner estuary and downstream of the river was conducted in October 2005. The majority of the total viral abundance was bacteriophage and only 5.4% of the total was algal virus. Total viral abundance varied with season and location, ranging from 6.75×105-1.68×107 particles/ml, and the virus:bacterium ratio (VBR) ranged from 1.52 to 72.02 with a mean of 8.7. In the present study, viral abundance peaked in both the summer and the winter, unlike the typical seasonal pattern reported in the literature, in which viral abundance peaks in the summer when bacterial hosts are also at their most abundant. However, the driving forces for the two peaks reported here were totally different, the summer viral abundance peak coupled with the development of bacterial hosts which were controlled largely by temperature year-round and by trophic state occasionally, while the winter one seemed to be multi-factor controlled. The host-phage interaction was no longer predominant in control of the winter viral abundance as bacterial abundance was lowest in this season. The winter low temperature would help maintain a high viral abundance as high temperatures might increase viral inactivation and viral decay; the VBR peak values actually occurred in the winter. More importantly, the high virus-containing freshwater discharge in winter due to a higher proportion of anthropogenic sewage relative to low natural flooding in winter run-off, turned out to be the first factor contributing to the high winter viral abundance and VBR values. In addition, the variation of intrusion of warm and relatively oligotrophic water from oceanic currents played a role alternating the distribution patterns of temperature, salinity and trophic conditions and consequently the distribution patterns of virus and bacteria seasonally and spatially. Dynamics of virus in the Yangtze river estuarine area is thus characterized by distinct seasonal and spatial variations due to natural forcing and by pronounced alternation of the regular patterns due to anthropogenic impacts.

71. Joo,J.**,** Gunny,M. **,** Cases,M.**,** Hudson,P.**,** Albert,R.**,** Harvill,E. (2006). **Bacteriophage-mediated competition in *Bordetella* bacteria.** *Proc. R. Soc. Lond. B Biol. Sci.* 273:1843-1848. **Abstract:** Apparent competition between species is believed to be one of the principal driving forces that structure ecological communities, although the precise mechanisms have yet to be characterized. Here we develop a model system that isolates phage-mediated interactions by neutralizing resource competition with a large excess of nutrients, and consists of two genetically identical *Bordetella* strains that differ only in that one is the carrier of phage and the other is susceptible to the phage. We observe and quantify the competitive advantage of the bacterial strain bearing the prophage in both invading and in resisting invasion by the bacterial strain sensitive to the phage, and use our experimental measurements to develop a mathematical model of phage-mediated competition. The model predicts, and experimental evidence confirms, that the competitive advantage conferred by the lysogenic phage depends only on the phage pathology on the sensitive bacterial strain and is independent of other phage and host parameters, such as the infection-causing contact rate, the spontaneous and infection-induced lysis rates and the phage burst size. This work combines experimental and mathematical approaches to the study of phage-driven competition, and provides an experimentally tested framework for evaluation of the effects of pathogens/parasites on interspecific competition.

72. Karam,J.D. (2005). **Bacteriophages: the viruses for all seasons of molecular biology.** *Virol. J.* 2:19. **Abstract:** Bacteriophage research continues to break new ground in our understanding of the basic molecular mechanisms of gene action and biological structure. The abundance of bacteriophages in nature and the diversity of their genomes

are two reasons why phage research brims with excitement. The pages of Virology Journal will reflect the excitement of the "New Phage Biology.".

73. Kasman,L.M. (2005). **Barriers to coliphage infection of commensal intestinal flora of laboratory mice.** *Virol. J.* 2:34. **Abstract:** BACKGROUND: Growth characteristics of coliphage viruses indicate that they are adapted to live with their *Eschericia coli* hosts in the intestinal tract. However, coliphage experimentally introduced by ingestion persist only transiently if at all in the gut of humans and other animals. This study attempted to identify the barriers to long term establishment of exogenous coliphage in the gastrointestinal (GI) tracts of laboratory mice. Intestinal contents were screened for the presence of coliphage and host bacteria, and strains of *E. coli* bacteria from different segments of the GI tract were tested for susceptibility to six common laboratory coliphages. RESULTS: Contrary to expectations, coliphage were not evident in the GI tracts of laboratory mice, although they were occasionally detected in feces. Commensal flora showed extreme variability within groups of mice despite identical handling and diet. Less than 20% of 48 mice tested carried *E. coli* in their gut, and of 22 commensal *E. coli* strains isolated and tested, 59% were completely resistant to infection by lambda, M13, P1, T4, T7, and PhiX174 coliphage. Lysogeny could not be demonstrated in the commensal strains as mitomycin C failed to induce detectable phage. Pre-existing immunity to phages was not evident as sera and fecal washes did not contain significant antibody titers to six laboratory phage types. CONCLUSION: Lack of sufficient susceptible host bacteria seems to be the most likely barrier to establishment of new coliphage infections in the mouse gut.

74. Khayat,R., Tang,L., Larson,E.T., Lawrence,C.M., Young,M., Johnson,J.E. (2005). **Structure of an archaeal virus capsid protein reveals a common ancestry to eukaryotic and bacterial viruses.** *Proc. Natl. Acad. Sci. USA* 102:18944-18949. **Abstract:** Archaea and their viruses are poorly understood when compared with the Eukarya and Bacteria domains of life. We report here the crystal structure of the major capsid protein (MCP) of the Sulfolobus turreted icosahedral virus, an archaeal virus isolated from an acidic hot spring (pH 2-4, 72-92 degrees C) in Yellowstone National Park. The structure is nearly identical to the MCP structures of the eukaryotic *Paramecium bursaria* Chlorella virus, and the bacteriophage PRD1, and shows a common fold with the mammalian adenovirus. Structural analysis of the capsid architecture, determined by fitting the subunit into the electron cryomicroscopy reconstruction of the virus, identified a number of key interactions that are akin to those observed in adenovirus and PRD1. The similar capsid proteins and capsid architectures strongly suggest that these viral capsids originated and evolved from a common ancestor. Hence, this work provides a previously undescribed example of a viral relationship spanning the three domains of life (Eukarya, Bacteria, and Archaea). The MCP structure also provides insights into the stabilizing forces required for extracellular hyperthermophilic proteins to tolerate high-temperature hot springs.

75. Kim,H.J., Kim,E.Y., Hong,Y., Rhee,J.H., Choy,H.E. (2006). **Alternative methods to limit extracellular bacterial activity for enumeration of intracellular bacteria.** *J. Microbiol. Meth.* 64:17-26. **Abstract:** The gentamicin survival assay, a method routinely used to estimate bacterial infection of eukaryotic host cells, depends on the presumed limited penetration of gentamicin across the eukaryotic cell membrane. However, some studies have suggested that gentamicin may in fact enter eukaryotic cells and kill intracellular bacteria. In this study we devised alternative methods to enumerate intracellular *Salmonellae* using a lytic bacteriophage, SP6, and an amino acid auxotroph, Pro- mutant, which replicates selectively within host cells in the presence of its uptake inhibitor, 3,4-dehydro-L-proline. The conventional gentamicin survival assay was systematically compared with the alternative methods for the enumeration of intracellular *Salmonellae*. We found that gentamicin decreases the survival of intracellular *Salmonellae* when added to extracellular media at concentrations above 20 microg/ml. The alternative methods do not suffer from this disadvantage, suggesting that they should be used to replace the gentamicin survival assay. In addition, the proline auxotroph method could be applied to detect bacterial release from host cells.

76. Ko,Y.T. (2005). **The receptor of an oyster juice-borne coliphage OJ367 in the outer membrane of *Salmonella derby*.** *J. Microbiol. Immunol. Infect. = Wei Mian Yu Gan Ran Za Zhi* 38:399-408. **Abstract:** The objective of this study was to identify the receptor of OJ367, an oyster juice-borne bacteriophage, in *Salmonella derby* ATCC 6960. The crude receptor outer membrane (OM) fraction was prepared and examined from the total cell envelope (TCE) by differential extraction with N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES)-MgCl2 and then with Triton-HEPES-ethylenediamine tetra-acetic acid buffers. The OM proteins (Omps) were isolated by diethylaminoethyl column chromatography to screen for receptor activity. A 45-kDa protein belonging to a minor Omp species, with phage neutralization ability, was eluted in a homogeneous form. It was a non-peptidoglycan-associated protein which was digestible by trypsin. Lipopolysaccharide had no influence on its receptor activity when coexistent in the diethylaminoethyl column fractions. An *S. derby* mutant resistant to lysis by phage OJ367 was isolated. The mutant not only showed decreased receptor activity in vitro when its TCE was tested but had an altered Omp profile. This implied that the 45-kDa Omp is involved as a receptor in coliphage binding; however, this role is affected by the expression of other Omps.

77. Korchak,G.I.**,** Skorokhod,I.N.**,** Surmasheva,E.V. (2006). **[Substantiation for the model value of somatic coliphage T2 in virological control of water preparation technology risk assessment].** *Gigiena i Sanitariia* 37-39.

78. Krylov,V.N.**,** Miller,S.**,** Rachel,R.**,** Biebl,M. **,** Pletneva,E.A.**,** Shuetz,M.**,** Krylov,S.V.**,** Shaburova,O.V. (2006). **[Ambivalent bacteriophages of different species active on *Escherichia coli* K12 and *Salmonella* sps. strains].** *Genetika* 42:159-168. **Abstract:** A study was made of several bacteriophages (including phages U2 and LB related to T-even phages of *Escherichia coli*) that grow both on *E. coli* K12 and on some *Salmonella* strains. Such phages were termed ambivalent. T-even ambivalent phages (U2 and LB) are rare and have a limited number of hosts among *Salmonella* strains. U2 and LB are similar to canonical *E. coli*-specific T-even phages in morphological type and size of the phage particle and in reaction with specific anti-T4 serum. Phages U2 and LB have identical sets of structural proteins, some of which are similar in size to structural proteins of phages T2 and T4. DNA restriction patterns of phages U2 and LB differ from each other and from those of T2 and T4. Still, DNAs of all four phages have considerable homology. Unexpectedly, phages U2 and LB grown on *Salmonella bungori* were unstable during centrifugation in a CsCl gradient. Ambivalent bacteriophages were found in species other than T-even phages and were similar in morphotype to lambdoid and other *E. coli* phages. One of the ambivalent phages was highly similar to well-known Felix01, which is specific for *Salmonella*. Ambivalent phages can be used to develop a new set for phage typing in *Salmonella*. An obvious advantage is that ambivalent phages can be reproduced in the *E. coli* K12 laboratory strain, which does not produce active temperate phages. Consequently, the resulting typing phage preparation is devoid of an admixture of temperate phages, which are common in *Salmonella*. The presence of temperate phages in phage-typing preparations may cause false-positive results in identifying specific *Salmonella* strains isolated from the environment or salmonellosis patients. Ambivalent phages are potentially useful for phage therapy and prevention of salmonellosis in humans and animals.

79. Lang,L.H. (2006). **FDA approves use of bacteriophages to be added to meat and poultry products.** *Gastroenterology* 131:1370. **Abstract:** [first paragraph] In the *Federal Register* of August 18, 2006, the US Food and Drug Administration (FDA) announced that it had approved the use of a bacteriophage preparation made from 6 individually purified phages (LMP-102) to be used on ready-to-eat (RTE) meat and poultry products as an antimicrobial agent against *Listeria monocytogenes*. The ruling came in response to a food additive petition submitted in 2002 from Intralytix, Inc. (Baltimore, MD), the biotech company that produces the bacteriophage.

80. Langmark,J.**,** Storey,M.V.**,** Ashbolt,N.J.**,** Stenstrom,T.A. (2005). **Biofilms in an urban water distribution system: measurement of biofilm biomass, pathogens and pathogen persistence within the Greater Stockholm Area, Sweden.** *Water Sci. Technol.* 52:181-189. **Abstract:** Distribution pipe biofilms can provide sites for the concentration of a wide range of microbial pathogens, thereby acting as a potential source of continual microbial exposure and furthermore can affect the aesthetic quality of water. In a joint project between Stockholm Water, the MISTRA "Sustainable Urban Water" program, the Swedish Institute for Infectious Disease Control and the Royal Technical University, Stockholm, the aim of the current study was to investigate biofilms formed in an urban water distribution system, and quantify the impact of such biofilms on potential pathogen accumulation and persistence within the Greater Stockholm Area, Sweden. When used for primary disinfection, ultra-violet (UV) treatment had no measurable influence on biofilm formation within the distribution system when compared to conventional chlorination. Biofilms produced within a model pilot-plant were found to be representative to those that had formed within the larger municipal water distribution system, demonstrating the applicability of the novel pilot-plant for future studies. Polystyrene microspheres (1.0 microm) and *Salmonella* bacteriophages demonstrated their ability to accumulate and persist within the model pilot-plant system, where the means of primary disinfection (UV-treatment, chlorination) had no influence on such phenomena. With the exception of aeromonads, potential pathogens and faecal indicators could not be detected within biofilms from the Stockholm water distribution system. Results from this investigation may provide information for water treatment and distribution management strategies, and fill key data gaps that presently hinder the refinement of microbial risk models.

81. Lee,L.H.**,** Lui,D. **,** Platner,P.J.**,** Hsu,S.F.**,** Chu,T.C.**,** Gaynor,J.J.**,** Vega,Q.C.**,** Lustigman,B.K. (2006). **Induction of temperate cyanophage AS-1 by heavy metal--copper.** *BMC Microbiol.* 6:17. **Abstract:** BACKGROUND: It has been reported that some marine cyanophage are temperate and can be induced from a lysogenic phase to a lytic phase by different agents such as heavy metals. However, to date no significant reports have focused on the temperate nature of freshwater cyanophage/cyanobacteria. Previous experiments with cyanophage AS-1 and cyanobacteria *Anacystis nidulans* have provided some evidence that AS-1 may have a lysogenic life cycle in addition to the characterized lytic cycle. RESULTS: In this study, the possible temperate *A. nidulans* was treated with different concentrations of heavy metal-copper. CuSO4 with concentrations of 3.1 x 10(-3) M, 3.1 x 10(-4) M, 3.1 x 10(-5) M and 3.1 x 10(-6) M were used to detect the induction of AS-1 from A. nidulans. The population of the

host, unicellular cyanobacteria *Anacystis nidulans*, was monitored by direct count and turbidity while the amount of virus produced was derived from plaque forming units (PFU) by a direct plating method. The ratio of AS-1 release from A. nidulans was also determined. From these results it appears that AS-1 lysogenic phage can be induced by copper at concentrations from 3.1 x 10(-6) M to 3.1 x 10(-4) M. Maximal phage induction occurred at 6 hours after addition of copper, with an optimal concentration of 3.1 x 10(-6) M. CONCLUSION: Cu2+ is a significant inducer for lysogenic cyanobacterial cells and consequently would be a potential control agent in the cyanobacteria population in fresh water ecosystems.

82. Leitet,C.**,** Riemann,L.**,** Hagström,Ä. (2006). **Plasmids and prophages in Baltic Sea bacterioplankton isolates.** *J. Mar. Bio. Assoc. UK* 86:567-575. **Abstract:** Plasmids and phages influence bacterial phenotype and may serve as vectors for transferring genes between bacteria. In the present study, we examined 130 marine bacterioplankton isolates for the presence of plasmids and prophages. Samples were obtained in spring, summer and autumn in the Baltic Sea proper. Plasmids and inducible prophages were found in 19% and 28% of the isolates, respectively. During spring, plasmids and prophages were 41-55% and 30% more common compared to the summer and autumn measurements and prevalence varied up to five-fold between bacterial phylogenetic groups, with the highest plasmid prevalence found in Bacteriodetes (41%), and lysogeny being common in α-, β-, and γ-Proteobacteria (32-50%). Plasmid genome sizes ranged from 1.5-15kb with most in the 2.1-4.0kb size-range. No plasmids showed identity to the broad-host-range incompatibility groups N and P. Phage genomes ranged in size from 8-87kb, with 57% being 35-45kb in size. Strain typing of phages with similar genome sizes by means of DP-RAPD (degenerated primer randomly amplified polymorphic DNA) showed that all were different (except two that were not resolved). In PFGE (pulsed-field gel electrophoresis) 34% of the lysates produced multiple bands. Transmission electron microscopy suggested that these originated from several phage morphotypes indicating that polylysogeny is common. The widespread distribution of small cryptic plasmids as well as of lysogeny and polylysogeny in Baltic Sea bacterioplankton may have important implications for bacterial phenotype and for lateral gene transfer; hence, the ecological significance of these vectors in marine environments requires further study.

83. Li,M.**,** Hu,H.Y.**,** Zhang,X.**,** Shen,H. (2006). **[Removal of coliphages by wastewater treatment processes].** *Huan Jing Ke Xue* 27:80-84. **Abstract:** The concentrations of somatic coliphages (SC) and F-specific RNA bacteriophages in effluent of three wastewater treatment plants in Beijing city were detected. Somatic coliphages and F-RNA bacteriophages in source wastewater were 6.25 x 10(3) - 1.34 x 10(4) PFU x mL(-1) and 2.4 x 10 - 2.4 x 10(3) PFU x mL(-1) respectively, and the corresponding average removal rates were 72.45% - 99.89 % and 57.84% - 93.06% by the wastewater processes, and which were lower than that of faecal coliforms. Biological aerated stage appeared to be the most efficient step in reducing the numbers of phages in wastewater, but not obviously in sand filter. The result of predicted concentrations of enteroviruses according to concentrations of F-RNA bacteriophages in water show that there are 0.65 - 15.8 PFU x L(-1) of the enteroviruses in final effluent.

84. Licis,N.**,** van Duin,J. (2006). **Structural constraints and mutational bias in the evolutionary restoration of a severe deletion in RNA phage MS2.** *J. Mol. Evol.* 63:314-329. **Abstract:** A 4-nucleotide (nt) deletion was made in the 36-nt-long intercistronic region separating the coat and replicase genes of the single-stranded RNA phage MS2. This region is the focus of several RNA structures conferring high fitness. One such element is the operator hairpin, which, in the course of infection, will bind a coat-protein dimer, thereby precluding further replicase synthesis and initiating encapsidation. Another structure is a long-distance base pairing (MJ) controlling replicase expression. The 4-nt deletion does not directly affect the operator hairpin but it disrupts the MJ pairing. Its main effect, however, is a frame shift in the overlapping lysis gene. This gene starts in the upstream coat gene, runs through the 36-nt-long intercistronic region, and ends in the downstream replicase cistron. Here we report and interpret the spectrum of solutions that emerges when the crippled phage is evolved. Four different solutions were obtained by sequencing 40 plaques. Three had cured the frame shift in the lysis gene by inserting one nt in the loop of the operator hairpin causing its inactivation. Yet these low-fitness revertants could further improve themselves when evolved. The inactivated operator was replaced by a substitute and thereafter these revertants found several ways to restore control over the replicase gene. To allow for the evolutionary enrichment of low-probability but high-fitness revertants, we passaged lysate samples before plating. Revertants obtained in this way also restored the frame shift, but not at the expense of the operator. By taking larger and larger lysates samples for such bulk evolution, ever higher-fitness and lower-frequency revertants surfaced. Only one made it back to wild type. As a rule, however, revertants moved further and further away from the wild-type sequence because restorative mutations are, in the majority of cases, selected for their capacity to improve the phenotype by optimizing one of several potential alternative RNA foldings that emerge as a result of the initial deletion. This illustrates the role of structural constraints which limit the path of subsequent restorative mutations.

85. Liu,B.**,** Wu,S.**,** Song,Q.**,** Zhang,X.**,** Xie,L. (2006). **Two novel bacteriophages of thermophilic bacteria isolated from deep-sea hydrothermal fields.** *Curr. Microbiol.* 53:163-166. **Abstract:** Bacteriophages of thermophiles are

of great interest due to their important roles in many biogeochemical and ecological processes. However, no virion has been isolated from deep-sea thermophilic bacteria to date. In this investigation, two lytic bacteriophages (termed *Bacillus* virus W1 and *Geobacillus* virus E1) of thermophilic bacteria were purified from deep-sea hydrothermal fields in the Pacific for the first time. *Bacillus* virus W1 (BVW1) obtained from *Bacillus* sp. w13, had a long tail (300nm in length and 15 nm in width) and a hexagonal head (70 nm in diameter). Another virus, *Geobacillus* virus E1 (GVE1) from *Geobacillus* sp. E26323, was a typical Siphoviridae phage with a hexagonal head (130 nm in diameter) and a tail (180 nm in length and 30 nm in width). The two phages contained double-stranded genomic DNAs. The genomic DNA sizes of BVW1 and GVE1 were estimated to be about 18 and 41 kb, respectively. Based on SDS-PAGE of purified virions, six major proteins were revealed for each of the two phages. The findings in our study will be very helpful to realize the effect of virus on thermophiles as well as the communities in deep-sea hydrothermal fields.

86. Lu,W.z., Yang,Q.x., Zhang,Y., Yang,M., Zhu,C.f. (2004). **[Inactivation of T4 phage in water environment using proteinase].** *Huan Jing Ke Xue* 25:93-96. **Abstract:** The inactivation effectiveness of proteinase to viruses was investigated by using T4 phage as a model virus. The results showed that the inactivation effectiveness of proteinase to T4 phage was obvious. In the optimum conditions and 67.5 u/mL concentration, the inactivation rate of proteinase K to T4 phage in sterilized water and in sewage achieved 99.4% and 49.4% respectively in an hour, and achieved >99.9% and 81.1% in three hours. The inactivation rate of the industrial proteinase 1398 to T4 phage in sterilized water achieved 74.4% in an hour. The effects of pH and temperature on the inactivation effectiveness was not evident.

87. Lu,Z., Altermann,E., Breidt,F., Predki,P., Fleming,H.P., Klaenhammer,T.R. (2005). **Sequence analysis of the *Lactobacillus plantarum* bacteriophage ΦJL-1.** *Gene* 348:45-54. **Abstract:** The complete genomic sequence of a *Lactobacillus plantarum* virulent phage ΦJL-1 was determined. The phage possesses a linear, double-stranded, DNA genome consisting of 36,677 bp with a G+C content of 39.36%. A total of 52 possible open reading frames (ORFs) were identified. According to N-terminal amino acid sequencing and bioinformatic analyses, proven or putative functions were assigned to 21 ORFs (41%), including 5 structural protein genes. The ΦJL-1 genome shows functionally related genes clustered together in a genome structure composed of modules for DNA replication, DNA packaging, head and tail morphogenesis, and lysis. This type of modular genomic organization was similar to several other phages infecting lactic acid bacteria. The structural gene maps revealed that the order of the head and tail genes is highly conserved among the genomes of several *Siphoviridae* phages, allowing the assignment of probable functions to certain uncharacterized ORFs from phage ΦJL-1 and other *Siphoviridae* phages.

88. Lucena,F., Ribas,F., Duran,A.E., Skraber,S., Gantzer,C., Campos,C., Moron,A., Calderon,E., Jofre,J. (2006). **Occurrence of bacterial indicators and bacteriophages infecting enteric bacteria in groundwater in different geographical areas.** *J. Appl. Microbiol.* 101:96-102. **Abstract:** AIMS: The aim of this research was to determine the suitability of coliphages (bacteriophages) for assessing the microbial quality of groundwater. METHODS AND RESULTS: The number of several bacterial indicators (faecal coliforms, *Escherichia coli*, enterococci and spores of sulfite-reducing clostridia) and bacteriophages (somatic coliphages, F-specific RNA bacteriophages and bacteriophages infecting *Bacteroides fragilis*) were determined in groundwater of aquifers in various geographical areas. Results show that the relative abundance, determined as percentages of positive detections, of the bacterial indicators and bacteriophages varies depending on the aquifer. CONCLUSIONS: A single bacterial indicator may not be enough to assess microbiological quality in certain aquifers. One bacterial indicator and a bacteriophage parameter provide more information than two bacterial indicators. SIGNIFICANCE AND IMPACT OF THE STUDY: Coliphages (CPH) provide different information from that provided by bacterial indicators on the microbial quality of groundwater in different geographical areas. Easy, fast and inexpensive methods for the detection of CPH are feasible in both industrialized and developing countries.

89. Lusiak-Szelachowska,M., Weber-Dabrowska,B., Gorski,A. (2006). **[The presence of bacteriophages in human feces and their potential importance].** *Polski merkuriusz lekarski* 21:381-383. **Abstract:** Bacteriophages are widely distributed throughout the environment as well as in the bodies of humans and animals (feces, urine, saliva, sputum). Higher presence of *Escherichia coli* phages compared with *Bacteroides fragilis* and *Salmonella* phages was noticed in the feces of healthy human individuals and patients, mainly those with gastro-intestinal tract diseases. A strict correlation exists between the number of bacteria and of phages in the feces of healthy individuals as well as of patients with different diseases. The presence of phages in human feces correlates with the character of the coexisting disease. The frequency of phages in the feces depends on the different indicator bacterial host strains and the numbers of indicator strains. The role of bacteriophages in protecting against pathogenic microorganisms and controlling bacterial flora in the human organism is of major significance.

90. Mahony,J., Deveau,H., Mc Grath,S., Ventura,M., Canchaya,C., Moineau,S., Fitzgerald,G.F., van Sinderen,D. (2006). **Sequence and comparative genomic analysis of lactococcal bacteriophages jj50, 712 and P008: evolutionary insights into the 936 phage species.** *FEMS Microbiol. Lett.* 261:253-261. **Abstract:** The complete genome sequences of three lactococcal 936-type bacteriophages, 712, jj50 and P008, were determined. Comparative genomic analysis of these phages with the previously sequenced 936-type phages, sk1 and bIL170, reveals a strict conservation of the overall genetic organization of this geographically diverse phage group. Genetic divergence was mainly observed in the early expressed region of the phage genomes, where a number of deletions, exchanges and insertions appear to have occurred. These genetic differences may be responsible for the observed differential sensitivity to the lactococcal DNA injection blocking protein, Sie(2009), and the abortive infection system, AbiA.

91. Mandilara,G., Mavridou,A., Lambiri,M., Vatopoulos,A., Rigas,F. (2006). **The use of bacteriophages for monitoring the microbiological quality of sewage sludge.** *Environ. Technol.* 27:367-375. **Abstract:** The use of bacteriophages as potential indicators of faecal pollution has recently been studied. The correlation of the number of bacterial indicators and the presence of three groups of bacteriophages, namely somatic coliphages (SOMCPH), F-RNA specific phages (FRNAPH) and phages of *Bacteroides fragilis* (BFRPH), in raw and treated sludge is presented in this study. Raw and anaerobically digested sewage sludge samples from two wastewater treatment plants in Athens were collected on a monthly basis, over a 2-year period, and analyzed for total coliforms, *E. coli*, intestinal enterococci and the three groups of bacteriophages. A clear correlation between the number of bacterial indicators and the presence of bacteriophages was observed. **E. coli** concentrations of > or =10(3) cfus g(-1) and <10(3) cfus g(-1) comprise a threshold for the presence of FRNAPH and BFRPH, respectively. Likewise, intestinal enterococci concentrations of > or =10(4) cfus g(-1) and <10(3) cfus g(-1) comprise a threshold for the presence of FRNAPH and BFRPH, respectively. In the case of SOMCPH, it was not possible to define a threshold, since they were detected with the lowest observed indicator concentrations in all samples.

92. Mandilara,G.D., Smeti,E.M., Mavridou,A.T., Lambiri,M.P., Vatopoulos,A.C., Rigas,F.P. (2006). **Correlation between bacterial indicators and bacteriophages in sewage and sludge.** *FEMS Microbiol. Lett.* 263:119-126. **Abstract:** The use of bacteriophages as potential indicators of faecal pollution has recently been studied. The correlation of the number of bacterial indicators and the presence of three groups of bacteriophages, namely somatic coliphages (SOMCPH), F-RNA-specific phages (FRNAPH) and phages of *Bacteroides fragilis* (BFRPH), in raw and treated wastewater and sludge is presented in this study. Raw and treated wastewater and sewage sludge samples from two wastewater treatment plants in Athens were collected on a monthly basis, over a 2-year period, and analysed for total coliforms, *Escherichia coli*, intestinal enterococci and the three groups of bacteriophages. A clear correlation between the number of bacterial indicators and the presence of bacteriophages was observed. SOMCPH may be used as additional indicators, because of their high densities and resistance to various treatment steps.

93. Marza,J.A.S., Soothill,J.S., Boydell,P., Collyns,T.A. (2006). **Multiplication of therapeutically administered bacteriophages in *Pseudomonas aeruginosa* infected patients.** *Burns* 32:644-646. **Abstract:** [first paragraph] Antibiotic resistant strains of bacteria are an increasing problem and *Pseudomonas aeruginosa* is one of the most resistant species [1]. No new classes of anti-pseudomonal agent have been developed for over 30 years. It is time to consider alternative strategies such as bacteriophage (phage) therapy.

94. McDaniel,L.D., delaRosa,M., Paul,J.H. (2006). **Temperate and lytic cyanophages from the Gulf of Mexico.** *J. Mar. Bio. Assoc. UK* 86:517-527. **Abstract:** The unicellular cyanobacterial species *Synechococcus* and *Prochlorococcus* are known to be vital components of marine ecosystems, especially in the vast oligotrophic areas. Lytic cyanophages infecting unicellular phytoplankton are prevalent and have been demonstrated to act as important constraints on community composition contributing to the seasonal succession in genotypes. Lysogeny in *Synechococcus* has been documented experimentally in natural environments by prophage induction. At this time it is completely unknown how prevalent lysogeny is among *Synechococcus* populations. This study was performed to document important features such as size, morphology and the incidence of the T4-like capsid portal protein gene (g20) in a group of lytic *Synechococcus* cyanophages (35 isolates) isolated from the Gulf of Mexico. A group of *Synechococcus* isolates (24 strains) were isolated concurrently to investigate the virulence and cross-infectivity of the lytic cyanophages and to determine the frequency of lysogeny by detection of inducible prophage. The host range of the cyanophages toward these *Synechococcus* strains ranged from 1 of 25 (host of isolation only) to 17 of 25 (68%). Of the 35 cyanophage isolates the large majority were myoviruses (94%) and only two (6%) were of the podovirus type. The expected polymerase chain reaction product for g20 was detected in 20 of the phages (63%). The presence of a detectable g20 was associated with low-infectivity cyanophages at the 90% confidence interval. The *Synechococcus* strains varied in their resistance to lytic infection from 11% to resistance to all of the phage isolates utilized in testing. The prevalence of inducible prophage-like particles was determined in the *Synechococcus* strains using mitomycin C and enumerating viruses by epifluorescence microscopy. A statistically

significant increase in viruses was detected in 11 of the strains (46%) in response to mitomycin C. There was no observed relationship between the occurrence of prophage induction in the *Synechococcus* isolates and their resistance to lytic infection. One putative lysogen was induced by continuous high light and contained a prophage-like particle with a single-stranded DNA (ssDNA) genome. Such a prophage-like particle is unlike any prophage described to date, implying that the process of lysogeny is unique in certain marine *Synechococcus* strains.

95. McLaughlin,M.R.**,** Balaa,M.F. (2006). **Enhanced contrast of bacteriophage plaques in *Salmonella* with ferric ammonium citrate and sodium thiosulfate (FACST) and tetrazolium red (TZR).** *J. Microbiol. Meth.* 65:318-323. **Abstract:** Visualization of bacteriophage plaques may be enhanced by addition of ferric ammonium citrate and sodium thiosulfate (FACST) or 2,3,5-triphenyltetrazolium chloride (tetrazolium red, TZR) to the soft agar layer of a traditional bacteriophage plaque assay. Background color from these reagents improved contrast between clear plaques and turbid host lawns in trypticase soy agar (TSA) plates. Enhancement by FACST is based on reaction with hydrogen sulfide gas (H2S) produced by some strains of bacteria and was tested here using H2S+ and H2S- strains of *Salmonella* enterica subsp. enterica with a bacteriophage (Podoviridae) isolated from swine lagoon effluent. Only the H2S+ strain produced dark brown-black color in FACST-amended agar. Both strains showed bright pinkish-red color in TZR-amended agar. Color intensity for both reagents decreased with decreasing concentrations of the reagents. Contrast in FACST-amended plates appeared greater than that with TZR, but diminished after 12 h, while contrast in TZR-amended plates remained constant. At the concentrations tested, neither reagent affected plaque counts in the H2S+ strain. The FACST should be useful in bacteriophage plaque assays with H2S+ strains of *Salmonella* and other H2S+ bacteria.

96. McLaughlin,M.R. (2006). **Factors affecting iron sulfide-enhanced bacteriophage plaque assays in *Salmonella*.** *J. Microbiol. Meth.* 67:611-615. **Abstract:** Reaction of ferric ions with hydrogen sulfide (H(2)S) enhances contrast of phage plaques in H(2)S+ *Salmonella*, but contrast diminishes in weak H(2)S+ strains. H(2)S was affected by concentrations of peptones, glucose, ferric ammonium citrate (FAC) and sodium thiosulfate (ST), and by FAC:ST ratio, temperature, pH, air, and host strain. Increasing peptone levels was most important for improving contrast in weak H(2)S+ strains.

97. McLaughlin,M.R.**,** Balaa,M.F.**,** Sims,J.**,** King,R. (2006). **Isolation of *Salmonella* bacteriophages from swine effluent lagoons.** *J. Environ. Qual.* 35:522-528. **Abstract:** Bacteriophages (phages) associated with *Salmonella* were collected from nine swine manure lagoons in Mississippi. Phages were isolated by an enrichment protocol or directly from effluent. For enrichment, chloroform-treated samples were filtered (0.22 mum) and selectively enriched by adding a cocktail of *Salmonella* strains in trypticase soy broth. After overnight incubation at 35 degrees C, chloroform was added and samples stored at 5 degrees C. Enriched samples were tested by double agar layer (DAL) plaque assay against individual *Salmonella* isolates. Phage titers of 2.9 x 10(8) to 2.1 x 10(9) plaque forming units (pfu) per mL were produced, but estimation of phage titers in lagoons was not possible. For direct isolation, effluent was clarified by centrifugation, filtered (0.22 microm), and used in DAL plaque assays to select single-plaque isolates for 15 *Salmonella* strains. Plaque counts varied among *Salmonella* strains and lagoons. The most sensitive strain for direct phage recovery was ATCC 13311. Phage titers estimated by direct isolation with ATCC 13311 ranged among lagoons from 12 to 148 pfu per mL. In limited host range tests, 66 isolates recovered by the enrichment protocol produced plaques only on Enteritidis and Typhimurium strains of *Salmonella* and none produced plaques on lagoon isolates of *Citrobacter*, *Escherichia*, *Proteus*, *Providencia*, or *Serratia*. Electron microscopy (EM) showed purified enrichment isolates had Podoviridae morphology (tailless 50-nm icosahedral heads with tail spikes). Electron microscopy of clarified concentrated effluent showed 5.5:1 tailless to tailed phages. The isolated phages have potential as typing reagents, specific indicators, and biocontrol agents of *Salmonella*.

98. McLeod,S.M.**,** Kimsey,H.H.**,** Davis,B.M.**,** Waldor,M.K. (2005). **CTXϕ and *Vibrio cholerae*: exploring a newly recognized type of phage-host cell relationship.** *Mol. Microbiol.* 57:347-356. **Abstract:** The genes encoding cholera toxin, one of the principal virulence factors of the diarrhoeal pathogen *Vibrio cholerae*, are part of the genome of CTXϕ, a filamentous bacteriophage. Thus, CTXϕ has played a critical role in the evolution of the pathogenicity of *V. cholerae*. Unlike the well-studied F pilus-specific filamentous coliphages, CTXϕ integrates site-specifically into its host chromosome and forms stable lysogens. Here we focus on the CTXϕ life cycle and, in particular, on recent studies of the mechanism of CTXϕ integration and the factors that govern lysogeny. These and other processes illustrate the remarkable dependence of CTXϕ on host-encoded factors.

99. Middelboe,M.**,** Jørgensen,N.O.G. (2006). **Viral lysis of bacteria: an important source of dissolved amino acids and cell wall compounds.** *J. Mar. Bio. Assoc. UK* 86:605-612. **Abstract:** Viral infection of bacteria causes release of dissolved organic matter (DOM), which is available for bacterial uptake. In aquatic environments, this virus-mediated transformation of living cells into dissolved and colloidal organic matter may be a quantitatively important process in the pelagic recycling of carbon and nutrients, but little is known about the amount, composition, or

bioavailability of viral lysates. By using a model system of a marine bacterium (*Cellulophaga* sp.) and a virus specific to this bacterium, the present study provides a first quantification of the input of dissolved free and combined amino acids (DFAA and DCAA) and bacterial cell wall compounds following viral lysis. The DCAA constituted 51-86% of the total virus-mediated organic carbon release of 1087-1825?gCl-1 (estimated biomass of the lysed bacteria), whereas DFAA and glucosamine each accounted for 2-3% of total lysate-C. The viral particles themselves constituted 4-6% of the released organic carbon, and altogether, the applied analyses thus identified 53-92% of the released lysates. Approximately 12% of the identified compounds were derived from bacterial cell wall peptidoglycan, including various D-isomers of DFAA and DCAA, glucosamine and diaminopimelic acid (DAPA). Although a portion of this cell wall material may have entered the pool of refractory material, a significant fraction of some peptidoglycan-derived components, e.g. 83% of the released D-DFAA, were removed from the dissolved phase during the last part of the incubations, suggesting that part of the cell wall material were utilized by the developing virus-resistant *Cellulophaga* population. Therefore, we suggest that virus-mediated DOM is a source of a variety of organic compounds, which contribute significantly to the pool of rapidly recycling material in the ocean.

100. Millard,A.D.**,** Mann,N.H. (2006). **A temporal and spatial investigation of cyanophage abundance in the Gulf of Aqaba, Red Sea.** *J. Mar. Bio. Assoc. UK* 86:507-515. **Abstract:** The aim of this study was to determine the abundance of cyanophages over an annual cycle in the Red Sea from the period April 1999 to December 1999 at a range of depths. Cyanophage numbers from 71 water samples were determined by the use of plaque assays using four different *Synechococcus* strains. The results indicate that cyanophage are found throughout the water column from surface waters to depths of 150m, with a discrete maximum in the number of cyanophages in the summer months of July, August and September at a depth of 30m. Eighty-seven cyanophages were isolated and characterized in terms of host range, genome size and possession of a myoviral portal vertex gene. Cyanophages were found to infect multiple strains of Synechococcus from different phylogenetic clades. The genome sizes of cyanophages were also found to be bigger than previously estimated.

101. Morgan,A.D.**,** Buckling,A. (2006). **Relative number of generations of hosts and parasites does not influence parasite local adaptation in coevolving populations of bacteria and phages.** *J. Evol. Biol.* 19:1956-1963. **Abstract:** A potential consequence of host-parasite coevolution in spatially structured populations is parasite local adaptation: local parasites perform better than foreign parasites on their local host populations. It has been suggested that the generally shorter generation times of parasites compared with their hosts contributes to parasites, rather than hosts, being locally adapted. We tested the hypothesis that relative generation times of hosts and parasites affect local adaptation of hosts and parasites, using the bacterium *Pseudomonas fluorescens* and a lytic phage as host and parasite, respectively. Generation times were not directly manipulated, but instead one of the coevolving partners was regularly removed and replaced with a population from an earlier time point. Thus, one partner underwent more generations than the other. Manipulations were carried out at both early and later periods of coevolutionary interactions. At early stages of coevolution, host and parasites that underwent relatively more generations displayed higher levels of resistance and infectivity, respectively. However, the relative number of generations that bacteria and phages underwent did not change the level of local adaptation relative to control populations. This is likely because generalist hosts and parasites are favoured during early stages of coevolution, preventing local adaptation. By contrast, at later stages manipulations had no effect on either average levels of resistance or infectivity, or alter the level of local adaptation relative to the controls, possibly because traits other than resistance and infectivity were under strong selection. Taken together, these data suggest that the relative generation times of hosts and parasites may not be an important determinant of local adaptation in this system.

102. Mudgal,P.**,** Breidt,F.J.**,** Lubkin,S.R.**,** Sandeep,K.P. (2006). **Quantifying the significance of phage attack on starter cultures: a mechanistic model for population dynamics of phage and their hosts isolated from fermenting sauerkraut.** *Appl. Environ. Microbiol.* 72:3908-3915. **Abstract:** We investigated the possibility of using starter cultures in sauerkraut fermentation and thereby reducing the quantity of salt used in the process. This, in turn, would reduce the amount of waste salt that would enter in our water resources. Phage, naturally present in sauerkraut fermentation, could potentially affect the starter cultures introduced. Thus, a mechanistic mathematical model was developed to quantify the growth kinetics of the phage and starter cultures. The model was validated by independent experiments with two *Leuconostoc mesenteroides* strains isolated from sauerkraut and their corresponding phage. Model simulations and experimental evidence showed the presence of phage-resistant cell populations in starter cultures which replaced phage-sensitive cells, even when the initial phage density (P(0)) and multiplicity of infection (MOI) were low (P(0) < 1 x 10(3) PFU/ml; MOI < 10(-4)) in the MRS media. Based on the results of model simulation and parameter optimization, it was suggested that the kinetic parameters of phage-host interaction, especially the adsorption rate, vary with the initial phage and host densities and with time. The model was validated in MRS broth. Therefore, the effects of heterogeneity and other environmental factors, such as temperature and pH, should be considered to make the model applicable to commercial fermentations.

103. Munn,C.B. (2006). **Viruses as pathogens of marine organisms—from bacteria to whales.** *J. Mar. Bio. Assoc. UK* 86:453-467. **Abstract:** Viruses are the most abundant members of marine ecosystems and play an enormous role in ocean processes through their interactions with all types of marine organisms. This short review provides examples of the dramatic increase in our knowledge of the diversity of marine viruses as pathogens of bacteria, protists, molluscs, crustaceans, cnidaria, reptiles, fish and mammals. Several examples are provided showing evidence of evolution of new strains, changes in virulence, and transfer of viruses between ecosystems. The natural and anthropogenic causes of these shifts are discussed. Despite considerable advances in recent years, knowledge of the importance of viruses in many important groups of marine organisms is lacking or incomplete. Suggestions for future investigations necessary to understand the dynamics of biogeochemical processes and the impacts of disease in our oceans are proposed.

104. Myrmel,M.**,** Berg,E.M.M.**,** Grinde,B.**,** Rimstad,E. (2006). **Enteric viruses in inlet and outlet samples from sewage treatment plants.** *J. Water Health* 4:197-209. **Abstract:** Samples collected every two weeks from the inlet and outlet of three sewage treatment plants were screened for the presence of noro-, rota-, astro-, adeno-, hepatitis A- and circoviruses by (RT)-nested PCR, and for F-specific bacteriophages by isolation in *Escherichia coli* Famp. Plants A and B were secondary treatment plants and plant C used primary treatment. Noroviruses were detected in 43%, 53% and 24% of the inlet samples and 26%, 40% and 21% of the outlet samples from plants A, B and C, respectively. Astroviruses, rotaviruses and adenoviruses were more prevalent. Adenoviruses were detected in 96% of inlet and 94% of outlet samples, supporting the potential of these viruses as indicators of viral contamination from sewage. Hepatitis A virus and circoviruses were found only rarely. Reduction of infective viral particles during sewage treatment was evaluated using F-specific bacteriophages. The phages were reduced by, respectively, 99%, 87% and 0% in plants A, B and C, which corresponded to the observed differences in reduction of norovirus positive samples between the same plants. The study shows that the high viral load in sewage results in a discharge to the environment of a large amount of virus despite sewage treatment. On the other hand, the advantage of a more advanced treatment is demonstrated.

105. Nagasaki,K.**,** Tomaru,Y.**,** Shirai,Y.**,** Takao,Y. **,** Mizumoto,H. (2006). **Dinoflagellate-infecting viruses.** *J. Mar. Bio. Assoc. UK* 86:469-474. **Abstract:** Dinoflagellates (Dinophyceae) are considered to be one of the most abundant and diverse groups of phytoplankton; however, the viral impact on dinoflagellates was not studied until recently. This review shows the present information concerning the viruses infecting dinoflagellates and the ecology relationships between the host and the virus. So far, two viruses have been isolated and characterized: a large DNA virus (HcV: *Heterocapsa circularisquama* virus) and a small RNA virus (HcRNAV: H. circularisquama RNA virus); both of which are infectious to the harmful bloom-forming dinoflagellate *H. circularisquama*.

106. Nappier,S.P.**,** Aitken,M.D.**,** Sobsey,M.D. (2006). **Male-specific coliphages as indicators of thermal inactivation of pathogens in biosolids.** *Appl. Environ. Microbiol.* 72:2471-2475. **Abstract:** Male-specific (F+) coliphages have been proposed as a candidate indicator of fecal contamination and of virus reduction in waste treatment. However, in this and earlier work with a laboratory thermophilic anaerobic digester, a heat-resistant fraction of F+ coliphage populations indigenous to municipal wastewater and sludge was evident. We therefore isolated coliphages from municipal wastewater sludge and from biosolid samples after thermophilic anaerobic digestion to evaluate the susceptibility of specific groups to thermal inactivation. Similar numbers of F+ DNA and F+ RNA coliphages were found in untreated sludge, but the majority of isolates in digested biosolids were group I F+ RNA phages. Separate experiments on individual isolates at 53 degrees C confirmed the apparent heat resistance of group I F+ RNA coliphages as well as the susceptibility of group III F+ RNA coliphages. Although few F+ DNA coliphages were recovered from the treated biosolid samples, thermal inactivation experiments indicated heat resistance similar to that of group I F+ RNA phages. Hence, F+ DNA coliphage reductions during thermophilic anaerobic digestion are probably related to mechanisms other than thermal inactivation. Further studies should focus on the group III F+ RNA coliphages as potential indicators of reductions of heat-resistant pathogens in thermal processes for sludge treatment.

107. Nasser,A.M.**,** Paulman,H.**,** Sela,O.**,** Ktaitzer,T.**,** Cikurel,H.**,** Zuckerman,I.**,** Meir,A.**,** Aharoni,A. **,** Adin,A. (2006). **UV disinfection of wastewater effluents for unrestricted irrigation.** *Water Sci. Technol.* 54:83-88. **Abstract:** Wastewater reuse in arid regions is important for the production of a water resource to be utilised for non-potable purposes and to prevent the environmental transmission of disease-causing agents. This study was conducted to evaluate the effect of water quality on the comparative disinfection efficiency of viruses, bacteria and spores by UV irradiation. Furthermore, the microbial quality of effluent produced by coagulation, high rate filtration (HRF) and either UV irradiation or chlorination was determined. Using low pressure collimated beam, a UV dose of 80 mWs/cm2 was needed to achieve a 3-log10 inactivation of either rotavirus SA-11 or coliphage MS2, whereas over 5-log10 inactivation of *E. coli* was reached with a dose of only 20 mWs/cm2. *B. subtilis* inactivation was found to be linear up to a dose of 40 mWs/cm2 and then a tailing up to a UV dose of 120 mWs/cm2 was observed. It is worth noting that effluent turbidity of < 5 NTU did not influence the inactivation efficiency of UV irradiation. Operation of a

pilot plant to treat secondary effluent by coagulation, HRF and UV disinfection at a UV dose of 80 mWs/cm2 resulted in the production of high quality effluent in compliance with the Israel standards for unrestricted irrigation (< 10 CFU/100 mL faecal coliform and turbidity of < 5 NTU). Sulphite reducing clostridia (SRC) were found to be more resistant than coliphages and F coliform for UV irradiation. The results of this study indicated that UV disinfection is suitable for the production of effluents for unrestricted irrigation of food crops.

108. Nolan,J.M.**,** Petrov,V.**,** Bertrand,C.**,** Krisch,H.M.**,** Karam,J.D. (2006). **Genetic diversity among five T4-like bacteriophages.** *Virol. J.* 3:30. **Abstract:** BACKGROUND: Bacteriophages are an important repository of genetic diversity. As one of the major constituents of terrestrial biomass, they exert profound effects on the earth's ecology and microbial evolution by mediating horizontal gene transfer between bacteria and controlling their growth. Only limited genomic sequence data are currently available for phages but even this reveals an overwhelming diversity in their gene sequences and genomes. The contribution of the T4-like phages to this overall phage diversity is difficult to assess, since only a few examples of complete genome sequence exist for these phages. Our analysis of five T4-like genomes represents half of the known T4-like genomes in GenBank. RESULTS: Here, we have examined in detail the genetic diversity of the genomes of five relatives of bacteriophage T4: the *Escherichia coli* phages RB43, RB49 and RB69, the *Aeromonas salmonicida* phage 44RR2.8t (or 44RR) and the *Aeromonas hydrophila* phage Aeh1. Our data define a core set of conserved genes common to these genomes as well as hundreds of additional open reading frames (ORFs) that are nonconserved. Although some of these ORFs resemble known genes from bacterial hosts or other phages, most show no significant similarity to any known sequence in the databases. The five genomes analyzed here all have similarities in gene regulation to T4. Sequence motifs resembling T4 early and late consensus promoters were observed in all five genomes. In contrast, only two of these genomes, RB69 and 44RR, showed similarities to T4 middle-mode promoter sequences and to the T4 motA gene product required for their recognition. In addition, we observed that each phage differed in the number and assortment of putative genes encoding host-like metabolic enzymes, tRNA species, and homing endonucleases. CONCLUSION: Our observations suggest that evolution of the T4-like phages has drawn on a highly diverged pool of genes in the microbial world. The T4-like phages harbour a wealth of genetic material that has not been identified previously. The mechanisms by which these genes may have arisen may differ from those previously proposed for the evolution of other bacteriophage genomes.

109. O'Flaherty,S.**,** Coffey,A.**,** Edwards,R.**,** Meaney,W.**,** Fitzgerald,G.F.**,** Ross,R.P. (2004). **Genome of staphylococcal phage K: a new lineage of myoviridae infecting Gram-positive bacteria with a low G-C content.** *J. Bacteriol.* 186:2862-2871. **Abstract:** Phage K is a polyvalent phage of the *Myoviridae* family which is active against a wide range of staphylococci. Phage genome sequencing revealed a linear DNA genome of 127,395 bp, which carries 118 putative open reading frames. The genome is organized in a modular form, encoding modules for lysis, structural proteins, DNA replication, and transcription. Interestingly, the structural module shows high homology to the structural module from *Listeria* phage A511, suggesting intergenus horizontal transfer. In addition, phage K exhibits the potential to encode proteins necessary for its own replisome, including DNA ligase, primase, helicase, polymerase, RNase H, and DNA binding proteins. Phage K has a complete absence of GATC sites, making it insensitive to restriction enzymes which cleave this sequence. Three introns (lys-I1, pol-I2, and pol-I3) encoding putative endonucleases were located in the genome. Two of these (pol-I2 and pol-I3) were found to interrupt the DNA polymerase gene, while the other (lys-I1) interrupts the lysin gene. Two of the introns encode putative proteins with homology to HNH endonucleases, whereas the other encodes a 270-amino-acid protein which contains two zinc fingers (CX2CX22CX2C and CX2CX23CX2C). The availability of the genome of this highly virulent phage, which is active against infective staphylococci, should provide new insights into the biology and evolution of large broad-spectrum polyvalent phages.

110. O'Flaherty,S.**,** Ross,R.P.**,** Flynn,J.**,** Meaney,W.J.**,** Fitzgeral,G.F.**,** Coffey,A. (2005). **Isolation and characterisation of two anti-staphylococcal bacteriophages specific for pathogenic *Staphylococcus aureus* associated with bovine infections.** *Lett. Appl. Microbiol.* 41:482-486. **Abstract:** Aims: The aim of this study was to isolate and characterize bacteriophages against bovine *Staphylococcus aureus* associated with mastitis.
Methods and Results: We describe the isolation of two anti-staphylococcal phages namely DW2 and CS1 from farmyard slurry. Both phages were characterized by electron microscopy and restriction analysis and shown to belong to the *Siphoviridae* family. CS1 and DW2 were lytic for representatives of all three clonal groups of Irish mastitis-associated staphylococci. These phages were compared with the previously characterized *Myoviridae* phage K. Infusion of a cocktail of all three phages at 10(8) PFU ml(-1) into live cow teats resulted in no detectable increase in somatic cell counts in milks indicating that the phages did not irritate the animal.
Conclusion: Two new anti-staphylococcal phages CS1 and DW2 were isolated and characterized and tested for immunogenicity in animal teats.
Significance and Impact of the Study: The phages isolated in this study are active against pathogenic *S. aureus* and may be incorporated into teat-dips or teat-washes as a non-antibiotic prophylaxis against staphylococcal bovine mastitis.

111. O'Flynn,G., Coffey,A., Fitzgerald,G.F., Ross,R.P. (2006). **The newly isolated lytic bacteriophages st104a and st104b are highly virulent against *Salmonella enterica*.** *J. Appl. Microbiol.* 101:251-259. **Abstract:** AIMS: To screen Irish faecal samples from a variety of sources with a view to isolating novel anti-*Salmonella* phages and to subsequently evaluate their lytic capability. METHODS AND RESULTS: Two novel anti-*Salmonella* phages st104a and st104b were isolated from a screening programme based on their lytic capability. The phages produced significantly larger plaques (2 mm) on the chosen indicator *Salmonella enterica* strain, DPC6046, when compared with the well-known control phage, Felix 01 (0.5 mm). Both phages st104a and st104b were found to have a broad host range within the *Salm. enterica* species. During in vitro trials, both phages (st104a and st104b) reduced *Salm. enterica* numbers more than 99% within 1 h. In vivo studies, involving the addition of the phage to porcine gastric juice (pH 2.5) demonstrated that phage st104a and phage Felix 01 were capable of surviving (10 and 30% survival respectively) the acidic conditions, unlike st104b, which was undetectable after 2 h exposure. CONCLUSIONS: Two novel lytic anti-*Salmonella* phages were isolated and characterized. SIGNIFICANCE AND IMPACT OF THE STUDY: With the exception of phage Felix 01, there has been relatively little phage therapy work performed using lytic *Salmonella* phage. In this study, the lytic phages st104a and st104b were isolated as a result of a faecal screening programme. Subsequently, phage st104a was found to have potential for biocontrol of *Salm. enterica* numbers if administered orally to pigs given their survival in porcine gastric juice, whereas, phage st104b may have potential in reducing cell numbers if applied by alternative approaches.

112. Parada,V., Herndl,G.J., Weinbauer,M.G. (2006). **Viral burst size of heterotrophic prokaryotes in aquatic systems.** *J. Mar. Bio. Assoc. UK* 86:613-621. **Abstract:** Viral burst size (BS), i.e. the number of viruses released during cell lysis, is a critical parameter for assessing the ecological and biogeochemical role of viruses in aquatic systems. Burst size is typically estimated by enumerating the viral particles in bacteria using transmission electron microscopy. Here, we review the average BS reported for different aquatic systems, present several hypotheses on the control of the BS and evaluate whether there are relationships between BS and bacterial activity parameters across systems. Based on reports from a variety of different aquatic environments, we calculated a mean BS of 24 and 34 for marine and freshwater environments, respectively. Generally, the BS increased with the trophic status of the environment and with the percentage of infected cells in marine populations. When diel dynamics were investigated or averages from large-scale environments were used, BS was positively related to bacterial production but no trend was detectable across systems. The across systems' finding that BS was significantly related to the frequency of infected cells (FIC) could be due to co-infection or superinfection. At any given site, BS seems to be influenced by a number of factors such as the size of the host cell and the viruses, the metabolic activity of the host and phage and host diversity. Thus, based on the available data collected over the past two decades on a variety of aquatic systems, some relations between BS and bacterial variables were detectable.

113. Parfitt,T. (2005). **Georgia: an unlikely stronghold for bacteriophage therapy.** *Lancet* 365:2166-2167. **Abstract:** The increasing failure of antibiotics to combat infections like multi-drug resistant *Staphylococcus aureus* has renewed interest in a long-forgotten treatment developed over 60 years ago in ex-Soviet Georgia. Tom Parfitt travelled to Tbilisi to witness the revival of bacteriophage therapy.

114. Park,D.K., Bitton,G., Melker,R. (2006). **Microbial inactivation by microwave radiation in the home environment.** *J. Environ. Health* 69:17-24. **Abstract:** The study reported here looked at the survival of microorganisms (heterotrophic plate counts, total coliforms, *E. coli*, and bacterial spores) in a consumer-type microwave oven. Kitchen sponges, scrubbing pads, and syringes were experimentally contaminated with wastewater and subsequently exposed to microwave radiation. At 100 percent power level, it was found that the heterotrophic plate count (i.e., total bacterial count) of the wastewater was reduced by more that 99 percent within 1 to 2 minutes, and the total coliform and *E. coli* were totally inactivated after 30 seconds of microwave radiation. Bacterial phage MS2 was totally inactivated within 1 to 2 minutes. Spores of *Bacillus cereus* were more resistant than the other microorganisms tested, and were completely eradicated only after 4-minute irradiation. Similar inactivation rates were obtained in wastewater-contaminated scrubbing pads. Microorganisms attached to plastic syringes were more resistant to microwave irradiation than those associated with kitchen sponges or scrubbing pads. It took 10 minutes for total inactivation of the heterotrophic plate count and 4 minutes for total inactivation of total coliform and *E. coli*. A 4-log reduction of phage MS2 was obtained after 2 minutes; 97.4 percent reduction was observed after 12 minutes. The authors also observed a higher inactivation of *B. cereus* spores in syringes placed in a ceramic container than of spores in syringes placed in a glass container. This finding could have some implications for the design of containers to be used in exposure of medical devices to microwave radiation. The article discusses the implications of these findings for consumer safety in the home environment.

115. Patten,N.L., Seymour,J.R., Mitchell,J.G. (2006). **Flow cytometric analysis of virus-like particles and heterotrophic bacteria within coral-associated reef water.** *J. Mar. Bio. Assoc. UK* 86:563-566. **Abstract:** Using flow cytometry, two distinct populations of virus-like particles (VLP) and heterotrophic bacteria were defined within

the 12cm water layer immediately overlying healthy, diseased and dead acroporid corals. Bacterial abundances were similar in overlying water for all coral types, however, VLP were 30% higher above diseased corals than healthy or dead corals. Mean virus to bacteria ratios (VBR) were up to 30% higher above diseased corals than above healthy or dead coral or in distant water. Concomitant with increasing VLP concentrations within 5cm of coral surfaces, VBR distributions were generally highest above healthy and diseased coral and depressed above dead coral. These results suggest fundamental shifts in the VLP and bacterial community in water associated with diseased corals.

116. Pelon,W.**,** Luftig,R.B.**,** Johnston,K.H. (2005). ***Vibrio vulnificus* load reduction in oysters after combined exposure to *Vibrio vulnificus*--specific bacteriophage and to an oyster extract component.** *J. Food Prot.* 68:1188-1191. **Abstract:** Oysters infected with *Vibrio vulnificus* can present a serious health risk to diabetic, immunocompromised, and iron-deficient individuals. Numerous studies have been conducted with the goal of eliminating this organism from raw oysters. We utilized two natural oyster-associated components: pooled *Vibrio vulnificus*-specific bacteriophage and an extract of the eastern oyster (*Crassostrea virginica*) that contains an antimicrobial component we named anti-*Vibrio vulnificus* factor, which is bactericidal for *V. vulnificus*. Although each component alone can reduce *V. vulnificus* numbers independently, the simultaneous use of both components in an in vitro system successfully more effectively reduced *V. vulnificus* bacterial loads.

117. Piuri,M.**,** Hatfull,G.F. (2006). **A peptidoglycan hydrolase motif within the mycobacteriophage TM4 tape measure protein promotes efficient infection of stationary phase cells.** *Mol. Microbiol.* 62:1569-1585. **Abstract:** The predominant morphotype of mycobacteriophage virions has a DNA-containing capsid attached to a long flexible non-contractile tail, features characteristic of the Siphoviridae. Within these phage genomes the tape measure protein (tmp) gene can be readily identified due to the well-established relationship between the length of the gene and the length of the phage tail--because these phages typically have long tails, the tmp gene is usually the largest gene in the genome. Many of these mycobacteriophage Tmp's contain small motifs with sequence similarity to host proteins. One of these motifs (motif 1) corresponds to the Rpf proteins that have lysozyme activity and function to stimulate growth of dormant bacteria, while the others (motifs 2 and 3) are related to proteins of unknown function, although some of the related proteins of the host are predicted to be involved in cell wall catabolism. We show here that motif 3-containing proteins have peptidoglycan-hydrolysing activity and that while this activity is not required for phage viability, it facilitates efficient infection and DNA injection into stationary phase cells. Tmp's of mycobacteriophages may thus have acquired these motifs in order to avoid a selective disadvantage that results from changes in peptidoglycan in non-growing cells.

118. Poon,A.F.Y.**,** Chao,L. (2006). **Functional origins of fitness effect-sizes of compensatory mutations in the DNA bacteriophage ϕX174.** *Evolution* 60:2032-2043. **Abstract:** Epistasis is an important and poorly understood aspect of mutations and strongly influences the evolutionary impact of genetic variation on adaptation and fitness. Although recent studies have begun to characterize the distribution of epistatic effects between mutations affecting fitness, there is currently a lack of empirical information on the underlying biological causes of these epistatic interactions. What are the functional constraints that determine the effectiveness of a compensatory mutation at restoring fitness? We have measured the effect-sizes of 52 compensatory mutations affecting nine different deleterious mutations in the major capsid and spike proteins of the DNA bacteriophage ϕX174. On average, an experimentally detectable compensatory mutation recovers about two-thirds of the fitness cost of the preceding deleterious mutation. Variation in fitness effect-sizes is only weakly associated with measures of the distance separating the deleterious and compensatory mutations in the amino acid sequence or the folded protein structure. However, there is a strong association of fitness effect-size with the correlation in the effects of the mutations on the biochemical properties of amino acids. A compensatory mutation has the largest effect-size, on average, when both the compensatory and deleterious mutations have radical effects on the overall biochemical make-up of the amino acids. By examining the relative contributions of specific biochemical properties to variation in fitness effect-size, we find that the area and charge of amino acids have a major influence, which suggests that the complexity of the amino acid phenotype is simplified by selection into a reduced number of phenotypic components.

119. Potgieter,N.**,** Mudau,L.S.**,** Maluleke,F.R.S. (2006). **Microbiological quality of groundwater sources used by rural communities in Limpopo Province, South Africa.** *Water Sci. Technol.* 54:371-377. **Abstract:** A survey of the microbiological quality of water from 194 boreholes (97 privately owned and 97 communal boreholes) in the rural Thitale-Hlanganani area of the Limpopo Province, South Africa was carried out between August 2002 and August 2003. Very little information on the microbiological quality of privately-owned boreholes in rural communities is available raising concerns about the safety of these groundwater supplies. In this study, levels of total coliforms, thermotolerant (faecal) coliforms, faecal enterococci, *Clostridium perfringens* (vegetative cells and spores) and somatic coliphages were determined for community and privately-owned borehole water. The average counts for total coliforms, faecal coliforms, faecal enterococci and *Clostridium perfringens* exceeded the South African

recommended guideline limits of 0-10 counts.100 ml(-1) for total coliforms and 0 counts. 100 ml(-1) for faecal coliforms, faecal enterococci and *Clostridium perfringens* respectively. Comparisons between the levels of indicator bacteria present in private and communal boreholes during dry seasons indicated a statistical difference for faecal enterococci bacteria (p = 0.005) and *Clostridium perfringens* (p = 0.08). Comparisons between the levels of indicator bacteria present in private and communal boreholes during rainy seasons indicated statistical differences between total coliforms (p = 0.05), faecal coliforms (p = 0.03) and Clostridium perfringens (p = 0.009) bacteria. No significant differences in the presence of somatic coliphages in both private and communal borehole water were see [*sic*]. The results indicated the need for environmental impact assessment studies to monitor the microbiological quality of groundwater sources in rural communities.

120. Power,M.L.**,** Ferrari,B.C.**,** Littlefield-Wyer,J.**,** Gordon,D.M.**,** Slade,M.B.**,** Veal,D.A. (2006). **A naturally occurring novel allele of *Escherichia coli* outer membrane protein A reduces sensitivity to bacteriophage.** *Appl. Environ. Microbiol.* 72:7930-7932. **Abstract:** A novel *Escherichia coli* outer membrane protein A (OmpA) was discovered through a proteomic investigation of cell surface proteins. DNA polymorphisms were localized to regions encoding the protein's surface-exposed loops which are known phage receptor sites. Bacteriophage sensitivity testing indicated an association between bacteriophage resistance and isolates having the novel ompA allele.

121. Prigent,M.**,** Leroy,M.**,** Confalonieri,F.**,** Dutertre,M.**,** DuBow,M.S. (2005). **A diversity of bacteriophage forms and genomes can be isolated from the surface sands of the Sahara Desert.** *Extremophiles* 9:289-296. **Abstract:** The surface sands of the Sahara Desert are exposed to extremes of ultraviolet light irradiation, desiccation and temperature variation. Nonetheless, the presence of bacteria has recently been demonstrated in this environment by cultivation methods and by 16S rDNA analyses from total DNA isolated from surface sands. To discern the presence of bacteriophages in this harsh environment, we searched for extracellular phages and intracellularly located phages present as prophages or within pseudolysogens. Mild sonication of the sand, in different liquid culture media, incubated with and without Mitomycin-C, was followed by differential centrifugation to enrich for dsDNA phages. The resulting preparations, examined by electron microscopy, revealed the presence of virus-like particles with a diversity of morphotypes representative of all three major double-stranded DNA bacteriophage families (Myoviridae, Siphoviridae and Podoviridae). Moreover, pulsed-field gel electrophoresis of DNA, extracted from the enriched bacteriophage preparations, revealed the presence of distinct bands suggesting the presence of putative dsDNA phage genomes ranging in size from 45 kb to 270 kb. Characterization of the bacteriophages present in the surface sands of the Sahara Desert extends the range of environments from which bacteriophages can be isolated, and provides an important point of departure for the study of phages in extreme terrestrial environments.

122. Prokopenko,E.I.**,** Shcherbakova,E.O.**,** Vatazin,A.V.**,** Budnikova,N.E.**,** Iankovoi,A.G.**,** Pasov,S.A.**,** Agafonova,S.G. (2005). **[Bacteriophages use in the treatment of pyoseptic complications in a female patient with renal allotransplant].** *Urologiia* 43-46.

123. Qimron,U.**,** Marintcheva,B.**,** Tabor,S.**,** Richardson,C.C. (2006). **Genomewide screens for *Escherichia coli* genes affecting growth of T7 bacteriophage.** *Proc. Natl. Acad. Sci. USA* 103:19039-19044. **Abstract:** Use of bacteriophages as a therapy for bacterial infection has been attempted over the last century. Such an endeavor requires the elucidation of basic aspects of the host-virus interactions and the resistance mechanisms of the host. Two recently developed bacterial collections now enable a genomewide search of the genetic interactions between *Escherichia coli* and bacteriophages. We have screened >85% of the *E. coli* genes for their ability to inhibit growth of T7 phage and >90% of the host genes for their ability to be used by the virus. In addition to identifying all of the known interactions, several other interactions have been identified. *E. coli* CMP kinase is essential for T7 growth, whereas overexpression of the *E. coli* uridine/cytidine kinase inhibits T7 growth. Mutations in any one of nine genes that encode enzymes for the synthesis of the *E. coli* lipopolysaccharide receptor for T7 adsorption leads to T7 resistance. Selection of T7 phage that can recognize these altered receptors has enabled the construction of phage to which the host is 100-fold less resistant.

124. Qureshi,H.**,** Saeed,S.**,** Ahmed,S.**,** Rasool,S.A. (2006). **Coliphage hsa as a model for antiviral studies/spectrum by some indigenous bacteriocin like inhibitory substances (BLIS).** *Pakist. J. Pharm. Sci.* 19:182-185. **Abstract:** Coliphage HSA was isolated from a raw sewage sample (collected from a local sewage treatment plant). The phage was analyzed by spot and tube lysis followed by plaque assay. Phage titre (plaque forming units i.e. PFU) was found to be 4.2 x 103 PFU/mL. Further purification of the phage was achieved by acid-precipitation method. Genomic identification of the coliphage HSA (done by fluorescent staining using acridine orange) revealed it to be a dsDNA bacterial virus. Staphylococcin188, Enterocins AAR-71, AAR-74, and Erwiniocin NA4 were screened for their antiphage activity by plaque assay. Accordingly, all the bacteriocin preparations possess demonstrable antiphage activity witnessed as a reduction in PFU after treatment. In the case of Staphylococcin

188, the number dropped up to 40 PFU/mL, Enterocin AAR-71 and Erwiniocin NA4 treatment reduced it to a zero PFU level, while Enterocin AAR-74 could reduce PFU to 50 (after addition of a constant volume, 500uL, of each of the crude bacteriocin preparations). Transmission Electron Microscopy studies revealed the phage to have an icosahedral head with a long tail and tail fibers.

125. Raven,J.A. (2006). **Aquatic viruses: the emerging story.** *J. Mar. Bio. Assoc. UK* 86:449-451. **Abstract:** It is likely that all living organisms can be infected by one or more viruses. One of the latest higher taxa to be converted from 'no characterized viruses' to 'well characterized viruses' are the diatoms (Bacillariophyceae, Heterokontophyta) with the recent publication of three papers characterizing an ssRNA and a ssDNA virus from two genera (*Chaetoceros* and *Rhizosolenia*) of marine planktonic diatom (Nagasaki et al., 2004, 2005; Bettarel et al., 2005). It would have been strange if viruses had not been able to exploit the dominant, in terms of global primary production, photosynthetic organisms in the ocean (assimilating perhaps as much as 20 Pg inorganic C into organic C per year), despite the less than completely convincing arguments assembled by Raven & Waite (2004) as to possible anti-viral defences unique to diatoms.

126. Raya,R.R.**,** Varey,P.**,** Oot,R.A.**,** Dyen,M.R. **,** Callaway,T.R.**,** Edrington,T.S.**,** Kutter,E.M.**,** Brabban,A.D. (2006). **Isolation and characterization of a new T-even bacteriophage, CEV1, and determination of its potential to reduce *Escherichia coli* O157:H7 levels in sheep.** *Appl. Environ. Microbiol.* 72:6405-6410. **Abstract:** Bacteriophage CEV1 was isolated from sheep resistant to *Escherichia coli* O157:H7 colonization. In vitro, CEV1 efficiently infected *E. coli* O157:H7 grown both aerobically and anaerobically. In vivo, sheep receiving a single oral dose of CEV1 showed a 2-log-unit reduction in intestinal *E. coli* O157:H7 levels within 2 days compared to levels in the controls.

127. Rees,C.E.D.**,** Dodd,C.E.R. (2006). **Phage for rapid detection and control of bacterial pathogens in food.** *Adv. Appl. Microbiol.* 59:159-186. **Abstract:** [first paragraph] In recent years there has been a revival of interest in the use of phage to treat bacterial infections, and research using phage therapy to overcome the problem of increasing levels of antibiotic resistance has become widely publicized (Sulakvelidze and Kutter, 2005). Accordingly, the idea that phage could be applied to food products as biocontrol agents has also received more interest among researchers. However, those working in the dairy industry are only too aware of the potential for these viruses to destroy populations of bacteria in milk-starter cultures. On the other hand, the use of phage is nothing new in the field of bacterial characterization, and phage typing schemes are routinely used in the subtyping of isolates of organisms such as *Salmonella enterica* (Anderson et al., 1977; Laszlo and Csorian, 1988), *Staphylococcus aureus* (Blair and Williams, 1961), *Listeria monocytogenes* (Rocourt, 1996), *Vibrio cholerae* (Basu and Mukerjee, 1968), and *Escherichia coli* O157:H7 (Khakhria et al., 1990). Over the last 10 years much work has been carried out to develop phage-based methods for rapid detection of pathogens in foods and, although the only commercially available test is that for detection of *Mycobacterium tuberculosis* in human sputum samples (Mole and Maskell, 2001), many new tests and applications for detection of pathogens in foods are currently being developed. This chapter will provide an overview of the recent developments in these fields and look at some new areas that may be developed into practical applications in the future.

128. Ripp,S.**,** Jegier,P.**,** Birmele,M.**,** Johnson,C.M.**,** Daumer,K.A.**,** Garland,J.L.**,** Sayler,G.S. (2006). **Linking bacteriophage infection to quorum sensing signalling and bioluminescent bioreporter monitoring for direct detection of bacterial agents.** *J. Appl. Microbiol.* 100:488-499. **Abstract:** AIM: To incorporate into the lambda phage genome, a luxI-based acyl-homoserine lactone (AHL) synthase genetic construct and exploit the autoamplified power of quorum sensing to translate a phage infection event into a chemical signature detectable by a lux-based bioluminescent bioreporter, with focus towards facile detection of microbial pathogens. METHODS AND RESULTS: The luxI gene from *Vibrio fischeri* was inserted into the lambda phage genome to construct a model phage-based biosensor system for the general detection of *Escherichia coli*. The AHL signalling molecules synthesized upon phage infection are detected by an AHL-specific bioluminescent bioreporter based on the luxCDABE gene cassette of *V. fischeri*. The assay generates target-specific visible light signals with no requisite addition of extraneous substrate. This binary reporter system was able to autonomously respond to lambda phage infection events at target *E. coli* concentrations ranging from 1 x 10(8) to 1 CFU ml(-1) within 1.5-10.3 h, respectively, in pure culture. When assayed against artificially contaminated lettuce leaf washings, detection within an *E. coli* inoculum range from 1 x 10(8) to 130 CFU ml(-1) was achieved within 2.6-22.4 h, respectively. CONCLUSIONS: The initial feasibility of binary phage-based reporter assays indicates that quorum sensing can be used to translate a phage infection event into an autoamplified chemical signature. SIGNIFICANCE AND IMPACT OF STUDY: With further modification, binary phage-based reporter assays may be capable of rapidly and cost effectively detecting pathogenic agents at very low population densities.

129. Rybniker,J.**,** Kramme,S.**,** Small,P.L. (2006). **Host range of 14 mycobacteriophages in *Mycobacterium ulcerans* and seven other mycobacteria including *Mycobacterium tuberculosis*--application for identification and**

susceptibility testing. *J. Med. Microbiol.* 55:37-42. **Abstract:** The host range of well-characterized mycobacteriophages, such as D29 and TM4, has been determined, together with that of more recently isolated mycobacteriophages, in *Mycobacterium ulcerans*, *Mycobacterium tuberculosis*, *Mycobacterium bovis* BCG, *Mycobacterium avium*, *Mycobacterium marinum*, *Mycobacterium scrofulaceum*, *Mycobacterium fortuitum* and *Mycobacterium chelonae*. Here, a set of virulent phages for *M. ulcerans*, a pathogen with a dramatic increase of incidence over the last decade, is demonstrated. In this work, a mycobacteriophage replication assay was adapted for the identification and rifampicin-susceptibility testing of *M. ulcerans*. Mycobacteriophages have generated a number of useful tools and enabled insights into mycobacterial genetics. With regard to the neglected pathogen *M. ulcerans*, the findings presented in this work allow the application of a large range of phage-based vectors and markers. The potential of phage therapy can now be evaluated for this extracellular pathogen.

130. Sandaa,R.A.**,** Larsen,A. (2006). **Seasonal variations in virus-host populations in Norwegian coastal waters: focusing on the cyanophage community infecting marine *Synechococcus* spp.** *Appl. Environ. Microbiol.* 72:4610-4618. **Abstract:** Viruses are ubiquitous components of the marine ecosystem. In the current study we investigated seasonal variations in the viral community in Norwegian coastal waters by pulsed-field gel electrophoresis (PFGE). The results demonstrated that the viral community was diverse, displaying dynamic seasonal variation, and that viral populations of 29 different sizes in the range from 26 to 500 kb were present. Virus populations from 260 to 500 kb and dominating autotrophic pico- and nanoeukaryotes showed similar dynamic variations. Using flow cytometry and real-time PCR, we focused in particular on one host-virus system: *Synechococcus* spp. and cyanophages. The two groups covaried throughout the year and were found in the highest amounts in fall with concentrations of 7.3 x 10(4) *Synechococcus* cells ml(-1) and 7.2 x 10(3) cyanophage ml(-1). By using primers targeting the g20 gene in PCRs on DNA extracted from PFGE bands, we demonstrated that cyanophages were found in a genomic size range of 26 to 380 kb. The genetic richness of the cyanophage community, determined by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified g20 gene fragments, revealed seasonal shifts in the populations, with one community dominating in spring and summer and a different one dominating in fall. Phylogenetic analysis of the sequences originating from PFGE and DGGE bands grouped the sequences into three groups, all with homology to cyanomyoviruses present in cultures. Our results show that the cyanophage community in Norwegian coastal waters is dynamic and genetically diverse and has a surprisingly wide genomic size range.

131. Sanogo,Y.O.**,** Dobson,S.L. (2006). **WO bacteriophage transcription in *Wolbachia*-infected *Culex pipiens*.** *Insect Biochem. Mol. Biol.* 36:80-85. **Abstract:** Bacteriophages are commonly found in association with free-living bacteria, both as exogenic phages (virions) and as prophages integrated into the bacterial genome. In contrast, the observation of bacteriophages associated with obligate intracellular bacteria has been described infrequently. An exception is provided by *Wolbachia* endosymbionts, which harbor multiple phage elements that have been designated as WO phage. *Wolbachia* are maternally inherited bacteria that occur in the cytoplasm of many invertebrates, where they often manipulate host reproduction. Previously, the WO phage orf7 locus and ankyrin repeat-encoding genes have been observed to represent sources of genetic diversity between *Wolbachia* (wPip) strains infecting mosquitoes of the *Culex pipiens* complex and have been suggested as potential participants in the reproductive manipulations. We have characterized WO phage associated with multiple *Wolbachia*-infected *Culex* strains and an uninfected strain using electron microscopy and RT-PCR. For each strain, different developmental stages were examined for transcription of three WO phage orf7 genes. The results provide evidence for the presence of both actively transcribed virions and inactive prophages. Variable orf7 transcription patterns are observed in comparisons of differing *Cx. pipiens* strains. Variability includes both mosquito stage-specific and sexually dimorphic orf7 expression patterns. This report provides additional support for the hypothesis that bacteriophages play an important role in *Wolbachia* and host evolution.

132. Sau,K.**,** Gupta,S.K.**,** Sau,S.**,** Ghosh,T.C. (2005). **Synonymous codon usage bias in 16 *Staphylococcus aureus* phages: implication in phage therapy.** *Virus Res.* 113:123-131. **Abstract:** To reveal the factors influencing architecture of protein-coding genes in staphylococcal phages, relative synonymous codon usage variation has been investigated in 920 protein-coding genes of 16 staphylococcal phages. As expected for AT rich genomes, there are predominantly A and T ending codons in all 16 phages. Both Nc plot and correspondence analysis on relative synonymous codon usage indicates that mutation bias influences codon usage variation in the 16 phages. Correspondence analysis also suggests that translational selection and gene length also influence the codon usage variation in the phages to some extent and codon usage in staphylococcal phages is phage-specific but not *S. aureus* -specific. Further analysis indicates that among 16 staphylococcal phages, 44AHJD, P68 and K may be extremely virulent in nature as most of their genes have high translation efficiency. If this is true, then above three phages may be useful for curing staphylococcal infections.

133. Seymour,J.R.**,** Seuront,L.**,** Doubell,M.**,** Waters,R.L.**,** Mitchell,J.G. (2006). **Microscale patchiness of virioplankton.** *J. Mar. Bio. Assoc. UK* 86:551-561. **Abstract:** The microscale spatial distributions of viruses were investigated in

three contrasting environments including oligotrophic open ocean, eutrophic coastal and estuarine habitats. The abundances of two discrete populations of both viruses and heterotrophic bacteria were measured at spatial resolutions of between 1 and 5cm using purpose-designed microscale sampling equipment and flow cytometric sample analysis. Within open water samples, virus distributions were characterized by non-normal distributions and by 'hotspots' in abundance where concentrations varied by up to 17-fold. In contrast to patterns generally observed at larger spatiotemporal scales, there was no correlation between bacterial and viral abundance or correspondence between bacteria and virus hotspots within these samples. Consequently, strong hotspots and gradients in the virus:bacteria ratio (VBR) were also apparent within samples. Within vertical profiles taken from above the sediment-water interface within a temperate mangrove estuary, distributions of planktonic viruses were characterized by gradients in abundance, with highest concentrations observed within the 1-2cm immediately above the sediment surface, and virus distributions were correlated to bacterial abundance ($P<0.01$). The patterns observed in these contrasting habitats indicate that microscale patchiness of virus abundance may be a common feature of the marine environment. This form of heterogeneity may have important implications for virus-host dynamics and subsequently influence microbial trophodynamics and nutrient cycling in the ocean.

134. Shen,Y.**,** Liu,Y. **,** Zhang,Y.**,** Cripe,J.**,** Conway,W.**,** Meng,J.**,** Hall,G.**,** Bhagwat,A.A. (2006). **Isolation and characterization of *Listeria monocytogenes* isolates from ready-to-eat foods in Florida.** *Appl. Environ. Microbiol.* 72:5073-5076. **Abstract:** Of 3,063 ready-to-eat food samples tested, 91 (2.97%) were positive for *Listeria monocytogenes*, and lineage 1 strains outnumbered lineage 2 strains 57 to 34. Seventy-one isolates (78%) exhibited multiple antibiotic resistance, and an *L. monocytogenes*-specific bacteriophage cocktail lysed 65 of 91 (71%) isolates. Determining phage, acid, and antibiotic susceptibility phenotypes enabled us to identify differences among strains which were otherwise indistinguishable by conventional methods.

135. Sheng,H.**,** Knecht,H.J.**,** Kudva,I.T.**,** Hovde,C.J. (2006). **Application of bacteriophages to control intestinal *Escherichia coli* O157:H7 levels in ruminants.** *Appl. Environ. Microbiol.* 72:5359-5366. **Abstract:** A previously characterized O157-specific lytic bacteriophage KH1 and a newly isolated phage designated SH1 were tested, alone or in combination, for reducing intestinal *Escherichia coli* O157:H7 in animals. Oral treatment with phage KH1 did not reduce the intestinal *E. coli* O157:H7 in sheep. Phage SH1 formed clear and relatively larger plaques on lawns of all 12 E. coli O157:H7 isolates tested and had a broader host range than phage KH1, lysing O55:H6 and 18 of 120 non-O157 *E. coli* isolates tested. In vitro, mucin or bovine mucus did not inhibit bacterial lysis by phage SH1 or KH1. A phage treatment protocol was optimized using a mouse model of *E. coli* O157:H7 intestinal carriage. Oral treatment with SH1 or a mixture of SH1 and KH1 at phage/bacterium ratios > or = 10(2) terminated the presence of fecal *E. coli* O157:H7 within 2 to 6 days after phage treatment. Untreated control mice remained culture positive for >10 days. To optimize bacterial carriage and phage delivery in cattle, *E. coli* O157:H7 was applied rectally to Holstein steers 7 days before the administration of 10(10) PFU SH1 and KH1. Phages were applied directly to the rectoanal junction mucosa at phage/bacterium ratios calculated to be > or = 10(2). In addition, phages were maintained at 10(6) PFU/ml in the drinking water of the phage treatment group. This phage therapy reduced the average number of E. coli O157:H7 CFU among phage-treated steers compared to control steers ($P < 0.05$); however, it did not eliminate the bacteria from the majority of steers.

136. Shirai,Y.**,** Takao,Y.**,** Mizumoto,H.**,** Tomaru,Y.**,** Honda,D.**,** Nagasaki,K. (2006). **Genomic and phylogenetic analysis of a single-stranded RNA virus infecting *Rhizosolenia setigera* (Stramenopiles: Bacillariophyceae).** *J. Mar. Bio. Assoc. UK* 86:475-483. **Abstract:** We report the first complete genome sequence of the marine diatom-infecting, positive-sense single-stranded RNA (ssRNA) virus, *Rhizosolenia setigera* RNA virus (RsRNAV). The genome is 8877 nucleotides (nt), polyadenylated, lacking a cap structure, and has two large open reading frames (ORFs): ORF-1 (4818 nt), a polyprotein gene coding for replicases, e.g. RNA helicase, RNA-dependent RNA polymerase (RdRp); and ORF-2 (2883 nt), a polyprotein gene coding for structural proteins. The ORFs are separated by a 323nt intergenic region (IGR), flanked by a 624nt 5?-untranslated region (UTR) and a 229 nt 3?-UTR. The deduced amino acid sequences for ORF-1 and ORF-2 respectively show considerable similarities to the non-structural and structural proteins of a marine raphidophyte-infecting virus HaRNAV (Heterosigma akashiwo RNA virus). Phylogenetic analyses of concatenated amino acid sequences of RNA helicase and RdRp domains supported the monophyly of RsRNAV, HaRNAV and a marine protist-infecting virus SssRNAV (Schizochytrium single-stranded RNA virus) with moderate bootstrap values of 79–83%, but not at the family level, whilst their monophyly was only weakly supported (50–55%) in the phylogenetic tree based on RdRp whole domain. As a result, comparison of the genome organization and sequence suggests RsRNAV is not a member of any currently defined virus family. In the RdRp tree, the positive-sense ssRNA viruses infecting Stramenopiles (RsRNAV, HaRNAV and SssRNAV) and Alveolata (HcRNAV (Heterocapsa circularisquama RNA virus)) were categorized into phylogenetically distant clades, which suggests a host/virus coevolution. Our study supports the hypothesis that a diverse array of ssRNA viruses exists in marine environments.

137. Sitohy,M., Chobert,J.M., Karwowska,U., Gozdzicka-Jozefiak,A., Haertle,T. (2006). **Inhibition of bacteriophage M13 replication with esterified milk proteins.** *J. Ag. Food Chem.* 54:3800-3806. **Abstract:** Esterified milk proteins [methylated (Met) or ethylated (Et) α-lactalbumin (ALA), β-lactoglobulin (BLG), and β-casein (BCN)], unmodified native milk proteins, and native basic proteins (calf thymus histone and hen egg white lysozyme) were tested for their antiviral activity against the bacteriophage M13 and for their influence on its replication (except BCN). All esterified milk proteins showed an antiviral activity against the bacteriophage M13, proportional to the extent of esterification and, hence, to the increased basicity of the modified proteins. Antiviral activity of 100% Met-BLG disappeared after its pepsinolysis but not after its trypsinolysis. The antiviral activity of Met-BLG was much higher than that of native basic proteins (histone and lysozyme). One hundred percent Met-BLG and 73% Et-BLG inhibited the replication of bacteriophage M13 completely, whereas 60% Met-ALA inhibited phage replication partially. Calf thymus histone inhibited the replication of bacteriophage M13 at a lower extent (20%) than Met- and Et-BLG (100% inhibition). Protein concentration, pH, and concentration of the Escherichia coli culture in the preincubation medium of the virus were other factors influencing antiviral activity. Interactions of esterified proteins with the phage DNA (phenol extracted) followed the same pattern as observed during studies of the inhibition of the phage replication: Met-BLG > Et-BLG > or = Met-ALA.

138. Smiddy,M., Kelly,A.L., Patterson,M.F., Hill,C. (2006). **High pressure-induced inactivation of Qβ coliphage and c2 phage in oysters and in culture media.** *Int. J. Food Microbiol.* 106:105-110. **Abstract:** High pressure (HP) treatment inactivates bacteria in shellfish, but its effects on viruses in shellfish have not yet been determined, although viral illness is frequently associated with shellfish consumption. The aim of this study was to investigate the baroresistance of two bacteriophage viruses, Qβ coliphage and c2 phage, in oysters and in culture media. High numbers (>or=10(7) ml(-1) or g(-1)) of both phages were obtained in culture media and in oysters. Samples were HP treated at 200-800 MPa at 20 degrees C for up to 30 min. Little or no inactivation of either phage was observed in oysters or in culture media after treatment at <or=400 MPa. High levels of inactivation of both phages in oysters and in culture medium were observed following treatment at 500-700 MPa. Titres of both phages were reduced to non-detectable levels (up to 8 log inactivation) in oysters and in GM17 broth (for c2 phage) after treatment at 800 MPa. The level of Qbeta coliphage in tryptone soya broth with yeast extract (10(10) PFU ml(-1)) was reduced by approximately 7 log units following treatment of 800 MPa. Levels of inactivation of both phages in oysters were similar to those in culture media. Increasing the duration of treatment at 550 or 600 MPa increased the level of inactivation of both phages in oysters. HP treatment may effectively inactivate phage in shellfish but HP-induced inactivation of human enteric viruses in oysters needs to be studied directly, to more accurately assess the ability of this technology to inactivate these viruses.

139. Smirnov,N.I., Zadnova,S.P., Toporkov,A.V. (2005). **[Effects of the recombinant plasmid carrying the genes of cholera prophages CTX and RS1 on the expression of virulence and immunogenicity genes in the cholera pathogen].** *Molekuliarnaia genetika, mikrobiologiia i virusologiia* 3-8. **Abstract:** Using toxin-coregulated adhesion pili (TCP), the etiologic agent of cholera is able to colonize human small intestine, where this pathogen proceeds with the production of the secreted cholera toxin (CT), inducing the development of severe diarrhea. At the same time, TCP and CT are not only the major factors of pathogenicity but also form a part of the group of key protective antigens. Immunoenzyme, immunoblotting, self-agglutination investigations, electron-microscopic studies, and electrophoretic assay of the outer membrane proteins showed that the recombinant plasmid carrying a number of cloned genes of two prophages, CTX and RS1, introduced into model *Vibrio cholerae* strains classical biovariant, resulted in the formation of strains with an enhanced rate of synthesis of three protective antigens: CT, TCP, and an outer membrane protein, OmpU. A simultaneous increase in the level of biosynthesis of the three antigens in *V. cholerae* was demonstrated to be specified by alterations in the expression of the toxR regulatory gene. Information was obtained suggesting that the transcriptional activity of toxR gene was dependent on the activity of rstC antirepressor gene derived from RS1 pro-phage and localized in the cloned fragment. Strains hyperproducing the three protective antigens can be used to construct more efficient non-living cholera vaccines, and to isolate the indicated proteins applicable to the development of diagnostic test-systems.

140. Stewart-Pullaro,J., Daugomah,J.W., Chestnut,D.E., Graves,D.A., Sobsey,M.D., Scott,G.I. (2006). **F+ RNA coliphage typing for microbial source tracking in surface waters.** *J. Appl. Microbiol.* 101:1015-1026. **Abstract:** AIMS: The utility of coliphages to detect and track faecal pollution was evaluated using South Carolina surface waters that exceeded State faecal coliform standards. METHODS AND RESULTS: Coliphages were isolated from 117 surface water samples by single agar layer (SAL) and enrichment presence/absence (EP/A) methods. Confirmed F+ RNA coliphages were typed for microbial source tracking using a library-independent approach. Concentrations of somatic coliphages using 37 and 44.5 degrees C incubation temperatures were found to be significantly different and the higher temperature may be more specific for faecal contamination. The EP/A technique detected coliphages infecting *Escherichia coli* Famp in 38 (66%) of the 58 surface water samples negative for F+ coliphages by the SAL method. However, coliphages isolated by EP/A were found to be less

representative of coliphage diversity within a sample. Among the 2939 coliphage isolates tested from surface water and known source samples, 813 (28%) were found to be F+ RNA. The majority (94%) of surface water F+ RNA coliphage isolates typed as group I. Group II and/or III viruses were identified from 14 surface water stations, the majority of which were downstream of wastewater discharges. These sites were likely contaminated by human-source faecal pollution. CONCLUSIONS: The results suggest that faecal contamination in surface waters can be detected and source identifications aided by coliphage analyses. SIGNIFICANCE AND IMPACT OF THE STUDY: This study supports the premise that coliphage typing can provide useful, but not absolute, information to distinguish human from animal sources of faecal pollution. Furthermore, the comparison of coliphage isolation methods detailed in this study should provide valuable information to those wishing to incorporate coliphage detection into water quality assessments.

141. Stummeyer,K.**,** Schwarzer,D.**,** Claus,H.**,** Vogel,U.**,** Gerardy-Schahn,R.**,** Muhlenhoff,M. (2006). **Evolution of bacteriophages infecting encapsulated bacteria: lessons from *Escherichia coli* K1-specific phages.** *Mol. Microbiol.* 60:1123-1135. **Abstract:** Bacterial capsules are not only important virulence factors, but also provide attachment sites for bacteriophages that possess capsule degrading enzymes as tailspike proteins. To gain insight into the evolution of these specialized viruses, we studied a panel of tailed phages specific for *Escherichia coli* K1, a neuroinvasive pathogen with a polysialic acid capsule. Genome sequencing of two lytic K1-phages and comparative analyses including a K1-prophage revealed that K1-phages did not evolve from a common ancestor. By contrast, each phage is related to a different progenitor type, namely T7-, SP6-, and P22-like phages, and gained new host specificity by horizontal uptake of an endosialidase gene. The new tailspikes emerged by combining endosialidase domains with the capsid binding module of the respective ancestor. For SP6-like phages, we identified a degenerated tailspike protein which now acts as versatile adaptor protein interconnecting tail and newly acquired tailspikes and demonstrate that this adapter utilizes an N-terminal undecapeptide interface to bind otherwise unrelated tailspikes. Combining biochemical and sequence analyses with available structural data, we provide new molecular insight into basic mechanisms that allow changes in host specificity while a conserved head and tail architecture is maintained. Thereby, the present study contributes not only to an improved understanding of phage evolution and host-range extension but may also facilitate the on purpose design of therapeutic phages based on well-characterized template phages.

142. Sullivan,M.B.**,** Lindell,D.**,** Lee,J.A.**,** Thompson,L.R.**,** Bielawski,J.P.**,** Chisholm,S.W. (2006). **Prevalence and evolution of core photosystem II genes in marine cyanobacterial viruses and their hosts.** *PLoS Biol.* 4:e234. **Abstract:** Cyanophages (cyanobacterial viruses) are important agents of horizontal gene transfer among marine cyanobacteria, the numerically dominant photosynthetic organisms in the oceans. Some cyanophage genomes carry and express host-like photosynthesis genes, presumably to augment the host photosynthetic machinery during infection. To study the prevalence and evolutionary dynamics of this phenomenon, 33 cultured cyanophages of known family and host range and viral DNA from field samples were screened for the presence of two core photosystem reaction center genes, *psbA* and *psbD*. Combining this expanded dataset with published data for nine other cyanophages, we found that 88% of the phage genomes contain *psbA*, and 50% contain both *psbA* and *psbD*. The *psbA* gene was found in all myoviruses and Prochlorococcus podoviruses, but could not be amplified from *Prochlorococcus* siphoviruses or *Synechococcus* podoviruses. Nearly all of the phages that encoded both psbA and *psbD* had broad host ranges. We speculate that the presence or absence of *psbA* in a phage genome may be determined by the length of the latent period of infection. Whether it also carries *psbD* may reflect constraints on coupling of viral- and host-encoded PsbA-PsbD in the photosynthetic reaction center across divergent hosts. Phylogenetic clustering patterns of these genes from cultured phages suggest that whole genes have been transferred from host to phage in a discrete number of events over the course of evolution (four for *psbA*, and two for *psbD*), followed by horizontal and vertical transfer between cyanophages. Clustering patterns of *psbA* and *psbD* from *Synechococcus* cells were inconsistent with other molecular phylogenetic markers, suggesting genetic exchanges involving *Synechococcus* lineages. Signatures of intragenic recombination, detected within the cyanophage gene pool as well as between hosts and phages in both directions, support this hypothesis. The analysis of cyanophage *psbA* and *psbD* genes from field populations revealed significant sequence diversity, much of which is represented in our cultured isolates. Collectively, these findings show that photosynthesis genes are common in cyanophages and that significant genetic exchanges occur from host to phage, phage to host, and within the phage gene pool. This generates genetic diversity among the phage, which serves as a reservoir for their hosts, and in turn influences photosystem evolution.

143. Suzuki,M.**,** Tawada,Y.**,** Kato,M.**,** Hori,H.**,** Mamiya,N.**,** Hayashi,Y.**,** Nakano,M.**,** Fukushima,R.**,** Katai,A.**,** Tanaka,T.**,** Hata,M.**,** Matsumoto,M.**,** Takahashi,M.**,** Sakae,K. (2006). **Development of a rapid strain differentiation method for methicillin-resistant *Staphylococcus aureus* isolated in Japan by detecting phage-derived open-reading frames.** *J. Appl. Microbiol.* 101:938-947. **Abstract:** AIMS: To develop a rapid genotyping method for investigating outbreaks of methicillin-resistant strains of *Staphylococcus aureus* (MRSA) isolated in Japan. METHODS AND RESULTS: Isolates were genotyped by detecting the keeping pattern of 16 open-reading frames (ORFs), a process

we call phage ORF typing (POT). Thirteen of the ORFs were selected from phage genomes and one from a genomic island SaGIm in the genome of strain Mu50. The other two ORFs, one from Tn554 and one from staphylococcal cassette chromosome mec (SCCmec) type II, were used as strain markers. Three hundred and sixty-eight isolates from five hospitals were classified into 133 types by POT, whereas they were classified into 139 types by pulsed-field gel electrophoresis (PFGE) subtyping. The discriminatory power of POT (D=0.989) was equal to that of PFGE subtyping (D=0.986). CONCLUSIONS: MRSA isolates collected in Japan can be genotyped by detecting the keeping pattern of phage-derived ORFs with a discriminatory power equal to that of PFGE subtyping. SIGNIFICANCE AND IMPACT OF THE STUDY: MRSA isolates can be genotyped rapidly by detecting phage-derived ORFs. As particular pandemic clones can be found in a specific region, a typing method localized to a pandemic clone may be effective for the rapid genotyping of MRSA during outbreaks.

144. Tao,L.**,** Pavlova,S.I., Kiliç,A.O. (2005). **Phages and bacterial vaginosis.** pp. 256-279 In Waldor,M.K., Friedman,D.I., and Adhya,S.L. (eds.), *Phages: Their Role in Pathogenesis and Biotechnology*. ASM Press, Washington DC. **Abstract:** [concluding paragraph] Undoubtably, phages are natural factors that suppress vaginal lactobacilli, and we have obtained extensive data to support this claim. However, a direct link between phages and BV has yet to be established. Of course, the most convincing evidence would be obtained by directly observing phage infections of vaginal lactobacilli in women at the early stage of BV. Unfortunately, this is a rather difficult task. when a woman realizes that she has developed BV and comes to a gynocologic clinic, her vaginal lactobacilli have already decreased in number by being depleted by an unknown factor, possible phage. To overcome this problem, future clinical studies should include a longitudinal sampling of healthy women with a high risk of developing BV or of women suffering from recurrent BV. An animal model is also important for testing Koch's postulate. Phages should be tested in animals to determine if they initiate BV and transmit the disease among different animals. Moreover, fundamental knowledge of *Lactobacillus* phages should be further advanced. Future studies should include establishing phage taxonomy, characterizing phage diversity, determining phage genomic sequences, and studying interactions bteween phages and lactobacilli. These advancements in basic sciences will help us to understand the role of *Lactobacillus* phages in the etiology of BV.

145. Templeton,M.R.**,** Andrews,R.C.**,** Hofmann,R. (2006). **Impact of iron particles in groundwater on the UV inactivation of bacteriophages MS2 and T4.** *J. Appl. Microbiol.* 101:732-741. **Abstract:** AIMS: To investigate the impact of iron particles in groundwater on the inactivation of two model viruses, bacteriophages MS2 and T4, by 254-nm ultraviolet (UV) light. METHODS AND RESULTS: One-litre samples of groundwater with high iron content (from the Indianapolis Water Company, mean dissolved iron concentration 1.3 mg l(-1)) were stirred vigorously while exposed to air, which oxidized and precipitated the dissolved iron. In parallel samples, ethylenediaminetetra-acetic acid (EDTA) was added to chelate the iron and prevent formation of iron precipitate. The average turbidity in the samples without EDTA (called the 'raw' samples) after 210 min of stirring was 2.7 +/- 0.1 NTU while the average turbidity of the samples containing EDTA (called the 'preserved' samples) was 1.0 +/- 0.1 NTU. 'Raw' and 'preserved' samples containing bacteriophage MS2 were exposed to 254-nm UV light at doses of 20, 40, or 60 mJ (cm(2))(-1), while samples containing bacteriophage T4 were exposed to 2 or 5 mJ (cm(2))(-1), using a low pressure UV collimated beam. The UV inactivation of both phages in the 'raw' groundwater was lower than in the EDTA-'preserved' groundwater to a statistically significant degree (alpha = 0.05), due to the association of phage with the UV-absorbing iron precipitate particles. A phage elution technique confirmed that a large fraction of the phage that survived the UV exposures were particle-associated. CONCLUSIONS: Phages that are associated with iron oxide particles in groundwater are shielded from UV light to a measurable and statistically significant degree at a turbidity level of 2.7 NTU when the phage particle association is induced under experimental conditions. SIGNIFICANCE AND IMPACT OF THE STUDY: While the particle association of the phage in this study was induced experimentally, the findings provide further evidence that certain particles in natural waters and wastewaters (e.g. iron oxide particles) may have the potential to shield viruses from UV light.

146. Templeton,M.R.**,** Andrews,R.C.**,** Hofmann,R. (2005). **Inactivation of particle-associated viral surrogates by ultraviolet light.** *Water Res.* 39:3487-3500. **Abstract:** This study investigated whether colloid-sized particles can enmesh and protect viruses from 254-nm ultraviolet (UV) light and sought to determine the particle characteristics (e.g. size, chemical composition) that are most relevant in causing a protective effect. Two viral surrogates (MS2 coliphage and bacteriophage T4), three types of particles (kaolin clay, humic acid powder, and activated sludge), two coagulants (alum and ferric chloride), two filtration conditions (none and 0.45 microm), and two UV doses (40 and 80 mJ/cm2 for MS2 coliphage; 2 and 7 mJ/cm2 for bacteriophage T4) were considered in a series of bench-scale UV collimated beam experiments. Transmission electron microscopy was used to qualitatively confirm the phage particle-association after coagulation. Humic acid and activated sludge floc particles shielded both viral surrogates to a statistically significant degree (with >99% confidence) relative to particle-free control conditions, while the kaolin clay particles provided no significant protection. The results of the study suggest that particles <2 microm in diameter are large enough to protect viruses from UV light and that particulate chemical composition

(e.g. UV-absorbing organic content) may be a critical factor in the survival of particle-associated viruses during UV disinfection.

147. Tian,F., Cheng,G.x., Wang,Z., Yang,J.q., Yang,J., Liu,S.j. (2006). **[Microenvironment of positive pressure powered air purifying medical protective equipment].** *Chin. J. Indust. Hyg. Occup. Dis.* 24:151-153. **Abstract:** OBJECTIVE: To study the filtration efficiency of a positive pressure powered air purifying medical protective equipment and the effect of the flow rate on the microenvironment of the equipment. METHODS: The filtration efficiency of high efficiency particulate air (HEPA) filter was measured with the biologic aerosol of simulating virus (*Escherichia coli* bacteriophage f(2)). The simulation work was done at the walk rate of 4 km/h in summer. The effect of the flow rate on the oxygen content, the carbon dioxide content, the temperature and the humidity of the microenvironment of the equipment was investigated. The clinical experiments were conducted in three appointed hospital for fighting against SARS. RESULTS: The HEPA filter could filtrate 99.99% simulating viruses in the air. When the flow rate ranged from 75 to 125 L/min, the microenvironment parameters of the equipment were: the oxygen content was between 19.6% and 20.1% (the physiological safety limit is more than 14.6%); the carbon dioxide content ranged from 0.43% to 0.57% (the physiological safety limit is less than 1.0%); the temperature was between 32.0 degrees C to 32.2 degrees C; the humidity ranged from 49.7% to 59.4% (the physiological safety limit is the temperature 31 degrees C and the humidity 85% or temperature 38 degrees C and humidity 50%). Each microenvironment parameter met the demand of a healthy person under the normal workload. In the clinical experiments, the doctors wearing the equipment who performed the tracheotomy for a SARS patient in a deep coma were not infected. CONCLUSION: The medical protective equipment can protect the doctor and nurse in SARS contaminated areas effectively and improve their work conditions.

148. Tinsley,C.R., Bille,E., Nassif,X. (2006). **Bacteriophages and pathogenicity: more than just providing a toxin?** *Microbes Infect.* 8:1365-1371. **Abstract:** An increasing number of pathogenicity factors carried by bacteriophages have been discovered. This review considers bacteriophage-bacterium interaction and its relation to disease processes. We discuss the search for new bacteriophage-associated pathogenicity factors, with emphasis on recent advances brought by the use of genomic sequence data and the techniques of genomic epidemiology.

149. Tukel,C., Sanlibaba,P., Ozden,B., Akcelik,M. (2006). **Identification of adsorption inhibition, restriction/modification and abortive infection type phage resistance systems in *Lactococcus lactis* strains.** *Acta Biol. Hung.* 57:377-385. **Abstract:** 98 *Lactococcus lactis* strains were isolated from traditional fermented milk products in Turkey tested against 60 lactococcal lytic phages to determine their resistance levels. While 82 *L. lactis* strains were sensitive against lactic phages at different levels, 16 *L. lactis* strains showed resistance to all phages tested. Types of phage resistance among 16 *L. lactis* strains were identified as phage adsorption inhibition in eight strains, restriction/modification in six strains and abortive infection (heat sensitive phage resistance) in two strains, using three broad-spectrum phages phi pll 98-32, phi pld 67-42 and phi pld 67-44.

150. van den Broek,P.J. (2005). **[Meningococci infection by a bacteriophage with a virulence factor].** *Nederlands tijdschrift voor geneeskunde* 149:2600-2602. **Abstract:** Meningococci are bacteria dreaded for their ability to kill young people. However, meningococci and humans usually live together peacefully. In a minority of cases, the co-existence results in disease. Recently, whole genome comparisons between hyperinvasive clones and clones not associated with disease revealed that a chromosomally integrated bacteriophage was related to invasiveness. Many examples of bacteriophage-encoded virulence factors are known--as such, this finding is not remarkable. However, the way this virulence factor was found is a nice example of unravelling the pathogenesis of infectious diseases in the genomic era.

151. Vantarakis,A., Venieri,D., Komninou,G., Papapetropoulou,M. (2006). **Hybridisation of F+ RNA coliphages detected in shellfish samples with oligonucleotide probes to assess the origin of microbiological pollution of shellfish.** *Water Sci. Technol.* 54:219-223. **Abstract:** Current measures for controlling the public health risks associated with bivalve molluscan shellfish consumption rely on the use of *Escherichia coli* to indicate the sanitary quality of shellfish harvesting areas. However, it has been demonstrated that *E. coli* is an inadequate indicator of the viral risk associated with shellfish. An alternative indicator, male-specific B+ coliphages, have been investigated as viral indicators of faecal contamination that may provide source-specific information for impacted environmental waters. This study compared the distribution of *E. coli* and F+ RNA bacteriophages in shellfish grown in harvesting areas of Greece and also examined the presence and proportions of the different subgroups of F+ RNA coliphages in shellfish. F+ RNA bacteriophages were present in shellfish at higher concentrations than *E. coli*. Elevated numbers of F+ RNA bacteriophages observed in the winter concur with the known increased viral risk associated with shellfish harvested at that time of year in Greece. The majority of F+ RNA coliphages detected in shellfish samples belonged to group IV which indicated the possible presence of animal faecal material in sample harvesting areas. Phages of groups II and III (human waste and human faecal material, respectively) were present at low levels. Finally, 8% of the phages hybridised were found to belong to group I. The presence of group IV showed

seasonal distribution (more in winter, less in summer) whereas the other groups did not show any difference. Monitoring of F+ coliphage subgroups may indicate the presence and major sources of microbial inputs to surface waters; however, environmental effects on the relative occurrence of different groups need to be considered.

152. Vidales-Contreras,J.A.**,** Gerba,C.P.**,** Karpiscak,M.M.**,** Acuna-Askar,K.**,** Chaidez-Quiroz,C. (2006). **Transport of coliphage PRD1 in a surface flow constructed wetland.** *Water Environ. Res.* 78:2253-2260. **Abstract:** A tracer study was conducted in a 3-ha surface flow constructed wetland to analyze transport performance of PRD1, an enteric virus model. The convection-dispersion equation (CDE), including a first-order reaction model, adequately simulated transport performance of PRD1 in the wetland under an average hydraulic loading rate of 82 mm/d. Convective velocity (v) and longitudinal dispersion coefficient (D) were estimated by modeling a conservative tracer (bromide) pulse through the wetland. Both PRD1 and bromide were simultaneously added to the entering secondary treated wastewater effluent. The mass of bromide and PRD1 recovered was 76 and 16%, respectively. The PRD1 decay rate was calculated to be 0.3/day. The findings of this study suggest that the CDE model and analytical moment equations represent a suitable option to characterize virus transport performance in surface flow constructed wetlands.

153. Vitiello,C.L.**,** Merril,C.R.**,** Adhya,S. (2005). **An amino acid substitution in a capsid protein enhances phage survival in mouse circulatory system more than a 1000-fold.** *Virus Res.* 114:101-103. **Abstract:** In experiments with germ free mice, free from adaptive antibodies to the bacterial virus lambda phage, titers of the virus in the circulatory system have been reported to decrease by more than 10(9)pfu within 48 h of intraperitoneal intravenous or oral administration. Based on these observations, serial passage techniques have been used to select lambda phage mutants, with 13,000-16,000-fold greater capacity to remain in the mouse circulatory system 24h after intraperitoneal injection. In these prior studies the "long-circulating" phage, designated lambdaArgo phage, had at least three mutations including one in the major phage capsid (E) protein, which resulted in the change of glutamic acid to a lysine at residue 158. In the current study, we demonstrate that this single specific substitution in the E protein is sufficient to confer the "long-circulating" phenotype. The isogenic pair of phage developed in this study consisting of the long-circulating marker-rescued lambdaArgo phage, and the parental wild type phage can be used for studies of viral recognition mechanisms of the innate immune system and for the development of more effective antibacterial therapeutic phage strains.

154. Walker,C.B.**,** Stolyar,S.S.**,** Pinel,N.**,** Yen,H.C.**,** He,Z.**,** Zhou,J.**,** Wall,J.D.**,** Stahl,D.A. (2006). **Recovery of temperate *Desulfovibrio vulgaris* bacteriophage using a novel host strain.** *Environ. Microbiol.* 8:1950-1959. **Abstract:** A novel sulfate-reducing bacterium (strain DePue) closely related to *Desulfovibrio vulgaris* ssp. *vulgaris* strain Hildenborough was isolated from the sediment of a heavy-metal impacted lake using established techniques. Although few physiological differences between strains DePue and Hildenborough were observed, pulse-field gel electrophoresis (PFGE) revealed a significant genome reduction in strain DePue. Comparative whole-genome microarray and polymerase chain reaction analyses demonstrated that the absence of genes annotated in the Hildenborough genome as phage or phage-related contributed to the significant genome reduction in strain DePue. Two morphotypically distinct temperate bacteriophage from strain Hildenborough were recovered using strain DePue as a host for plaque isolation.

155. Wang,J.**,** Hu,B.**,** Xu,M.**,** Yan,Q.**,** Liu,S.**,** Zhu,X.**,** Sun,Z.**,** Tao,D.**,** Ding,L. **,** Reed,E.**,** Gong,J.**,** Li,Q.Q.**,** Hu,J. (2006). **Therapeutic effectiveness of bacteriophages in the rescue of mice with extended spectrum beta-lactamase-producing *Escherichia coli* bacteremia.** *Int. J. Mol. Med.* 17:347-355. **Abstract:** The emergence of multidrug-resistant bacteria has become a global crisis. Accumulating evidence shows that bacteriophages (phages) can rescue animals from a variety of lethal infections and be effective in treating drug-resistant infections in humans. Enterobacteriaceae, producing extended spectrum beta-lactamase enzymes (ESBLs), are resistant to a broad range of beta-lactamase antibiotics. One of the most common ESBL-producing gram-negative bacilli in Enterobacteriaceae is *Escherichia coli*. Since ESBL-producing *E. coli* poses a formidable challenge in the management of critically ill patients with bacterial infections, we undertook this study to explore the possible therapeutic utility of phages to control ESBL-producing *E. coli* infections. The phage O9882 used in this study was isolated from our hospital sewage and has lytic activity against a broad range of clinical isolates of ESBL-producing *E. coli*. ESBL-producing *E. coli* strains (n=30) were isolated in the clinic, and one of them was used to induce bacteremia in a murine model. Bacteremia was established by intraperitoneal (i.p.) injection of 3 x 10(7) CFU/ml, the minimum lethal dose (MLD) of bacterium in this animal model. Mice infected with the MLD of this strain alone died within 14 h, whereas a single i.p. inoculation of O9882 (MOI > or =10(-4)) given 40 min after the bacterial challenge led to 100% survival at 24-168 h, compared to 0% survival of saline-treated controls. Protection was obtained even when administration of the phage was delayed up to 60 min after the bacterial infection and the survival rate of infected animals was 60% at 168 h. Furthermore, it was shown that the therapeutic efficacy of O9882 in lethal systemic infection in our model is due to the functional capability of the phage and not the nonspecific immune effects. Our data both in vitro and in vivo revealed that: i) the protection of mice from death

occurred only in animals infected with selected bacterial strains and the virulent phage specific to them; ii) when the phages were heat-inactivated, survival of the infected mice was strikingly decreased to 0; and iii) the level of antibody against the phage was not substantially elevated when the bacteremic animals were protected by the phage. The present findings indicate that phages can effectively rescue our mouse model from bacteremia and death, and thus provide the rationale and framework to evaluate the therapeutical efficacy of lytic phages against fatal ESBL-producing *E. coli* infections in humans.

156. Weitz,J.S.**,** Hartman,H. (2006). **Phage in the time of cholera.** *The Lancet infectious diseases* 6:257-258. **Abstract:** [first paragraph] Bacteriophage (bacterial viruses) were heralded as revolutionary therapeutic agents soon after the discovery by Felix d'Herelle in 1917 of an "invisible microbe" capable of lysing bacteria.1 Bacteriophage appeared to be effi cient killers of their bacterial hosts- we now know that their life history is far more complex than fi rst assumed2-and so the effort to use phage as curatives or prophylaxis spread quickly to research institutes in Europe, North America, and Asia.3 d'Herelle himself spearheaded many of these eff orts, the most famous of which was the initiation of an extensive campaign to use phage in the treatment and prevention of cholera in colonial India. The authors of one such study4 conclude by noting that "the results establish sufficient probability in favour of a significant effect of the administration of bacteriophage to form a basis of practical policy in the treatment and prevention of cholera in villages".

157. Wilhelm,S.W.**,** Carberry,M.J.**,** Eldridge,M.L.**,** Poorvin,L.**,** Saxton,M.A.**,** Doblin,M.A. (2006). **Marine and freshwater cyanophages in a Laurentian Great Lake: evidence from infectivity assays and molecular analyses of g20 genes.** *Appl. Environ. Microbiol.* 72:4957-4963. **Abstract:** While it is well established that viruses play an important role in the structure of marine microbial food webs, few studies have directly addressed their role in large lake systems. As part of an ongoing study of the microbial ecology of Lake Erie, we have examined the distribution and diversity of viruses in this system. One surprising result has been the pervasive distribution of cyanophages that infect the marine cyanobacterial isolate *Synechococcus* sp. strain WH7803. Viruses that lytically infect this cyanobacterium were identified throughout the western basin of Lake Erie, as well as in locations within the central and eastern basins. Analyses of the gene encoding the g20 viral capsid assembly protein (a conservative phylogenetic marker for the cyanophage) indicate that these viruses, as well as amplicons from natural populations and the ballast of commercial ships, are related to marine cyanophages but in some cases form a unique clade, leaving questions concerning the native hosts of these viruses. The results suggest that cyanophages may be as important in freshwater systems as they are known to be in marine systems.

158. Williamson,S.J.**,** Paul,J.H. (2006). **Environmental factors that influence the transition from lysogenic to lytic existence in the ϕHSIC/*Listonella pelagia* marine phage-host system.** *Microb. Ecol.* 52:217-225. **Abstract:** The marine phage varphiHSIC has been previously reported to enter into a pseudolysogenic-like interaction with its host *Listonella pelagia*. This phage-host system displays behaviors that are characteristic of both pseudolysogeny and lysogeny including a high rate of spontaneous induction and chromosomal integration of the prophage. To determine what parameters may influence the transition from lysogenic to lytic existence in the varphiHSIC/L. pelagia phage-host system, cultures of this organism were incubated under different environmental conditions, while host cell growth and bacteriophage production were monitored. The environmental parameters tested included salinity, temperature, a rapid temperature shift, and degree of culture aeration. The highest titers of phage were produced by HSIC-1a cells grown in high-salinity nutrient artificial seawater media (67 ppt with a natural salinity equivalent of 57 ppt) or those cultured in highly aerated nutrient artificial seawater media (cultures shaken at 300 rpm). Conversely, the lowest titers of phage were produced under low salinity or rate of aeration. In general, conditions that stimulated growth resulted in greater lytic phage production, whereas slow growth favored lysogeny. These results indicate that elevated salinity and aeration influenced the switch from lysogenic to lytic existence for the phage varphiHSIC. These results may have implications for environmental controls of the lysogenic switch in natural populations of marine bacteria.

159. Wilson,W.H.**,** Schroeder,D.C.**,** Ho,J.**,** Canty,M. (2006). **Phylogenetic analysis of PgV-102P, a new virus from the English Channel that infects *Phaeocystis globosa*.** *J. Mar. Bio. Assoc. UK* 86:485-490. **Abstract:** A new virus that infects the harmful algal bloom-forming microalga *Phaeocystis globosa* was isolated from surface water in the English Channel off the coast of Plymouth, UK, in May 2001. Phylogenetic analysis of the DNA polymerase gene revealed the virus isolate, designated PgV-102P, belongs to the family Phycodnaviridae, a group of large double-stranded DNA viruses known to infect algae. Basic characterization of PgV-102P revealed it was a lytic virus with a relatively slow culture lysis period of 10-days. The genome size (176kbp) and capsid diameter (98nm) of PgV-102P fall at the bottom end of the range expected for phycodnaviruses. Interestingly, PgV-102P did not cluster with other *P. globosa* viruses; instead, it was more closely related to other prymnesioviruses that infect the marine prymnesiophyte *Chrysochromulina brevifilum*. We discuss the effectiveness of DNA polymerase as a diagnostic

marker. Although it is ideal for determining what family or even genus an algal virus belongs to, it is clear that the DNA polymerase gene does not have sufficient resolution when looking for relationships within algal virus genera.

160. Xie,H., Zhuang,X., Kong,J., Ma,G., Zhang,H. (2005). **Bacteriophage Esc-A is an efficient therapy for *Escherichia coli* 3-1 caused diarrhea in chickens.** *J. Gen. Appl. Microbiol.* 51:159-163. **Abstract:** The bacteriophage Esc-A was isolated from sewage by using the intestinal pathogenic *Escherichia coli* 3-1 as the host. Toxicity in chickens showed its safety as a bio-product. Phage therapy against diarrhea in chickens indicated that Esc-A could decrease the death rate more efficiently compared with antibiotic treatments.

161. Yacoby,I., Shamis,M., Bar,H., Shabat,D., Benhar,I. (2006). **Targeting antibacterial agents by using drug-carrying filamentous bacteriophages.** *Antimicrob. Agents Chemother.* 50:2087-2097. **Abstract:** Bacteriophages have been used for more than a century for (unconventional) therapy of bacterial infections, for half a century as tools in genetic research, for 2 decades as tools for discovery of specific target-binding proteins, and for nearly a decade as tools for vaccination or as gene delivery vehicles. Here we present a novel application of filamentous bacteriophages (phages) as targeted drug carriers for the eradication of (pathogenic) bacteria. The phages are genetically modified to display a targeting moiety on their surface and are used to deliver a large payload of a cytotoxic drug to the target bacteria. The drug is linked to the phages by means of chemical conjugation through a labile linker subject to controlled release. In the conjugated state, the drug is in fact a prodrug devoid of cytotoxic activity and is activated following its dissociation from the phage at the target site in a temporally and spatially controlled manner. Our model target was *Staphylococcus aureus*, and the model drug was the antibiotic chloramphenicol. We demonstrated the potential of using filamentous phages as universal drug carriers for targetable cells involved in disease. Our approach replaces the selectivity of the drug itself with target selectivity borne by the targeting moiety, which may allow the reintroduction of nonspecific drugs that have thus far been excluded from antibacterial use (because of toxicity or low selectivity). Reintroduction of such drugs into the arsenal of useful tools may help to combat emerging bacterial antibiotic resistance.

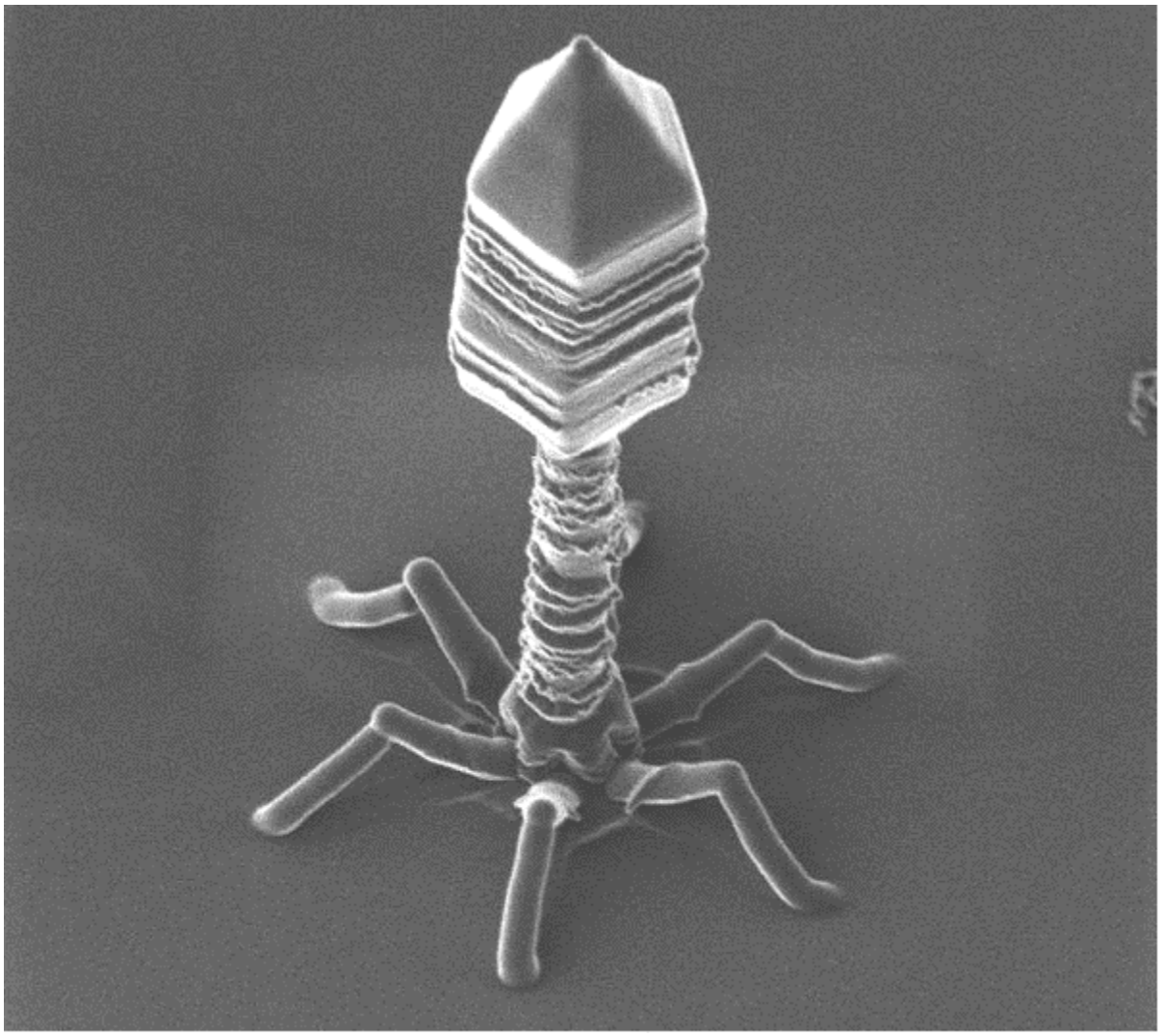
162. You,L., Yin,J. (2006). **Evolutionary design on a budget: robustness and optimality of bacteriophage T7.** *Systems Biol.* 153:46-52. **Abstract:** Exploring how biological systems have been 'designed' by evolution to achieve robust behaviours is now a subject of increasing research effort. Yet, it still remains unclear how environmental factors may contribute to this process. This issue is addressed by employing a detailed computer model for the intracellular growth of phage T7. More than 150 000 in silico T7 mutants were generated and the rates and efficiencies of their growth in two host environments, namely, a realistic environment that offered finite host resources for the synthesis of phage functions and a hypothetical environment where the phage was supplied infinite host resources, were evaluated. Results revealed two key properties of phage T7. First, T7 growth was overall robust with respect to perturbations in its parameters, but fragile with respect to changes in the ordering of its genetic elements. Secondly, the wild-type T7 had close to optimal fitness in the finite environment. Furthermore, a strong correlation was found between fitness and growth efficiency in the finite environment. The results underscore the potential importance of the environment in shaping robust design of a biological system. In particular, the strong correlation between fitness and growth efficiency suggests that T7 may have evolved to maximise its growth rate by minimising waste of finite resources.

163. Yu,M.X., Slater,M.R., Ackermann,H.W. (2006). **Isolation and characterization of *Thermus* bacteriophages.** *Arch. Virol.* 151:663-679. **Abstract:** One-hundred-fifteen bacteriophage strains were isolated from alkaline hot springs in Iceland, New Zealand, Russia (Kamchatka), and the U.S.A. The phages belonged to the *Myoviridae*, *Siphoviridae*, *Tectiviridae*, and *Inoviridae* families. Over 50% of isolates were isometric or filamentous. One type of siphovirus had giant tails of over 800 nm in length. Phages were further characterized by host range, genome size, DNA restriction endonuclease digestion patterns, and temperature and pH sensitivity. Myoviruses and tectiviruses had a worldwide distribution. Most phages were narrowly host-specific and all were highly resistant against heating and alkaline and acidic pH. This is the first time that tectiviruses and filamentous phages are reported for bacteria of the *Thermus-Deinococcus* phylum. The presence of tectiviruses, inoviruses, and myoviruses is attributed to acquisition from ancestral gamma-proteobacteria by horizontal gene transfer.

164. Zhang,X., Kong,J., Qu,Y. (2006). **Isolation and characterization of a *Lactobacillus fermentum* temperate bacteriophage from Chinese yogurt.** *J. Appl. Microbiol.* 101:857-863. **Abstract:** AIMS: The aim of this study was to investigate the properties of temperate bacteriophage of *Lactobacillus fermentum*, based on its morphology, restriction patterns, protein profile and the impact on the growth of host strain. METHODS AND RESULTS: With Mitomycin C, seven temperate phages were induced from Lactobacilli derived from Chinese yogurt. The temperate phages induced belong to the most common Bradley's group B, having hexagonal head and long, noncontractile tail. They were furthermore confirmed to be the same bacteriophage by identical restriction patterns. SDS-PAGE profile showed that the phage studied had one major structure protein about 31.9 kDa. The presence of the prophage influenced the cell shape and colony size of its lysogenic strain. CONCLUSIONS: The phage obtained

had similar, but not complete identical properties with other *L. fermentum* phages reported. It influenced the growth behaviour of its lysogenic strain. SIGNIFICANCE AND IMPACT OF THE STUDY: This study provides some information about bacteriophages occurring in the Chinese yoghurt manufacture and contributes to our knowledge on the bacteriophage diversity in the dairy industry.

(see www.phage.org/beg_mission_statement.htm for
why papers covering more than just bacteriophages are included)



From the 2005 Micrograph Contest of the International Conference of Electron, Ion, and Photon Beam technology and Nanofabrication
http://www.zyvexlabs.com/EIPBNuG/2005MicroGraph.html

Yes, this is a microscopic and almost-diamond-like rendiction of phage T4!

Quoting the page the image is found on:

TITLE: Artificial Nano “T4 Bacteriophage”
Description: “T4 Bacteriophage” is a virus like the robot in the living body.
Artificial nano “T4 Bacteriophage” was fabricated by FIB-CVD on Si surface.
Size of the artificial nano “T4 Bacteriophage” is about ten times as large as the real virus.
It is made of Diamond-like Carbon. It is likely to begin to walk in the nano space!!
Magnification: 25,000X
Instrument: SII NanoTechnology Inc. / SMI2050MS2
Submitted by: Reo Kometani & Shinji Matsui (University of Hyogo)