BIOLOGY TERM PAPER

Phage Therapy: Phages against Bacteria

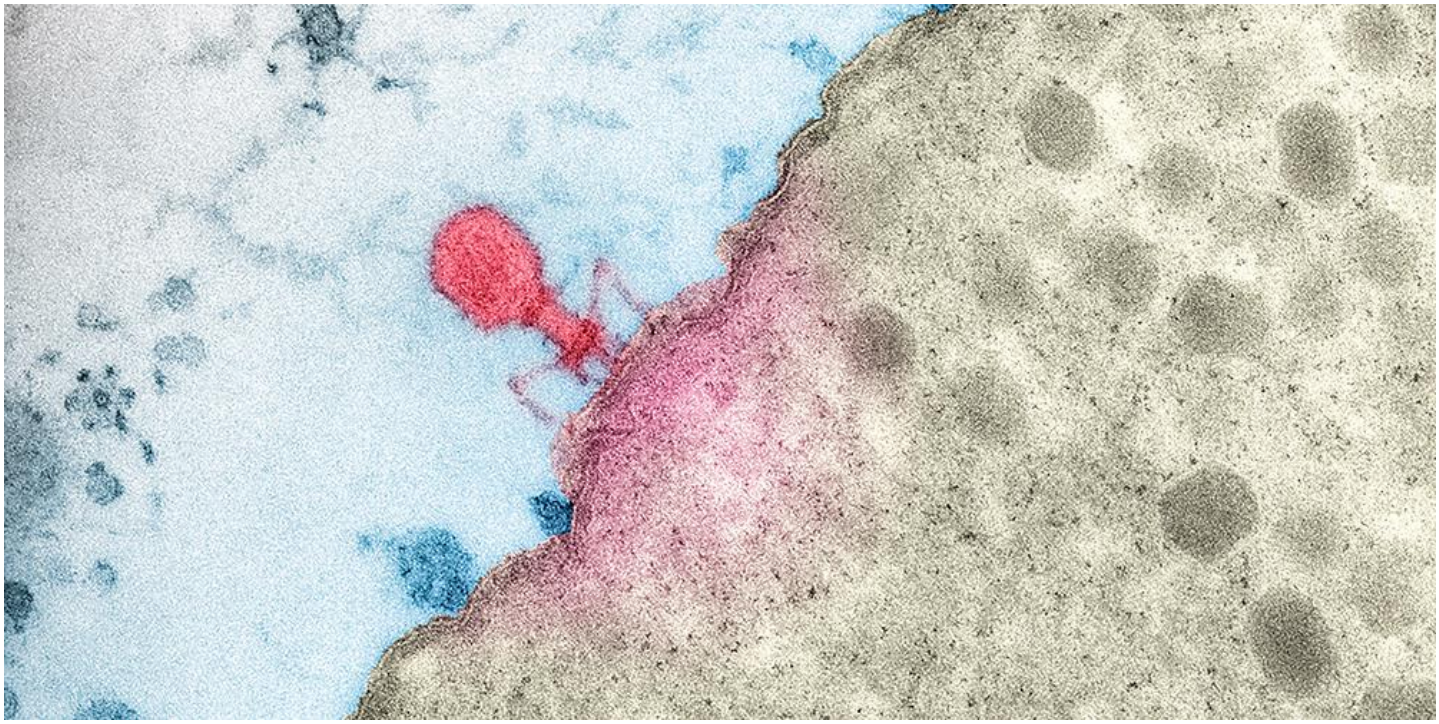

Figure 0: Picture of a phage

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Content:

Preface:

Our motivation:

The given task was writing a term paper about genetic engineering in microbiology. At first, we had big plans for writing this paper about antibiotic-resistant bacteria. We started to do our research but then focussed a commonly found organism[i], the bacteriophage, or phage for short. This ubiquitous virus, which infects bacteria and uses them as a host immediately caught our interest. They replicate very fast, so they can respond to evolutionary pressure, and can even be used for medical purposes.

We had seen different documentaries on the web, which discussed whether these viruses could save our lives or not, and so we decided to dive deeper into this topic and do our own research. We now dedicated our work to the aim of finding out how bacteriophages can be used against antibiotic-resistant bacteria, and how we can engineer and modify their health benefitting properties.

What is especially interesting:

We find the concept of using living organisms to fight diseases extremely fascinating. Furthermore, the process of phage reproduction amazed us.

The two types of phage therapy with which we deal in our paper are Phage Display and Bacterial Lysis. These two methods seem both very promising for further development in the fight against diseases. In the case of the Phage Display method, the striking and promising part is the engineering part itself. The fact that we can infuse phages with a modified DNA with which we can trick the bacteria to produce a curative effect all by itself. On the other hand, the Bacterial Lysis technique seems captivating and is definitely seen as a potential research topic, because as far as we know, this method consists of trial and error, thus more clinically supervised trials have to be conducted.

Our questions regarding the chosen topic:

After a little research, some questions came to our minds. We tried to answer a part of them by ourselves, the other part we asked Dr. Alexander Harms who works at the Biozentrum of the University of Basel and Dr. Ann-Charlott Salabarria from San Diego State University. After first reading about this topic, we naturally asked ourselves first how this therapy works. Thus, our research about phages began by finding out what they are and how they can be used to treat diseases.

Our first impression of the topic was, that it was a rather new and a not broadly used method. However, we were not sure if this impression was right because we are not acquainted with the research of medical topics. Although we got better at researching, we still ended up asking that question to Dr. Harms and Dr. Salabarria from whom we thought they might have a better overview than we have.

Before we had to find a topic for our biology term paper, we have never heard of Phage Therapy. We soon found out that part of the reason we have never heard of it is, that in most places it is not used as this treatment yet because the research is still ongoing. As phages mutate so quickly, it is not possible to produce ‘standardised’ batches needed for clinical trials. Because of that, we wanted to find out what needs to happen for Phage Therapy to be commercialized as a treatment. Every medicine has it good and bad sides, therefore we didn't expect phage therapy to be any different. We wanted to find out what is especially good about treating a disease with phage therapy and what its downsides could be.

Introduction:

The context of the chosen topic and recent events:

Considering we found out about phages and the research that is being done and has been done in the fight against MDR (multidrug resistance) Bacteria, the question of why has phage therapy not yet become a significant tool in modern medicine, despite it being such a promising treatment method, is the leading motivation to investigate more about the engineering of such treatments.

The (recent) scientific history:

In ancient times, people used to treat infections by using the curing properties of fungi, moulds, and plants. The problem being was, that they couldn't tell what the crucial curative component really was. But then in 1928 the Scottish physician Alexander Fleming first extracted a bactericidal substance and called it Penicillin after the fungi he extracted it from[ii,iii]. This ground-breaking invention changed our everyday lives significantly. We soon could cure bacterial infections extremely efficiently. This worked really well for some time but eventually, we got to see nature at its finest work. By killing a lot of bacteria, we gave the bacteria that were by coincidence resistance to antibiotics a big evolutionary advantage. Those bacteria that were resistant had a better chance of reproducing and spreading their DNA to other bacteria. Because bacteria have the ability to transfer their genes to each other, such important genes as being antibiotic resistance can spread extremely quickly. Our way of treating ill people, by bringing them all to hospitals, only adds to the problem. Hospitals are the single place where we use a lot of antibiotics while also having a lot of disease-causing bacteria. That makes hospitals the ideal place to create resistant bacteria. At the beginning of the use of antibiotic treatments, the compounds were used much too often (including in animal feedstuffs to promote weight gain), which led to an acceleration of the rate at which bacteria acquire resistance to antibiotics. We are constantly searching for new antibiotics because bacteria have become resistant to the old ones. And because we cannot really predict evolution, we are always one step behind in the fight against bacteria. As larger our population gets and as more antibiotics we use, the more bacteria will be resistant to them. That makes this topic more important today than it has ever been.

The discovery of the bacteriophage started in 1896 when Ernest Hankin, an English bacteriologist, reported that something very small in the Ganges River acted against the bacterium Vibrio cholerae (cholera), however, he could not find out what it was. 20 years later the bacteriologist Frederick Twort also discovered that something small was able to infect and kill bacteria, but he was not sure if it was an enzyme a virus or even the bacterium itself. Finally, in 1917 French-Canadian microbiologist Félix d'Hérelle discovered that it was actually a parasitic virus that killed the bacteria and he decided to call that virus bacteriophage[iv]. As soon as this was made public microbiologists started to research how this virus could be used for treating diseases caused by bacteria. Because bacteriophages store information on how to kill bacteria in their DNA it means that they also automatically evolve every time bacteria get immune against a certain type of bacteriophages. This could solve the problem we today have with antibiotics resistance. Unfortunately, in the past phage therapy research had difficulty attracting funding. Nobody really saw a use in investing when antibiotics were already proven to work. It was used in Russia during WWII but due to language barriers and the Cold War, the treatment never spread to western countries. Only Georgia took on the Russian research and is one of the few places where phage therapy is commercially available.

Application of the technique:

To date, phages are used to control and counteract diseases. These sicknesses - such as for example Salmonella, Campylobacter, E. coli and Listeria – can make people severely ill and even in some cases have resulted in the hospitalization of the affected person. Infection can even lead to death. These foodborne pathogens have an origin in animal- and plant-based products which we consume on daily basis.

Phage therapy can, for example, prevent bacteria from causing food poisoning in humans. The main reason why these bacteriophages are so incredibly effective and efficient is that the virus only affects bacteria and isn't harmful to humans, animals, or plants.

For now, only some few Eastern European countries permit commercially available phage therapy for direct use on humans. The rest of the world hasn't yet approved or licensed any treatment, mainly because we don't know enough how exactly different phage cocktails works and behave inside different patients. As long as we do not have a good understanding of these patterns, we will always face a risk of unexpected treatment failure. This risk is way too high and not considering it would be negligent. Therefore, this new technique of treatment will most likely take more time to optimize hence to become available.

What has to be done then? The most important thing which must be done is the standardization throughout preclinical and clinical studies. Based on this goal, we still must figure out some central questions:
- does the human immune system recognize the phage and could that cause side effects by mounting a viral response against the phage?
- how do you select the best phages for therapy and phage cocktail design?
- do phages and antibiotics work in synergy or against one another?

In trials, phages can be taken up through different routes. We have six different ways to take up xenobiotics: the topical route, the oral route, the intraoperative route, the intravenous route, the intrarectal route, and the inhalation route. In all of these routes, the number of phages used reaches from 9.0×10^5 PFU (Plaque Forming Unit), orally, up to 6.3×10^{10} PFU of 40 daily infusions which lasted 30 min[v]. For some diseases, there is a specific treatment route, but in general, researchers haven't found the optimal way and the right concentration nor the perfect system of evaluation of the right phages to use.

Are there any alternative treatments?

The interesting part about phage therapy is it is the alternative. We have already stated the problems that come with the use of antibiotics. The problems of the current way to treat bacterial diseases are what make phage therapy important[vi].

However, phage therapy being an alternative also causes problems. The biggest problem is funding. Even the established methods like vaccines and antibiotics have financial problems because in the best case you only must take them rarely (so that sales and return on investment will be low). Most of the medical funding goes toward anti-depressants, anti-anxiety medication, etc. because these are taken regularly, meaning investors can make a lot of profit. But if our antibiotic system crashes, we will have no other choice than funding an alternative, but ideally, it should not get that far.

Description of engineering technique:

What are phages and how do they operate?

With the rise of the new century came a lot of appreciation for bacteriophages. With phages in the uprise came the idea of using them in a therapeutical manner to treat infectious diseases with it. Today, where so many MDR (multidrug-resistant) bacteria are becoming commonplace, and antibiotics lose efficiency, phages could be the solution[vii].

> "Phages are naturally occurring viruses that infect both gram-positive and gram-negative bacteria. As such, they are generally unaffected by antibiotic resistance and are able to target bacteria encased in biofilms. A substantial volume of preclinical data and an increasing body of clinical evidence indicate the immense therapeutic potential across a wide range of infectious diseases."[1]

[1] https://www.sciencedirect.com/science/article/pii/S0149291820303489 [11.01.2021]

Phage Therapy or bacteriophage therapy is a type of therapy that uses viruses to combat and treat bacterial infections; the bacterial viruses that are used, go by the name of bacteriophages or just phages. Bacteriophages themselves do not harm humans or animals and plants in any way, they are, however, extremely effective in attacking bacteria. Phages are commonly used to treat and combat bacterial infections.

Bacteriophages may be found in soil, water, sewage and essentially all places bacteria live and can reproduce by binary fusion. These bacteriophages, or as previously stated viruses, are what represses bacteria growth in nature.

The bacteriophage consists of three basic parts. The head, a sheath and tail fibres. When the phage is near a possible host, the tail fibres allow the phage to attach to the bacterial cell[viii]. The DNA, which is stored in the head, travels down the sheath and is injected inside the host cell. Following this, the transmitted phage DNA hijacks the cellular machinery and starts replicating new proteins. When these proteins are completed and assembled, new phages will begin to form inside the bacterial cell, the bacteria literally burst from all the phages which lyses the bacterial cell rendering it dead. This process resets the phage life cycle. The phages, after successfully killing all bacteria, can then lay dormant until they are activated again.

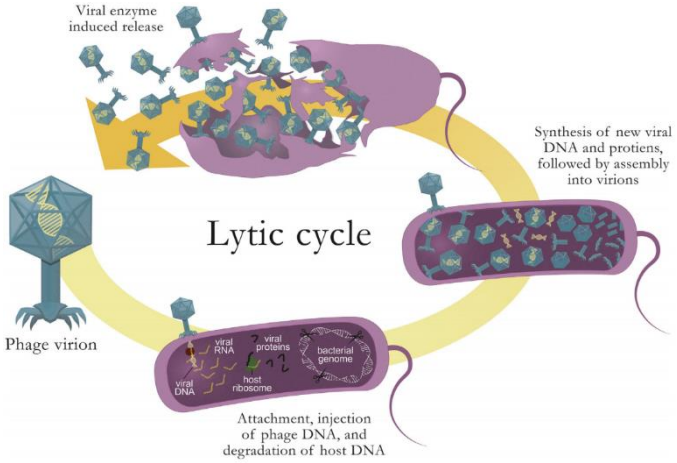

Figure 1: Description of the lytic cycle

The Bacterial Lysis technique:

The term Phage Therapy is very broad, and, in our case, it is used to describe a treatment method with phages as the main active agent.

Bacterial Lysis, as the name implies, is based on the concept of letting bacteria explode. In this method selected phages which are specialized in inserting their genotype into the corresponding bacteria are injected so they can reproduce. When they do so, the bacteria which is rendering the patient sick is spiked with the proteins from the phage, and therefore killing by itself [Figure 1]. With the constantly decreasing number of bacteria in the body, the phage population increases. With now fewer bacteria available to be attacked by the phages, it takes longer to eliminate the disease itself. Luckily, after the initial phase where phages did most of the job, the body's immune system can step in and join the fight.

When researchers try to integrate phages for health benefits, they have two options. They can either apply a Monophage or a Polyphage Therapy. The meaning behind the names is evident. In a Monophage Therapy, only one kind of phage genotype is used for the treatment. This is mostly used in experiments for demonstrative and educational purposes. In this case, phages are examined and tested for the development in an experimental model. The disadvantages of only using one single kind of phage are that it requires a precise, near-perfect match between the pathogen and the phage. For that reason, it isn't used in clinical trials, because the likelihood of this perfect match is near none. On the other hand, Polyphage Therapy, also known as Phage Cocktails, utilize a combination of different phage genotypes. On the contrary to Monophage Therapy, uses many different phages, which together target multiple genotypes of a single bacteria species or multiple species. The main problem of both methods is, as stated before, that we don't yet know how different phages correlate with the effects on the bacteria genomes and the foundation delivered by the patient's body.

With the help of two different proteins, Viron-associated peptidoglycan hydrolases, or short VAPGH, and polysaccharide depolymerase, which some phages possess, phages can work together in combination and

even decompose biofilms. These enzymes enable them to absorb, invade, and disintegrate the host bacteria. In view of these special functions of the proteins, researches now can explore and expand the development made into novel antibiotics, adjuvants for antibiotics, and bacterial biofilm disruptants.

Phages as a potential antibiotic adjuvant:

The figures below show the four main mechanisms [Figure 2] of antibiotic resistance[ix]. In this method, figure A, bacteria can either (1) excrete the antibiotic through the activation of the efflux pumps[x], or (2) decrease the uptake of antibiotics by simply mutation and changing the permeability of the outer membrane which leads to an uptake shortage. The third way (3) is to modify the target of the antibiotic, and lastly, (4) bacteria can inactivate the antibiotic through enzymes[xi]. If bacteria can initiate one of these four steps, they are of some kind resistant against the treatment method or medium.

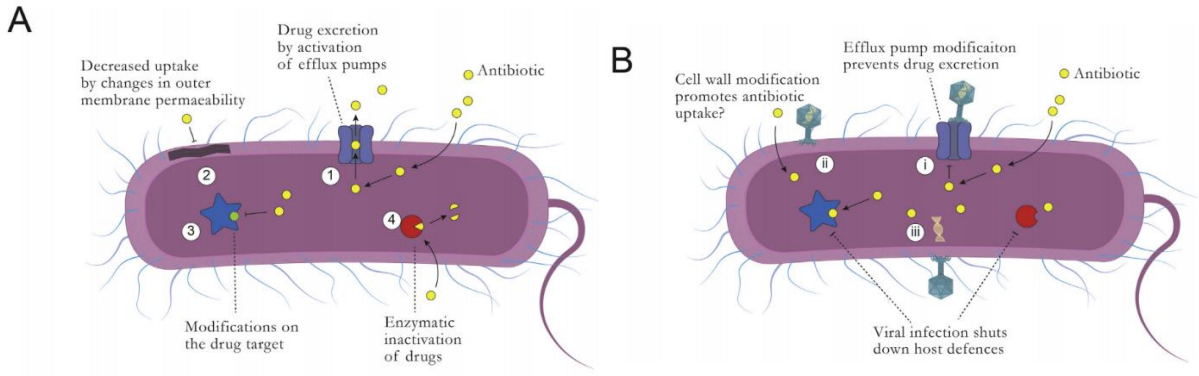

Figure 2: Phages as antibiotic adjuvants

To resensitize these MDR bacteria, phages can, figure B, potentiate the antibiotics. For example, they can (i) alter the physiological activities exhibited by the phage by a direct blockade or mutation of the drug efflux mechanism. Phages can even (ii) enhance the drug uptake by mutation of the cell wall, and (iii) inhibit the antibiotic-resistant elements.

What happens if phages mutate?

In fact, mutation phages are a problem. In the same way, bacteria mutate to become drug-resistant, it can become resistant against the phages' genotype. That is the reason why in trials researches use phage cocktails, so if it mutates, there is still a possibility to combat efficiently with the other phagial components in the cocktail. If the phages show no efficacy, it is possible, other than with antibiotics, adapt the phages on bacterial strains in the lab. The phages can even be re-evaluated by the display technique. Always manually re-establishing the efficiency of a single phage is time-consuming, and very costly. Phages have one big advantage over antibiotics. They can mutate correspondingly to the mutating bacteria. Maybe the phages lose a fraction of their performance potential, but they can still harm the bacteria to make them vulnerable enough for the body's immune system or the antibiotics to show effects. By designing phage cocktails, or always treating with antibiotics simultaneously, the chance of resistance can be decreased from the start.[xii]

The Phage-Display technique:

Phages can not only be used to simply kill bacteria. The chemistry Nobel price of 2018 was given to George Smith and Gregory Winter for finding a smarter, more effective way of fighting bacteria with phages[xiii]. In this method, called Phage Display, the Phages DNA can be altered in a way that they start producing antibodies for certain bacteria. This is done by site-directed mutagenesis[xiv,xv,xvi].

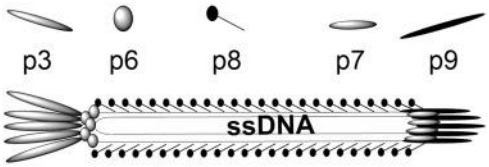

Figure 3: Model of M-13 Phage with visualisation of the different proteins

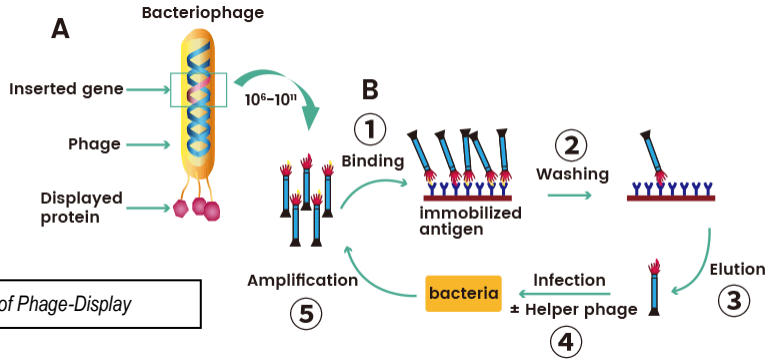

Figure 4: Steps of Phage-Display

In this procedure, an altered RNA primer is introduced, causing DNA-Polymerase to replicate mutated base pairs. This leads to the phage (most commonly the M-13 phage[xvii]) producing exactly the proteins wanted. For the proteins to be useful, the proteins need to be on the outside of the phage. For this reason, the capsid proteins (also called coat proteins) p8 and p3 are changed to exhibit the wanted antibodies.

The reason for using M-13 Phages is for them replicating using the lysogenic instead of the lytic cycle. The difference between them is, that the M-13 Phages integrate their DNA into the DNA of the host instead of causing the host DNA to fall apart. Therefore, the DNA of the phage gets replicated every time the bacterium divides. Eventually, the lysogenic cycle finds the same end as the lytic cycle by the phage DNA eventually being expressed therefor producing M-13 Phages that escape the bacteria by killing it. Because the bacterium has divided many times before it gets killed, the amount of phages now present is bigger than if the phage used the lytic cycle, which is very useful for continuing the process. The used host bacteria are E. coli.

In this way antibodies thought to be more effective than the antibodies used by the immune system can be 'synthesized'. To test whether they are, a phage library is assembled. This library contains a variety of phages all producing different antibody-proteins. To test which version of the antibody is the best, the mutated phages are mixed with the bacteria it should stick to. After washing the phage library, only the phages that stick best to the bacteria are left. With elution, the leftover phages can be separated from the bacteria. The phages now must replicate in order to be analysed. By analysing the phages genes one can figure out which version of an antibody it produced and therefore now know what the best antibody for a specific bacterium is.

Discussion:

Progress made with the application of the chosen technique:

Since 1930, in Georgia, a medical centre provides Phage Therapy[xviii]. The Eliava Phage Therapy Centre provides therapy over the counter. They mostly treat urinal and other simpler bacterial infections. They also claim to have a very high rate of success treating lung infection and inflammation of the bone marrow (osteomyelitis). Some pharmaceutical companies started developing using the enzyme endolysin, which phages use to penetrate the bacterium's cell membrane, in order to treat burns in the early stages.

Apart from the eastern European countries which have licenced treatments, researchers in the west are still on the search to find the formula. Most research in this area is academic. The main problem being is that almost no university is well equipped enough to conduct studies of a higher volume. The race of researchers is tight, but nowhere near the finish line, but to bring up a couple of big names in this game, here are three big researchers: Stephen T. Abedon, Robert Schooley, and Dr. Li Deng. Some university research groups even split off and became independent. The best example of such a new company is Tailor Labs. This kind of partition is becoming more frequent.

What are future research steps?

The main step to be overcome is to reach the level of consistency needed for medical approval to licence the product, to make phage-based therapies commercially available. Phage therapy is not yet developed to its final potential. Therefore, the most important future steps are simply to do more research. We have to find better and faster screening methods, which allow us to produce a better and more precise phage mixture for a more efficient treatment. More clinical trials must be done to be able to predict the pattern based on which we can forecast the effects of different phages and methods on different diseases in different human bodies.

To this day there are still no large-scale studies about the different treatments that include phages. Because of this, there are a lot of questions yet to be answered and possible answers yet to be confirmed. For this to happen, standardized protocols must be written so that phage production can become uniform and therefore studies can get comparable.

For phages to be an adjuvant for antibiotics or other drugs, a great deal of research about potential risks and ideal dosages must be carried out. Also, it is not yet known how our immune system reacts to phages and if that could be a potential problem. In general, one could say that the differences of ex vivo (outside organism) and in vivo (inside organism) still are unknown at that they need to be tested to use phage therapy more often.

Summary:

Antibiotics have revolutionized medicine and saved millions of lives. But through the excessive use of them, super bacteria have been created. Bacteria that are immune to antibiotics are causing problems more and more frequently. For every new resistant bacterium, a new antibiotic must be developed. Unfortunately, until a new antibiotic is created, those bacteria can cost many lives. But there could be another way.

Certain types of viruses have specialized, over the span of millions of years, on taking certain bacteria as a host and thereby killing them. Those viruses can be very useful for medicine, but we are still far away from realizing their complete potential. While antibiotic-resistant bacteria are becoming more and more of a problem, the amount of research is also growing. We now can not only just let phages do their natural job, but we can also use them as an adjuvant for other drugs to weaken bacteria or to deactivate their immunity to antibiotics, or these can be used to find more effective antibodies, by changing their genes so that the desired protein is produced when the virus is replicated, which, in the end, can be used to create more powerful vaccines.

Although the discovery of phages and the idea of using them for medicine is quite old, there is much current research and even fewer studies of applied phages therapy. But once phage therapy is more commonly used it could revolutionise the medical system.

Annex:

Interview with Dr. Salabarria:

Hallo Sebastian & Constantin,

Hier sind meine Antworten auf eure Fragen, falls irgendetwas unklar ist meldet euch gerne wieder bei mir! Es hat was laenger gedauert bis ich Zeit hatte Sie zu beantworten wegen dem Umzug, aber ich hoffe es ist noch nicht zu spaet!

_____

When will Phage therapy become a commercially available/viable treatment in cases of sicknesses due to bacteria and what needs to be done/needs to happen that it comes to this?

While phage therapy holds great potential and I as a researcher am very excited about it we still do not know a lot about how phage therapy interacts with the patients immune system, if combination therapy with antibiotics or other drugs hold any risk and what doses are effective in treating bacterial infections. There is no standardized protocol or standard yet to produce phages for therapy so the first step is to establish one protocol that will be used by all labs. Then what needs to be done is thorough preclinical and clinical studies with standardized phage solutions that answer some of the following questions:

- does the human immune system recognize the phage and could that cause side effects by mounting a viral response against the phage?

- how do you select the best phages for therapy and phage cocktail design?

- do phages and antibiotics work in synergy or against one another?

What is the next step? (In a scientific and in a political manner)

I cannot speak for the political side as this is not my expertise. However scientifically we need to optimise the process of phage production so that it is economically feasible to produce large quantities of phages and that they are clinically safe for the patient population. Currently phage for experimental treatments are produced largely by academic research labs that are poorly equipped too produce phages on such a large scale. Slowly companies such as Taylor labs are rising up from academic labs to fill in this gap (https://www.bcm.edu/research/labs-and-centers/research-centers/tailor).

Who is the leading researcher in phage therapy?

There are a lot of rising players right now but to name a few important names I would say Stephen T. Abedon Robert Schooley, MD, or to name a germany based one Dr. Li Deng (https://www.helmholtz-muenchen.de/viro/research/emmy-noether-research-group-virus-in-nature-and-health/index.html)

How does one deal with mutation of phages regarding the phage selection?

Mutations that deactivate phage effectiveness against bacteria are of course a problem. Similar strategies that we use with other antibacterial medications can be utilized such as using multiple phages at once in a patient, therefore increasing the chance that if a bacteria evolves to become resistant to one phage that the second (or third or fourth phage) still kills the bacterial strain. Other than antibiotics we can also adapt phages on bacterial strains in the lab. This is basically a small scale evolution experiment in which we artificially give the advantage to the phage and thereby reestablish effectiveness, however this would be time consuming and costly to do on a large scale for each individual patient so the design of phage cocktails with multiple phage strains against one bacteria is the better option.

If bacteria mutate to being resistant to certain types of phages and one has to wait until phages mutate to being effective again, wouldn't that mean that we are always one step behind the bacteria, in the same way, we are with antibiotics?

Similar to my answer above I would say that while bacterial mutations that give the bacteriophage resistance can be a problem the big difference to antibiotics is that the phage itself is adaptable. In addition, often when the bacteria mutates to be resistant to phages this mutation will also affect other characteristics of the bacteria e.g. it will re-establish sensitivity to certain antibiotics or make the bacteria more susceptible to the immune system in the patient. In those ways even though the

phage is no longer killing the bacteria it can still facilitate a removal of the bacteria from the patient by making the bacteria vulnerable to other medications or the patient's immune system. Or as mentioned above by designing phage cocktails, or always treating with antibiotics simultaneously we decrease the change of resistance occurring from the start.

If phage therapy is more effective in an advanced state of sickness, does that mean that a patient has to wait until being severely sick to receive effective phage therapy? And if so, does this lead to moral conflicts?

Luckily no. Currently this is the case yes, but that has to do with the fact that phage therapy is not a FDA or EU approved therapy that is commercially available. The cases being treated right now are exceptions and those are usually limited to clinical trials or cases that have no other options (aka advanced sickness state). Once Phage therapy is more widespread I envision it being accessible to early disease stages as well. For example in eastern europe there are centers (such as the Eliava Phage Therapy Center) that routinely treat urinary tract infections with a phage cocktail.

Interview with Dr. Harms:

Dear Sebastian,

thank you for your email and your interest in our research. I have to answer you from my private email address because your address is apparently blacklisted on the spam list of the university (which caused some problems and my late answer). The answers to your questions:

0) Your mail left me slightly confused - what is your evidence that I participated in the paper "Phage Therapy in the Resistance Era", given that I am clearly not in the author list? But it is indeed a nice article, even though I have not written it ;).

1) Phage therapy is already widely commercially available, e.g., in Georgia. It is just not widely available in Western countries because it stopped being commonly used here (for a couple of reasons) after antibiotics became available. Right now, I see two major problems: We don't know enough how exactly works inside patients, and as long as we don't get a better understanding about this we will always face a risk of unexpected treatment failure. As an example, it's not totally clear how phages get to the place where the bacteria are or how the very different physiology of bacteria in vivo compared to in vitro affects the efficacy of the treatment. Furthermore, phages cannot simply be manufactured like drugs, which means that the classical path of getting them approved by the authorities (e.g., SwissMedic in Switzerland) for broad use is not really applicable.

2) The next steps are to tackle the problems mentioned above by 1) doing more fundamental research in combination with 2) more controlled clinical trials in order to 3) find a good way of making phage therapy easier and more predictable. All this together will make it easier to find good solutions for the approval of phage therapy by the authorities.

3) There is no single "leading" researcher in phage therapy - this is not how science works. Important researchers in Switzerland are, e.g., Prof. Martin Loessner in Zürich or Prof. Gregory Resch in Lausanne.

4) I assume you mean how to deal with bacterial mutants that become resistant to phages. This is not really a problem, for a couple of reasons. Usually, phage therapy uses cocktails of phages that bind to different receptors etc. on the bacterial cell surface which makes it much more difficult for the bacteria to become resistant to the treatment. In addition, most spontaneous bacterial resistance mutants have severe fitness defects and decreased virulence or are even avirulent. As an example, many phages target long glycans on the cell surface of bacteria (the so-called "O-antigen") for host recognition and one most frequent way of becoming resistant to these phages is therefore to get rid of these glycans. However, losing the protective O-antigen coat makes bacteria much more sensitive to, e.g., host immunity.

5) No, for the reasons outlined above - there is no need to wait for anything. And if the patient's bacterial infection really gets totally resistant to the treatment, you can always easily isolate new phages from the environment that kill the bacteria (unless the patient is severely ill in which case you might not have the time for that, see (6)). The problems are rather not with resistance but on different levels. For example, what do we do if patients are infected with different strains or even species of pathogenic bacteria? Any given phage commonly has a very narrow host range, meaning that phages isolated to kill strain A of your pathogen are generally not very likely to infect strain B. Also, the fact that in vivo inside the patient the whole

environment is very different from the in vitro setups commonly used to select phages for treatment is obviously a problem that requires further investigation.

6) It is wrong to assume that phage therapy works better in severe infections than in light infections. Like other treatments, it is better to treat a patient before she / he is overwhelmed by bacterial infection. One aspect distantly related to your question is that in Western countries phage therapy is often used as a "last resort" when all other treatment attempts with antibiotics have failed. In this case, it is often possible to try phage therapy on a case-by-case basis even without general formal approval of this way of treatment.

I hope that this was helpful - best wishes,

Alexander

References:

Reference of figures:

Figure 0: Picture of a phage. Vahlensieck, Yvonne. Bakterien mit Viren bekämpfen. URL: https://www.unibas.ch/de/Aktuell/Uni-Nova/Uni-Nova-134/Uni-Nova-134-Bakterien-mit-Viren-bekaempfen.html [03.02.2021]

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