Exact structure of bacteriophage T4 and infection: Stopping *Escherichia coli*

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Experimental–theoretical works explain the energetics of the packing of a virus with deoxyribonucleic acid (DNA) and the injection of the DNA into a cell. Washing has a limited effect on enteric viruses in food. Alternatives are needed to acidic matrixes. Feline calicivirus is not a good control model. Various protocols are being prepared. New challenges are: new methods, impact of new technologies, significance of detection, new virus models and emergent–re-emergent viruses. The forthcoming step is to re-develop the virus to direct it and contain the most common contaminants in food: salmonella, listeria, staphylococcus and *Mycobacterium tuberculosis*.

Key words: *T4 bacteriophage, Escherichia coli, O_{157}:H_7, food contamination, food epidemic*

INTRODUCTION

Today, more than 50 years after Max Delbrück's *phage group*, it turns out that the bacterial viruses (commonly called *bacteriophages* or *phages*) are again occupying centre stage, but this time in a rather different context.[1] Using 21st-Century ideas and methods, researchers have been able to measure the physical properties of phages as the inanimate, mechanical objects that they are.

With much energy required to confine the genome, there is a correspondingly large pressure in the capsid, *ca*. 40atm.

What are the biological consequences of a highly pressurized capsid? To appreciate this question, consider the typical life cycle of a bacterial virus, *e.g.*, a salmonella cell 30min after being infected by bacteriophage P22. Consider the situation in which two P22 phages have bound to receptors in the outer membrane of the bacterium. That triggers the opening of the capsid and releases the genome. Unlike the situation in which the capsid is ruptured and the deoxyribonucleic acid (DNA) spills out in all directions, here the head and tail remain intact and the DNA is ejected along its length *via* the tail into the bacterial cell. That kind of controlled and directed release is essential to the survival of the virus because its genome can only be replicated, and its genes translated into protein products, if its DNA gets inside the host cell. The genome replication and expression of viral protein result in the production of a large number of new viral particles. They accumulate to high densities, as witnessed by their formation of an ordered array in the cell's interior before they are released when the cell ruptures. Xiang *et al*. analyzed the structural changes of bacteriophage ϕ29 upon DNA packaging and release.[2] Cohen *et al*. studied the shared catalysis in virus entry and bacterial cell wall depolymerization.[3]

The scenario above was established after many years of work by the phage group, culminating in Alfred Hershey and Martha Chase's famous 1952 experiment in which the protein shell was definitively shown to remain outside the host cell, with only the viral genome entering. The demonstration confirmed DNA as the *transforming principle* of life because it, not proteins, carries the genetic information. Less conspicuously, the experiment raised the physical question of what drives the injection of the phage genome into the cell, an issue that lay dormant for decades because of the ensuing rush to control the more fundamental questions of molecular biology, *e.g.*, how DNA replication and protein syntheses occur.

Since the find of viruses in the 19th Century, bacteriophages (which are DNA viruses) have been used against bacteria. After the discovery of penicillin (Fleming, 1928) and globallization of the progressive use of antibiotics, the employment of bacteriophages against bacteria was given up in the Occidental Countries. However, the rise of bacterial resistance to antibiotics caused the use of alternative weapons, *e.g*., bacteriophages as antibacterial. Bacteriophages have been successfully used to localized infections in burns, control of cholera–typhus by sprinkling water tanks, against salmonella in farming animals (antibiotics are forbidden because of resistances that pass to humans), *etc*. Notice that the main difference from antibiotics is that bacteriophages replicate. Bacteriophages have potential advantages over antibiotics: viruses are innocuous to all but their target (*e.g*., bacteriophages are harmless against the intestinal flora), the dose is lower because viruses go to there where there are more bacteria, viruses act with maximal concentration where there are more bacteria, viruses evolve, *etc*. From the bacteriophage group we are interested in the ones of lytic type. The latency period is 0.5–2h. There is interest in food science and technology in administering bacteriophages to farm animals, *e.g*., chicken, pig, *etc*., to fight salmonella. It is interesting to know the optimal latency period in bacteriophages and the role of the age structure of infection.

RESULTS AND DISCUSSION

Figure 1 shows the exact structure of the T4 bacteriophage and the infective process.

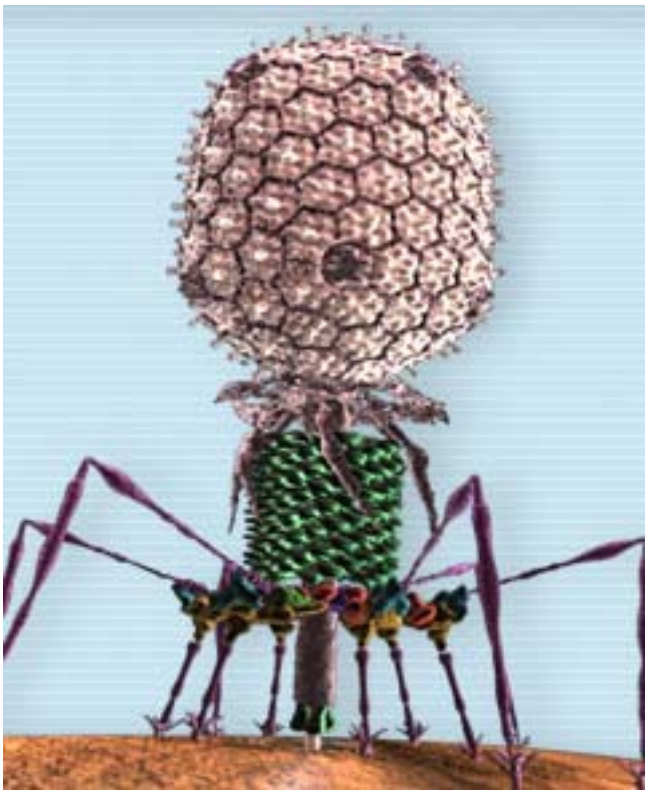

Fig. 1. Exact structure of the T4 bacteriophage and infective process.[18]

Figure 2 shows the three-dimensional (3D) visualization of genetically engineered endobacteria phage T4, which can detect and detain a virulent cell of *Escherichia coli* that causes countless epidemics of diseases originated in contaminated foods. The phage attacks pathogen cells and inject them its DNA, which makes contaminated foods luminescent or coloured in red.

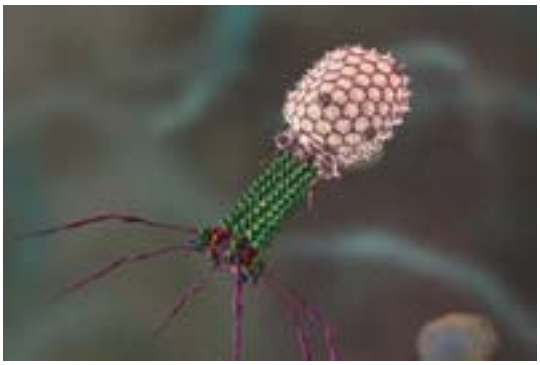

Fig. 2. 3D visualization of genetically engineered enterobacteria phage T4.[18] Image created by Seyet LLC in collaboration with the Michael G. Rossmann laboratory at Purdue University.

Viruses are forced intracellular parasites. Enteric viruses from animal origin do not usually affect humans. Most enteric viruses are of ribonucleic acid (RNA). For many enteric viruses the transmission route is faecal–oral; the rest, which are minority, need a carrier, *e.g*., contaminated water, food, blood, *etc*. Enteric viruses resist months, *e.g*., in shellfish, *etc*. Shellfish filters water and accumulates suspension materials including viruses. In fresh foods, *e.g*., berries (raspberry, strawberry, *etc*.), vegetables (greenness, garden produce, *etc*.), *etc*., enteric viruses come from irrigation water or harvesting (harvester). The foods prepared in buffets can be infected with virus by the preparation process. Some haemoderivatives can be infected with hepatitis A virus (HAV). When it is compared between classes noroviruses infect with great frequency *via* foods, *e.g*., shellfish, frozen raspberry, *etc*. The HAV infects with little frequency *via* these foods but causes great mortality. Rotaviruses and hepatitis C (HCV) and E (HEV) viruses infect frequently *via* water. For some emergent viruses, *e.g*., stationary, A and avian influenzas, there is no prove that they are transmitted *via* foods. The characteristics of enteric viruses are that they do not multiply in foods, need 10^4–10^8 particles to infect, are favoured in cold (winter) and resist the intestinal tract (acidity, *etc*.). A solution consistent in heating the food at 95ºC for 1.5min was reported. However, some problems remain to be solved, *e.g*., HAV is excreted by humans 1–2 weeks before the symptoms appear. In ancient times with poor hygienic conditions HAV infected children, which was slight and immunized them. Nowadays with better hygienic conditions HAV infects nonimmunized adults, which results grave. Noroviruses are excreted by humans 1–2 weeks after the symptoms disappear, whereas workers return to work after only two days. Therefore, it is imperative to keep good hygiene practices avoiding the transmission of diseases. When compared with other causes of infection, noroviruses are rather frequent (70% of infections), but they are difficult to diagnose or remain without diagnosis. On the other hand, campylobacter, *E. coli* and salmonella are less frequent.

Sánchez *et al*. studied the capsid region involved in HAV binding to glycophorin A of the erythrocyte membrane.[4] They discovered a novel $CD4^+$ T-helper lymphocyte epitope in the VP3 protein of HAV.[5] They molecularly characterized HAV isolates from a transcontinental shellfish-borne outbreak.[6] They analyzed the genome variability and capsid structural constraints of HAV.[7] They evidenced for quasispecies distributions in the human HAV genome.[8]

Nestlé Company detected enteric viruses in bottled waters. Beuret *et al*. informed Norwalk-like virus sequences detected by reverse transcription-polymerase chain reaction (RT-PCR) in mineral waters imported into or bottled in Switzerland.[9] They analyzed Norwalk-like virus sequences in mineral waters by one-year monitoring of three brands.[10] Lamothe *et al*. reported RT-PCR analysis of bottled and natural mineral waters for the presence of noroviruses.[11] Sánchez *et al*. noticed that the presence of norovirus sequences in bottled waters is questionable: it is frequent to detect genes of enteric viruses in bottled waters but not infective viruses.[12] Therefore bottled waters are not a health risk. However, other foods or their raw materials are a health risk. Nestlé considered three types of foods: shellfish (with absence of enteric viruses in Nestlé by thermal treatment), fresh foods (*e.g*., berries and vegetables) and prepared foods. Formerly there was no analytic method and a new one had to be tuned. This new method had to be rather sensitive, *e.g*., shellfish in samples tied to outbreaks contains only 10^3–10^5 copies·g^{-1}. The impact of technological processes is important. The respiratory feline calicivirus (FCV) was used as a control. However, respiratory viruses are sensitive to p*H* (inactivated by acid, *e.g*., strawberry, raspberry, *etc*.), whereas gastrointestinal viruses are not: *e.g*., strawberry and raspberry deactivate respiratory viruses whereas they do not deactivate gastrointestinal viruses, which are difficult to eliminate. Some treatments (*e.g*., water chlorinated at 200 p.p.m., $ClO_{2(g)}$, lyophilización, *etc*.) can be either effective or not, depending on the matrix and the virus.

Costafreda *et al*. informed the development, evaluation and standardization of a real-time TaqMan RT-PCR assay for quantification of HAV in clinical and shellfish samples.[13] Butot *et al*. studied the attachment of enteric viruses to bottles.[14] Some treatments are effective in some cases: lyophilización plus thermal treatment at 80ºC (a standard against fungi and ants), 100ºC, 120ºC and vapour bleaching at 75ºC for 2.5min. In summary the effectiveness of a treatment depends on the matrix and the virus.

DIFFERENTIATION OF *Escherichia coli* FROM COLIFORMS

Escherichia coli detection is possible because the bacterium ferments dextrose (D-glucose) by producing mixed acids (*e.g*., lactic, acetic and formic acids) that can then be made visible with the addition of the indicator methyl red.[15] There are many other methods of detection to indicate the presence of *E. coli*; *e.g*., Voges and Proskauer found a test to detect acetoin and 2,3-butanediol produced when Klebsiella and *Enterobacter* ferment glucose. They found that under alkaline conditions, these two compounds oxidize themselves into diacetyl. Diacetyl then reacts with creatine (a guanidine derivative) and appears as a pinkish-red compound, or it reacts with α-naphthol and appears cherry-red in colour.

Some other characteristic enzymes can also be detected by their interactions. Tryptophanase cleaves tryptophan into pyruvate, indol and ammonia; by using reagents (Kovac's and dimethylcarbazole), researchers can detect indole production. β-Galactosidase is detected with 2-nitrophenyl β-D-galactopyranoside, a chromogenic substrate that turns yellow after cleavage has occurred. Furthermore, the ability to reduce nitrate to nitrite can be detected with the addition of sulphanilic acid and β-naphthylamine, which results in a red precipitate (prontosil). Finally, lysine is degraded by *E. coli* to cadaverine by the lysine decarboxylase. Because this is an alkaline reaction, the indicator (bromocresol purple) will change colour from yellow to purple.

Interesting differentiation results are obtained with the inoculation of TSI Agar slants. Because of the formation of acid during fermentation of lactose, sucrose and glucose, the p*H* level usually drops. However in the case of oxidative decarboxylation of peptone alkaline products the p*H* rises. This increase is indicated by phenol red, which changes colour in acidic surroundings from red-orange to yellow; upon alkalinization it turns deep red. *Escherichia coli* shows an acid reaction (yellow) and gas formation in the butt of the test tube and an acid reaction (yellow) on the slant surface.

EXPERIMENTAL

To prevent *E. coli* from reaching consumers, scientists developed a method of detecting and detaining one of its more common virulent strains, serotype O_{157}:H_7. Shiga toxin-producing *E. coli* O_{157}:H_7 (STEC O_{157}) recently emerged as a cause of diarrhoea, haemorrhagic colitis and haemolytic uremic syndrome (HUS).[16] The phylogenetic classification of *E. coli* O_{157}:H_7 strains of human and bovine origin was carried out using a novel set of nucleotide polymorphisms.[17] This bacterium is found in contaminated foods, *e.g.*, beef, vegetables and unpasteurized milk, and is estimated to infect more than 70 000 people a year. The technique could help food-processing companies track the *E. coli*'s source and even put an end altogether to outbreaks of food-borne illness.

Intelliphage, the company that developed the method, was co-founded by Dr. Bruce M. Applegate, an associate professor of food science at Purdue University and research associate Lynda Perry. The business created a nonpathogenic virus that, when incorporated into food contaminated with *E. coli,* will bind to the strain and turn the food red or make it luminesce. The transformation will alert food processing companies that their product is unfit for human consumption and must be recalled.

Applegate genetically engineered the enterobacteria phage T4 virus to target the pathogenic bacteria by growing it in a controlled environment within a nonpathogenic *E. coli*

host. When introduced into food, the phage uses long tail fibres to identify the *E. coli* cell surface receptors. A signal is sent to the baseplate of the phage, which releases short tail fibres that connect permanently to the *E. coli* cell. Upon attachment the baseplate changes and the fibres induce glycoprotein V, which punctures the outer membrane of the *E. coli*. Once the cell wall and plasma membrane are broken down, deoxyribonucleic acid from the phage is transmitted into the cell.

Prior methods of confirming the presence of *E. coli* in food took more than 24hr, but the new technique can detect the bacteria within 4hr. It can identify one bacteria cell in only 25g of food furthering its early detection capability. Injecting the T4 phage does not destroy the *E. coli* but instead detains it so that, when food companies make recalls, it is easy to track the contamination's source.

Many laboratories grow the T4 phage in an *E. coli* host, which involves large quantities of the bacteria and potentially exposes the public to large amounts of it accidentally. Intelliphage devised a way to avoid this threat by growing the phage within a nonpathogenic strain.

Food processing companies can benefit from the method without spending money on additional equipment because most companies already are equipped with luminometers.

CONCLUSIONS

From the present results and discussion the following conclusions can be drawn.

1. Experimental works can measure, and theory explain, the energetics beyond the packing of a virus with deoxyribonucleic acid and the injection of the deoxyribonucleic acid into a cell.

2. The infection process is not yet an image: it is a story if one is sometimes able to understand completely its meaning. The picture gives an a wonderful view of how the T4

bacteriophage makes its work, which is really a nanomachine. The forthcoming step is to re-develop the virus to direct it and contain the bacteria salmonella, listeria, staphylococcus and *Mycobacterium tuberculosis*, which are the most common contaminants in food.

3. Washing has a limited effect on enteric viruses in food.

4. Alternatives are needed to acidic matrixes, *e.g*., raspberry, *etc*.

5. Feline calicivirus is not a good control model.

6. Various protocols are being prepared for shellfish, water, hard surfaces (*e.g*., apple, pear, *etc*.), salad, *etc*.

7. The new challenges are: new methods, impact of the new technologies, significance of the detection, new models of virus and emergent and re-emergent viruses. With the new methods of polymerase chain reaction able to detect one only copy, many samples would result positive. Therefore the question that should be stated is: is the copy infectious?

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