Viral Host linteractions

Master project

Importance: The genomes of phages (viruses of bacteria) are highly diverse, but largely understudied. They represent a black box of genes of unknown functions, which could potentially have significant biotechnological value and elucidate important virus-host interactions[1].

Background: The genomes of a designated group of marine and non-marine phages (*Podoviridae*) with both temperate and lytic lifestyles infecting a range of bacteria all encode a peculiar operon of potential peptidoglycan modification genes located in between genes involved in transcription, DNA metabolism, and replication. The gene products of this operon are hypothesised to play an important role during infection and host take-over by either modifying the host cell surface to prevent superinfection or by modifying or reversing modification of host enzymes or other cell components[2, 3].

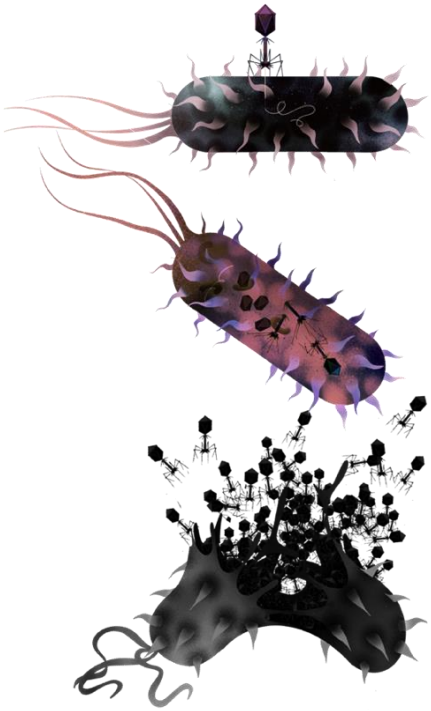


Figure 1 Graphic illustration of a lytic phage infection cycle, by Andreas Y. Pilskog.

The aim of this project is to examine the potential roles and biological consequences of these phage gene products, successful results may lead to a scientific publication.

Techniques: DNA work, epigenetics, 3rd generation sequencing, whole genome amplification, transposon-based random knock-out mutagenesis *in vitro,* cloning techniques (CRISPR-Cas9 and Gibson assembly), electroporation, PCR, phage characterisation and biological consequence assays.

You are more than welcome to contact Lars Hestbjerg Hansen at lhha@plen.ku.dk or Nikoline Olsen at sno@plen.ku.dk.

Relevant literature:

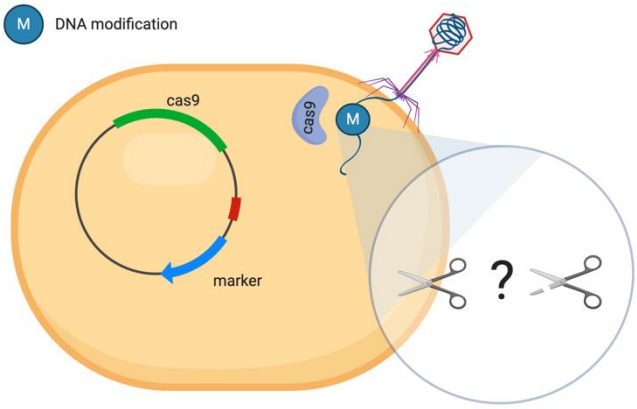
1. Salmond, G. P. C. & P. C. Fineran. A century of the phage: past, present and future. *Nature Reviews Microbiology.* 2015, vol. 13, pp.: 777-786. https://doi.org/10.1038/nrmicro3564**.**

2. Duhaime MB, Solonenko N, Roux S, Verberkmoes NC, Wichels A, Sullivan MB. Comparative Omics and Trait Analyses of Marine *Pseudoalteromonas* Phages Advance the Phage OTU Concept. *Front Microbiol*. 2017;8:1241. Published 2017 Jul 6. doi:10.3389/fmicb.2017.01241

3. Hardies SC, Hwang YJ, Hwang CY, Jang GI, Cho BC. Morphology, physiological characteristics, and complete sequence of marine bacteriophage φRIO-1 infecting Pseudoalteromonas marina. *J Virol*. 2013;87(16):9189-9198. doi:10.1128/JVI.01521-13.

Do novel DNA modifications protect phage from CRISPR-cas9 system?

In the continuous battle between bacteria and phages, bacteria are constantly evolving defenses mechanisms like restriction-modification systems or CRISPR-cas. To escape these defenses, phages use multiple strategies to counterattack and one of the most widespread strategy is to modify their DNA. We recently showed that newly discovered 7-deazaguanine modifications can protect phage DNA from restriction endonucleases (1).



Furthermore, we are able to detect in which places in the phage genome these modifications take place (2). Combining these two discoveries, we are now ready to check if these modifications also provide protection against CRISPR-cas systems. In this project you will test if newly discovered DNA modifications provide protection for cas9 protein. You will be able to learn and apply number of microbiological and molecular techniques like phage microbiology, cloning, plasmid transformation, antibiotic selections and DNA sequencing. This project offers a great chance for a high-impact publication. Don't miss this opportunity!

For more information or questions, please contact the supervisor:

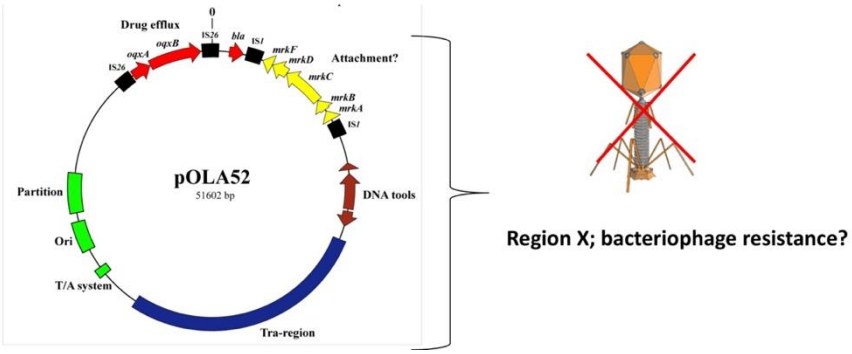
Assistant professor Witold Kot (wk@plen.ku.dk)

Relevant literature:

1. Hutinet G., Kot W., Cui L. *et al.* 7-Deazaguanine modifications protect phage DNA from host restriction systems. *Nature Communication* **10,** 5442 (2019) doi: 10.1038/s41467-019-13384-y
2. Kot W., Olsen S.N., Nielsen T.K. *et al.* Detection of preQ$_0$deazaguanine modifications in bacteriophage CAjan DNA using Nanopore sequencing reveals same hypermodification at two distinct DNA motifs. *Nucleic Acids Research* **48**, 18, (2020) doi : 10.1093/nar/gkaa735

Unraveling the secret behind plasmid-mediated resistance towards bacterial viruses

Background: Extracellular DNA elements that are self-replicating, such as plasmids, often provide favorable features to the host bacteria. One such plasmid is pOLA52, originally isolated from bacteria in swine manure. This plasmid is known to increase the biofilm forming ability of the host, as well as to grant resistance towards the antibiotic, olaquindox, extensively used as a growth promoter in pigs. Interestingly, bacterial strains carrying pOLA52 also display an increased resistance towards bacteriophages (i.e. bacterial viruses) as compared to their isogenic counterparts not carrying the plasmid. The genetic region(s) of the plasmid that allow this extraordinary ability are, however, unknown.



Aim and Experimental approach: In this project we would like to determine which genetic part(s) of the pOLA52 plasmid that are responsible for increasing the host resistance towards bacteriophages. Here, random knock-out mutations will be constructed throughout the genetic sequence of pOLA52 using a transposon-based insertion system *in vitro*. This creates a selection of various pOLA52 plasmid mutants. Inserting these plasmid variants into bacterial host cells prior to exposure to bacteriophages, will reveal which genetic region(s) carried by pOLA52 that are important to grant the host resistance towards bacteriophages.

Importance: Increasing our current understanding of plasmid-mediated traits that grant resistance towards bacterial viruses is considered highly valuable and might serve as a powerful tool in the development/selection of bacteriophages as biocontrol agents.

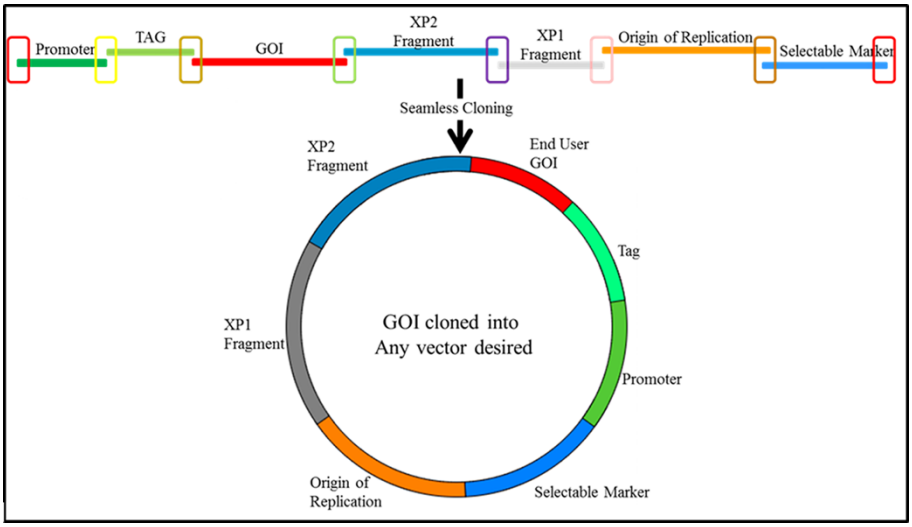
Techniques: DNA work, bacterial genetics, bacteriophages, molecular microbiology, transposon-based random knock-out mutagenesis *in vitro*, electroporation, PCR, DNA sequencing, transcriptomics, bacteriophage screening assays.

Supervisors: Prof. Lars Hestbjerg Hansen (lhha@plen.ku.dk)
Assistant Prof. Leise Riber (lriber@plen.ku.dk)
Assistant Prof. Witold Kot (wt@plen.ku.dk)

Relevant literature: Norman et al. (2008). Nucleotide sequence of pOLA52: A conjugative IncX1 plasmid from *Escherichia coli* which enables biofilm formation and multidrug efflux. Plasmid **60:**59-74.

Design and synthesize DNA building blocks for stitching together the ultimate bacterial cloning vector

Background and Aim: Today, several varieties of molecular tools are available for cloning and gene editing in bacterial species. However, most of these tools work only truly efficient in the model organism, *Escherichia coli*. In this project we would like to develop synthesized cloning and gene editing vectors for efficient use in other bacterial species, such as *Pseudomonas syringae* and possibly *Sphingomonas spp*. The basic idea is to design the desired vector(s) based on all the necessary DNA building blocks needed for replication, maintenance and cloning/gene editing purposes in the target strains. For a 'smart' design one could consider constructing basic vector(s) that allow the possibility of adding/replacing/deleting specific synthetic building blocks in order to obtain the perfectly adapted cloning vector for any given application. The vector design will be based on information gathered from the composition of many sequenced plasmids found in *P. syringae*, as well as on inspiration obtained from standard DNA building blocks, such as BioBricks. The finale vector layout will be commercially synthesized, and a "Proof of concept" will include testing the stability, copy number etc. of the newly constructed vector.



Assembly of DNA building blocks into functional multi-device vector systems. Figure is from Braman & Sheffield (2019). PLoS ONE. https://doi.org/10.1371/journal.pone.0199653.

Importance: Successful synthetic cloning vectors will make gene editing possible in non-model organisms, such as *P. syringae* and *Sphingomonas spp*., in which molecular work so far has been limited. Designing basic synthetic vectors with the possibility of exchanging DNA building blocks also allows for the creation of advanced gene editing CRISPR-Cas systems (for example see reference: *Halter and Zahn (2018). J. Industrial Microbiol. Biotechnol.* ***45****:153-163*).

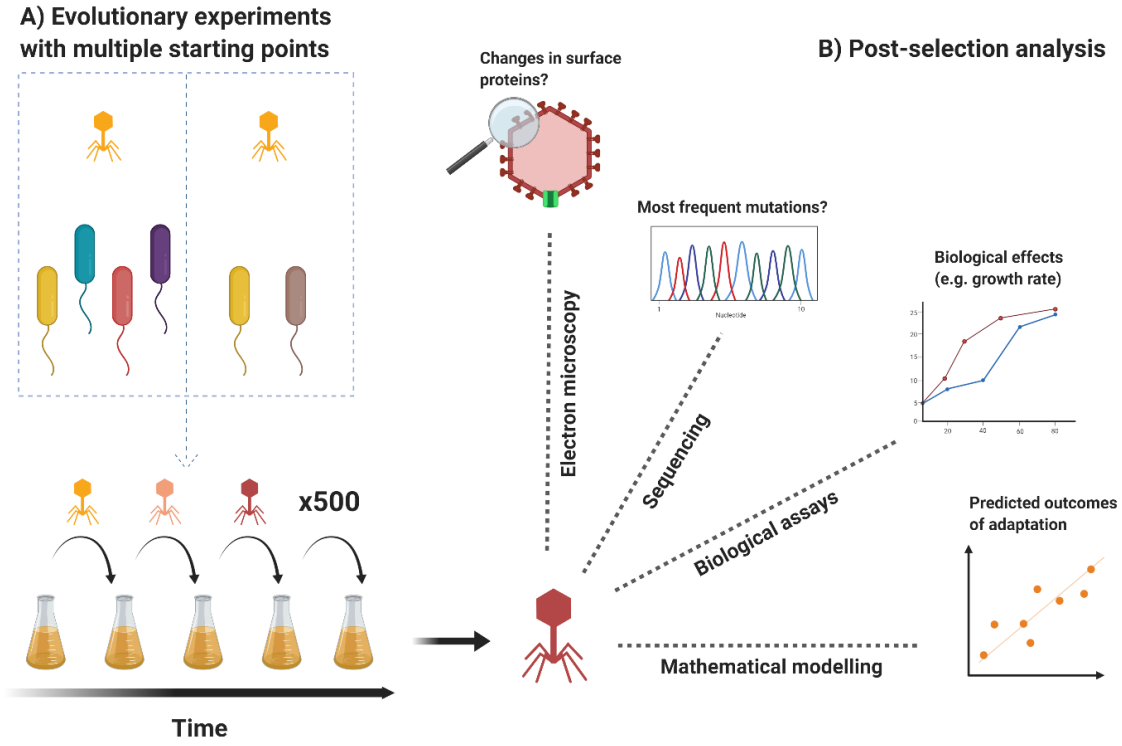
Techniques: DNA work, plasmid biology, bacterial genetics, molecular microbiology, bioinformatic analyses, DNA cloning, restriction enzymes, electroporation, plasmid stability assays, qPCR for copy number determination, gene editing.

Supervisors: Prof. Lars Hestbjerg Hansen (lhha@plen.ku.dk) and Assistant Prof. Leise Riber (lriber@plen.ku.dk).

This Project is carried out at the Environmental Microbial Genomics Group, MEB, PLEN, KU

Viral evolution

Occasionally a virus will mutate in a way that allows it to infect a new organism. This is known as host range mutations. This event can have a large impact on both the virus and its new host(s). Once a virus has become able to infect a new host it undergoes rapid evolution where it adapts to its new environment/host.
In this project, we wish to better understand this process and what adaptations a virus makes to adapt to a new host. We will do this by setting up evolutionary experiments where we create the optimal conditions to witness this host range change in the laboratory. For ethical and safety reasons we have chosen to work with viruses that infect bacteria (bacteriophages) as the model organisms. This allows us to replace test animals with bacteria, allowing for faster and safer experiments without worries about the welfare of the test subject. If we get a better understanding of viral host range change we might be able to predict what viruses are more likely to perform this switch and perhaps take preemptive action to prevent host range change in the future.



As a student in this project you will; set up experiments to study the evolution of viruses in the laboratory, perform DNA isolation and DNA sequencing of viruses, determine growth criteria of bacteriophages (host range, burst size, latent period). Although the overall goal of the project is decided beforehand, there are many ways to approach this and the MSc student will have the option to tailor the project based on his/her own ideas.

If you have any questions or want to hear more about the projects in the environmental microbial genomics group, you are more than welcome to contact Lars Hestbjerg Hansen at lhha@plen.ku.dk or Alexander Byth Carstens at alexander.carstens@plen.ku.dk

UNIVERSITY OF COPENHAGEN
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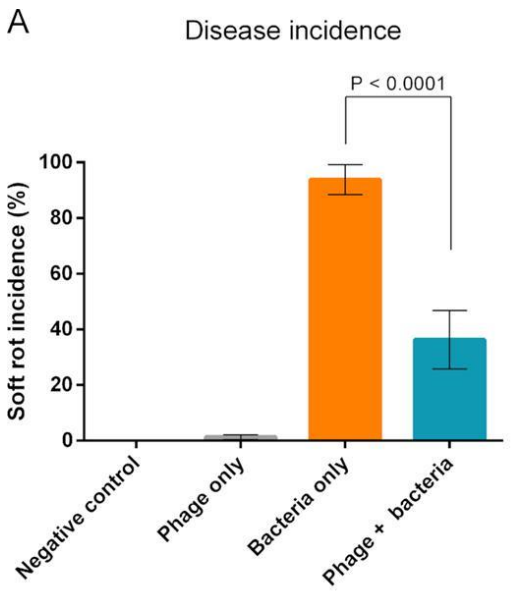
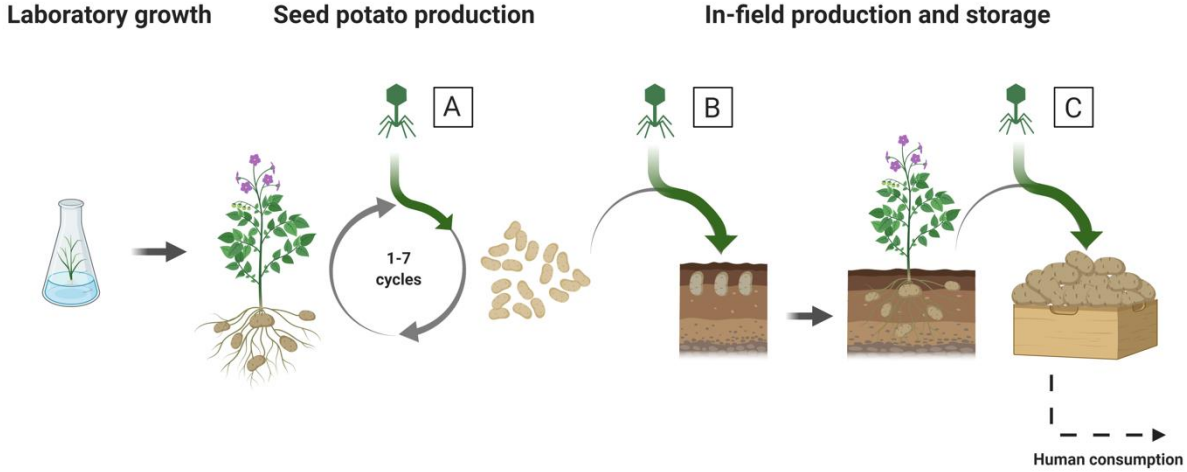
Can phages replace pesticides?

(MSc and BSc)
Bacterial plant diseases are a major problem in agriculture worldwide. To combat plant pathogenetic bacteria we use pesticides and heavy metals, with detrimental effects on the environment and human health. In this project, we seek to develop bacteriophages (phages) as a green alternative to pesticides and heavy metal sprays in agriculture. Phages are the natural enemies of bacteria in nature but unlike chemical pesticides and heavy metals, they are biological organisms and do not harm the environment.





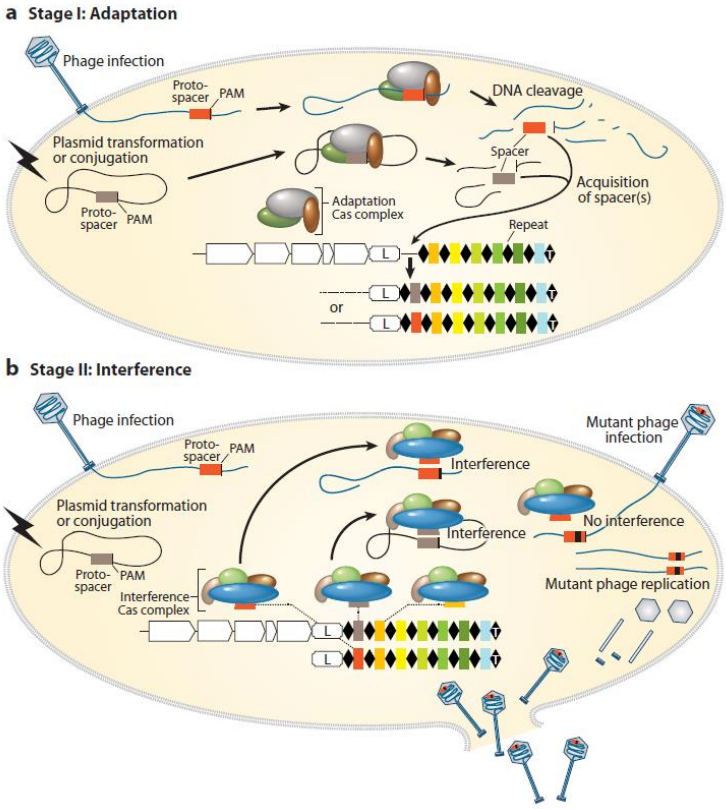
We have serval ongoing projects regarding the use of phages to replace pesticides and the student will have the option to join an existing project or tailor their own project within this overall topic. Tools often used in this type of work include; DNA sequencing, phage isolation, phage characterization and bioinformatics.

If you have any questions or want to hear more about the projects in the environmental microbial genomics group, you are more than welcome to contact Lars Hestbjerg Hansen at lhha@plen.ku.dk or Alexander Carstens alexander.carstens@plen.ku.dk

How do bacteriophages infecting Erwinia amylovora cope with the CRISPR defense?

Background: CRISPR-Cas systems have recently gained immense attention due to its applications in gene editing. However, CRISPR-Cas most likely evolved to protect bacteria from invading genetic elements, such as plasmids and bacterial viruses (bacteriophages). In connection to the global increase in antibiotic resistance, interest in using phages to combat bacterial pathogens has resurged. Nevertheless, in many cases, the phage-bacteria interactions are poorly understood, including the mechanisms phages utilize to evade anti-phage systems such as CRISPR-Cas. In this project, you will focus on a collection of phages infecting a bacterial plant pathogen *Erwinia amylovora*, which has an active CRISPR-Cas system that has not been studied extensively.

Aims and Experimental Approach:
This project will consist of two parts, and is well suited for students who want to collaborate on a project, but it is not required. The student(s) on this project will work to establish a functional platform to work efficiently with CRISPR-Cas in *E. amylovora,* using its native type I-E CRISPR-Cas systems. Using molecular cloning and other techniques, they will investigate the necessary components for the system to function and if possible establish an endogenous CRISPR-Cas editing platform in this organism. Building on this platform, the student(s) will study the interactions between the different groups of phages and *E. amylovora*, to elucidate how modified bases or other counter-defense mechanisms interferes with CRISPR-Cas in this context.

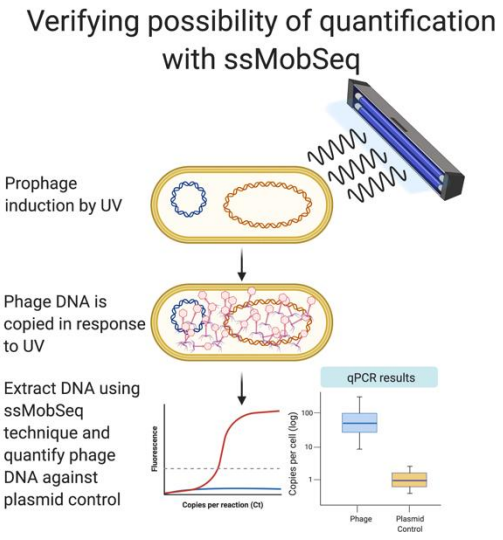


Deveau et al. 2010. *Annual Review of Microbiology*, doi: 0.1146/annurev.micro.112408.134123

Importance: The knowledge acquired in these projects will guide decisions on the choice of phages in a phage therapy context. It will provide knowledge on CRISPR-Cas outside of a gene-editing context, giving a much-needed perspective on the ecological consequences of CRISPR.

Supervisors: Prof. Lars Hestbjerg Hansen (lhha@plen.ku.dk), Assistant Prof. Witold Kot (wt@plen.ku.dk), PhD Student Amaru Djurhuus (amaru@plen.ku.dk)

Using single-strain mobilome sequencing to quantify prophage induction in *E. coli*



Bacterial genomes consist of one or more large chromosomes and then a dynamic pool of mobile genetic elements (MGEs), including plasmids, transposons, integrons, and bacteriophages. Most of these MGEs appear as circular DNA molecules at some point of their lifecycle. Many bacteriophages can integrate into their host chromosome as prophages and lie dormant here until a signal, such as UV-induced DNA damage, instructs the prophage to become active (induction). The sum of MGEs in a cell is often termed the "mobilome" and it can be directly investigated using the state-of-the-art method Single-Strain Mobilome Sequencing (ssMobSeq). Broadly, this method relies on enzymatic digestion of the chromosome(s), leaving only smaller MGEs which can then be sequenced using the Illumina platform. The ssMobSeq method is very new but holds great promise with regards to directly surveying mobilomes and how MGEs shape bacterial genetics.

In the proposed project, you will apply ssMobSeq on the *E. coli* type strain MG1655 which has an integrated prophage that becomes active upon UV treatment and creates many copies of its own DNA. You will test if ssMobSeq can be used to actually directly quantify the number of phage genome molecules by comparing the ssMobSeq results to a "ground truth" qPCR assay on the same samples. As a control, you will have inserted, using electroporation, a single-copy plasmid into strain MG1655 that will serve as a known copy number molecule. During the project, you will learn and apply advanced molecular techniques, including plasmid electroporation, DNA digestion by exonuclease, qPCR, Illumina DNA sequencing, and bioinformatic analyses.

For more information or questions, please contact the supervisors: Postdoc Tue Kjærgaard Nielsen tkn@plen.ku.dk or Prof. Lars Hestbjerg Hansen lhha@plen.ku.dk

The Smallest of the Small

Bachelor / Master project

The aim of this project is to perform a thorough characterization of a small group of so far undescribed *Salmonella* phages (virus of bacteria) representing a completely new genus within the subfamily *Picovirinae,* family *Podoviridae* of the order *Caudovirales* (the tailed phages).

Background: The *Picovirinae* subfamily consists of only nine phage species characterised by their special tail structure, their small genomes (16-20 kb) with only 20-29 CDSs and inverted terminal repeats (ITR) encoding a rare protein primed polymerase (type B). The *Salmonella* phages Astrid, assan and astrithr have low nucleotide similarity with phages in databases (<39%) and no sequence similarity with classified *Picovirinae.* But based on their genome compositions with ITR and type B polymerases, Astrid, assan and astrithr are the first *Picovirinae* isolated on *Salmonella*, but with even shorter genomes (11.6-11.7 kb) and encoding only 15 potential genes.

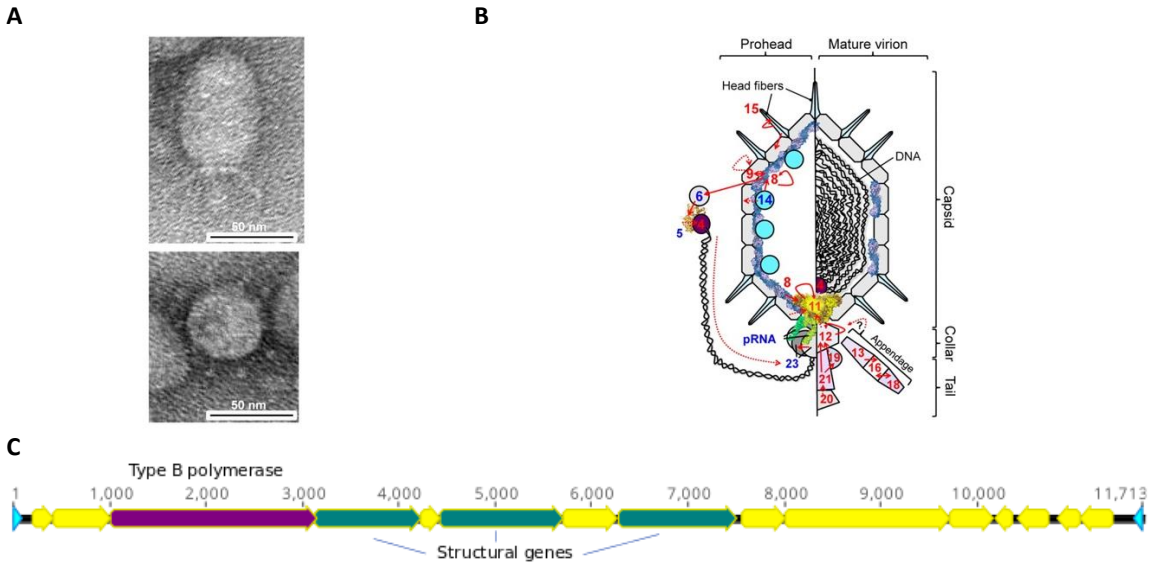


Figure 2. A Electron micrographs (lateral and top-down) of the *Picovirinae* Streptocpcoccus phage Cp-1 modified from Häuser *et al.,* 2011.**B** Schematic model of the Cp-1 virion from Häuser *et al.,* 2011. **C** The genome of Salmonella phage Astrid, yellow arrows represent hypothetical genes, blue arrows are inverted terminal repeats (ITR).

Techniques: DNA work; primer design, PCR, sequencing, bioinformatics. Microbiology; phage characterisation, imaging (transmission electron microscopy TEM), host range analysis, adsorption, burst size and latency period assays, investigations of the effect of co-infection with other phages (*Jerseyvirus*) or phage therapy related assays. The obtained results are expected to be included in a scientific publication presenting the novel *Picovirinae* genus.

Contact: Lars Hestbjerg Hansen at lhha@plen.ku.dk or Nikoline Olsen at sno@plen.ku.dk

Relevant literature:

Häuser, R., M. Sabri & P. Uetz (2011). The Proteome and interactome of *Streptococcus pneumonia* Phage Cp-1. Journal of Bacteriology. https://doi.org/10.1128/JB.01481-10.

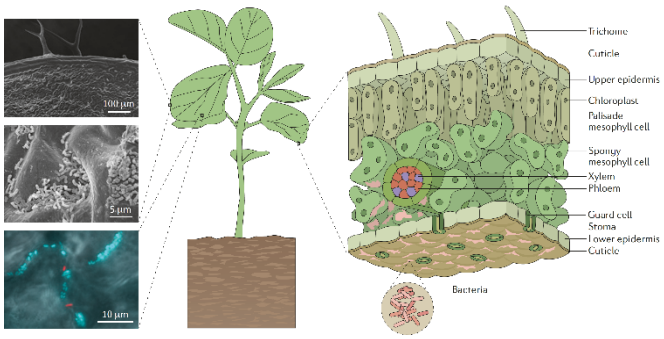
Kleppen HP, Holo H, Jeon SR, Nes IF, Yoon SS. Novel Podoviridae family bacteriophage infecting Weissella cibaria isolated from Kimchi. Applied and Environmental Microbiology. 2012 Oct;78(20):7299-7308. DOI: 10.1128/aem.00031-12.

This Project is carried out at the Environmental Microbial Genomics Group, MEB, PLEN, KU

Master Project

Genomics of Novel Light-harvesting Bacteria on Plant Leaves

Plant life is not possible without microbes. Plants are populated by microorganisms both below and above the ground. The phyllosphere comprises the aerial parts of plants and is dominated by the leaves. Astonishingly, the global leaf area is estimated approximately twice as large as the land surface, accommodating up to 10^{26} bacterial cells. We are just starting to understand this vast and rich microbial diversity thanks to modern genomics tools.



Microbes attached to plant surfaces, dominated by leaves, are termed phyllosphere[1,2]. There microbes play a vital role in plant health.

Among these microbes, we are interested in a group of bacteria that can harvest sunlight as the energy source. Our recent work revealed that there are abundant and culturable photosynthetic bacteria dwelling on wheat leaves in Denmark[3]. In this project, we will further explore the evolutionary genomics of these photosynthetic bacteria and their interactions with plants. Modern microbiological techniques will be applied, including mass spectroscopy, bacterial infra-red imaging, Nanopore sequencing, and bioinformatics.

Location and Vision

The project will be carried out at Prof. Lars H. Hansen's lab in the Department of Plant and Environmental Sciences KU with co-supervision by Associate professor Yonghui Zeng. The project is expected to start between before August 2021.The student is expected to perform fieldwork, bacterial cultivation, molecular identification of isolates, genome sequencing, and sequence data analysis. The successful results may lead to a scientific publication.

Contact

Lars Hestbjerg Hansen, lhha@plen.ku.dk or Yonghui Zeng, zeng@plen.ku.dk

References

1. Vorholt, J. A. (2012). Microbial life in the phyllosphere. *Nature Reviews Microbiology*, 10(12), 828-840.
2. Vacher, C., Hampe, A., Porté, A. J., Sauer, U., Compant, S., & Morris, C. E. (2016). The phyllosphere: microbial jungle at the plant–climate interface. *Annual Review of Ecology, Evolution, and Systematics*, 47, 1-24.
3. Zervas, A., Zeng, Y., Madsen, A. M., & Hansen, L. H. (2019). Genomics of aerobic photoheterotrophs in wheat phyllosphere reveals divergent evolutionary patterns of photosynthetic genes in *Methylobacterium* spp. *Genome Biology and Evolution*, 11(10), 2895-2908.

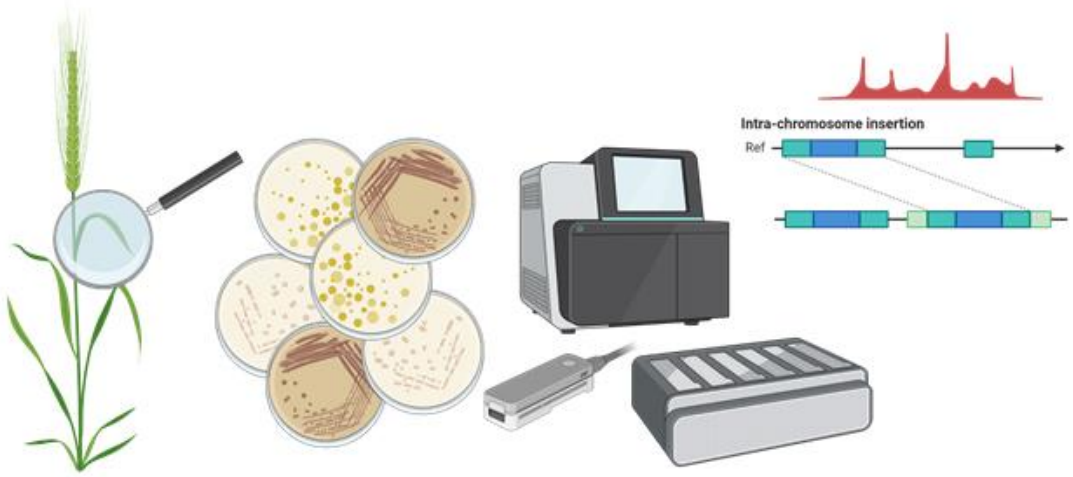
Flag-Leaf Bacterial Inventory: Isolation, Collection and Characterization of Wheat-Leaf Bacteriome

Background: Wheat is one of the most important crop worldwide. The filling of the grains within the spike is mostly due to the photosynthetic contribution of a specific leaf; the so called flag-leaf. This leaf has been extensively studied during the years, targeting specific microbes in order to characterize the pathogens that affected its development. However, not much is known about the overall bacterial distribution and composition on this leaf. In this project, we aim to build an extensive collection of isolates representing the epiphytic and endophytic bacterial community. The isolates will be sequenced using our in-house sequencing facilities. Combining all the genomes obtained we will access a previously unedited taxonomical and functional diversity of such important leaf.The student will have the possibility to tailor the study of the genomes based on his/her own ideas and preferences

Techniques: In this project we will make an extensive use of classical microbiology isolation techniques, covering different growth media and conditions to obtain the largest diversity across the flag-leaf bacteriome. From selected isolates we will extract the DNA and prepare sequencing libraries for Illumina and Nanopore sequencing. The genomes obtained will be analysed by using bioinformatics tools for assembly, alignment and annotation.

Importance: Characterizing potentially new bacterial strains on one of the most important leaves worldwide.

Supervisors: Prof. Lars Hestbjerg Hansen (lhha@plen.ku.dk)
Dr. Alex Gobbi (alex.gobbi@plen.ku.dk)
Contact us if you have any question and you want to know more about it

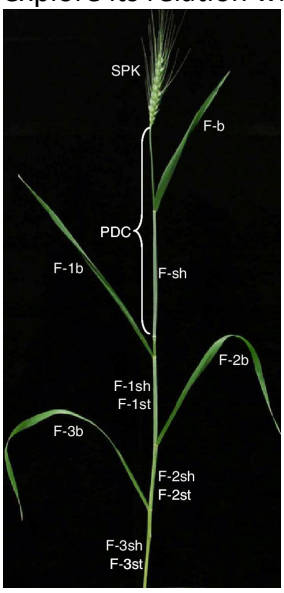


Microbial shifts on wheat phyllosphere under double-fertilization regimes

Background: Wheat is one of the most economical important crop worldwide. From the flour obtained by grinding its grains, you can produce bread, pizza and pasta but also grain distilled spirits such as vodka. Yet, many studies based on high throughput DNA sequencing focused on specific tissues of wheat (spike, flag-leaf or roots) but none of them investigated the overall microbial composition associated with all the different plant tissues. This project will produce the most accurate characterization of the wheat microbial community and will shed light on the impact of a double-fertilization treatment on the associated microbiome.

Techniques: This project will make an extensive use of amplicon sequencing and qPCR and the candidate will be taught on how to perform the complete sample processing, from DNA extraction to library preparation (including PCR, indexing, clean-up with magnetic beads and pooling) and finally DNA sequencing. The candidate will be introduced also to the use of the robot-handling liquids (Opentrons) in our lab.

Importance: Using high throughput DNA sequencing, characterizing the tissue-specific microbial community of wheat and explore its relation with the fertilization-treatment.



Supervisors:
Prof. Lars Hestbjerg Hansen (lhha@plen.ku.dk)
Dr. Alex Gobbi (alex.gobbi@plen.ku.dk)

References:

- Wheat Microbiome: Present Status And Future Perspective; M. K. Solanki et al. (eds.), Phytobiomes: Current Insights and Future Vistas, 2020.
- Characterization of the Wood Mycobiome of *Vitis vinifera* in a Vineyard Affected by Esca: Spatial Distribution of Fungal Communities and Their Putative Relation With Leaf Symptoms. Del Frari G, Gobbi A. et al (2019). *Front. Plant Sci.* 10:910. doi: 10.3389/fpls.2019.00910

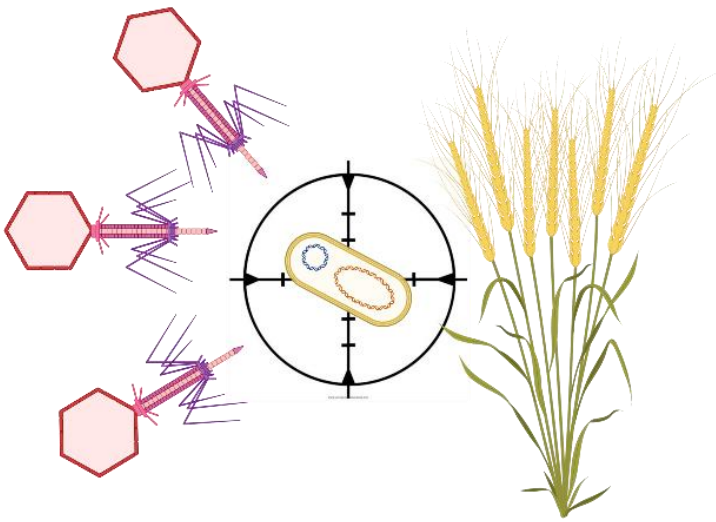
Follow the Flag: Hunting Season Open for Bacteriophage on Wheat-Flag leaves

Background: Wheat is one of the most important crop worldwide. As for many other crops, bacterial disease represent a major threat in agriculture. Therefore, an increasing amount of studies had been targeting the pathogens that could negatively affect the plant development, reducing the yield. Bacterial agent, mostly belonging to the species of *Pseudomonas*, *Clavibacter* and *Xanthomonas*, causes a few known diseases on wheat. Instead of fighting them using pesticides or heavy metals, in this project we will try to isolate bacteriophages to selectively snipe the pathogens responsible for *leaf-blight*, *sheath-rot*, *leaf-streak* and *glume-rot*. The successful isolation of new strains of bacteriophages will be enough to produce a scientific publication on a peer-reviewed international journal.

Techniques: In this project, the candidate will isolate phages from wheat phyllosphere or other ecosystems (to be decided) and use the double-agar overlay system to visualize potential phages against targeted bacteria. The isolates will be characterized through DNA sequencing and bioinformatics analyses. Wheat-virome sequencing could be also performed if it meets the preference of the student.

Importance: Characterizing new bacteriophages from an important and understudied environment while looking for a sustainable alternative to traditional method to fight plant pathogens

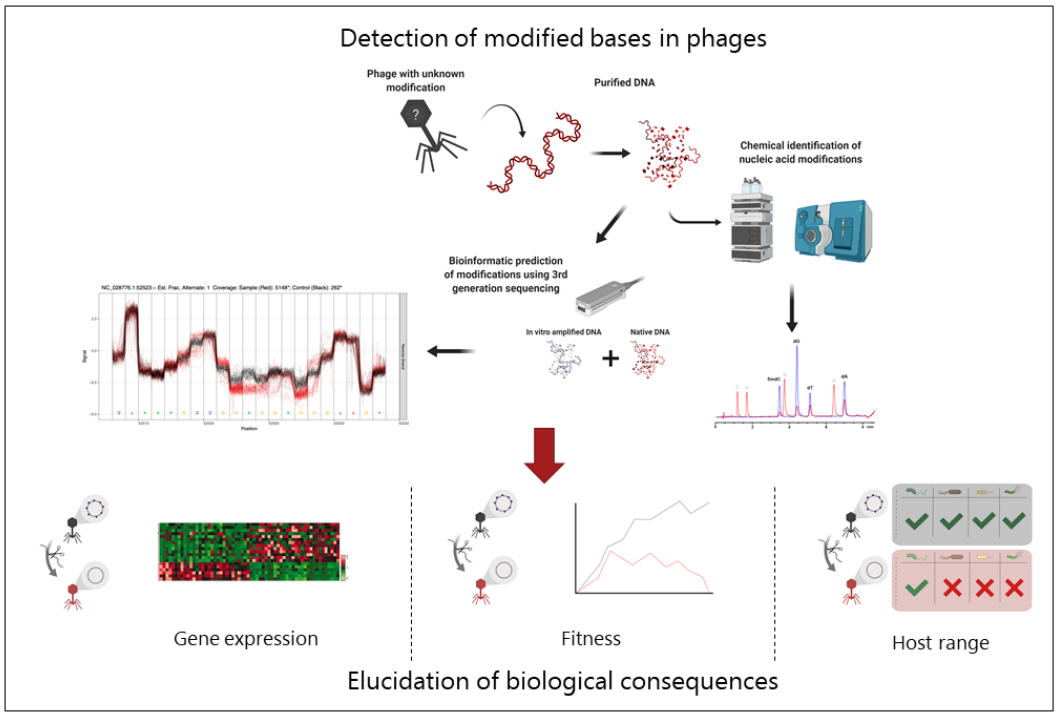
Supervisors: Prof. Lars Hestbjerg Hansen (lhha@plen.ku.dk), Dr. Alex Gobbi (alex.gobbi@plen.ku.dk)
Contact us if you have any question and you want to know more about it



Biological consequences of viral DNA modification systems

Background: The evolutionary arms race between bacterial viruses (phages) and bacteria in many ways shape their interactions. An important event during phage-bacteria interactions is the entry of phage DNA into the bacterial cell to facilitate the lytic cycle of the phage. Consequently, bacteria have evolved defense systems such as CRISPR-Cas and restriction modifications to protect themselves against invading nucleic acids. However, phages have evolved elaborate counter-defense mechanisms in order to circumvent these systems, including DNA modifications. However, very little is currently known about the biological consequences of these DNA modifications.

Aim and Experimental Approach: To assess the biological consequences of DNA modifications, we would like to compare different parameters important to the phage life cycle, such as burst size and host range between a wild-type phage, and a modification-deficient mutant. To do this, we will employ both cutting-edge techniques based on 3rd generation sequencing to detect DNA modifications and CRISPR/Cas9-mediated cloning and more to generate phage mutants, before finally performing biological assays to determine the effects on biological parameters of the phages.



Importance: With the resurgence of phages as potential candidates for treating diseases in humans and as alternatives to pesticides in agriculture, it will be imperative to understand the interactions between phages and bacteria in detail, so that we can employ them in a more informed manner.

Techniques: DNA work, phage and bacterial genetics, 2nd and 3rd generation sequencing, molecular microbiology, phage characterization, CRISPR/Cas9 cloning, Gibson assembly.

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Viral epigenetics

MSc project

A novel phage DNA hypermodification?

Phages (virus of bacteria) by far have the greatest diversity of modified bases in their genomic DNA, assumed to be involved in defence against host restriction enzymes and CRISPR-Cas systems, regulation of genome cleavage, stable packaging of virions, regulation of mismatch repair and timing of replication. Known base-modifications include both simple methylations (m6A, m4C, m5C) and more complex hypermodifications like the glycosylations (glycosyl-hmC) of T4-like phages[1], the arabinosylations (ara-hmC) of the *mosigvirus*[2] and the widespread recently discovered 7-deazaguanine derived modifications (dG^+, dADG, $dpreQ_0$, $dpreQ_1$)[3], but many more are expected to exist. Escherichia phage ukendt has modified bases in at least four DNA sequence motifs of which only three can be explained by known modifications (methylations). In this project the student will examine the nature of the unknown potential hyper-DNA-modification of phage ukendt. The project is expected to result in a joint scientific publication.

Techniques: DNA sequencing (Nanopore), whole genome amplification, epigenetics, bioinformatic analyses, , restriction enzymes, electroporation, cloning techniques (CRISPR-Cas9 and Gibson assembly), gene editing and phage-microbial work including characterisation and bio-consequence assays such as host range and adsorption analyses.

A

Motif	ModFrac %	% of bases	Putative enzyme
GATC	30	0.24 (A)	Dam?
CCAGG	22	0.25 (C)	Dcm (1)?
CCTGG	25	0.4 (C)	Dcm (2)?
GTAC	70	?	??

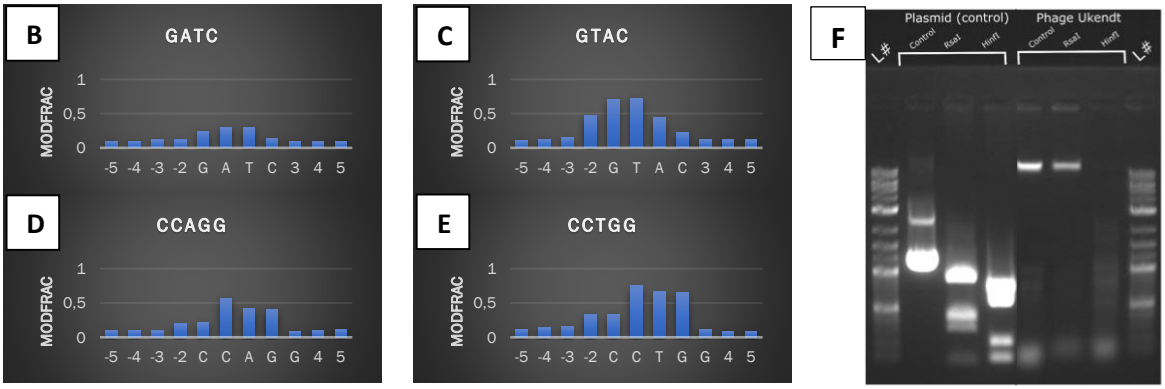

Fig 1. (A) Modified motifs (Nanopore sequencing, Tombo software). ModFrac: percentage of sites modified. Percentage of bases: the amount of the predicted bases modified as compared to the whole genome content of this base. (**B**) Dam-like modification pattern with the GATC motif. (**C**) The unknown modification with the GTAC motif. (**D-E**) The two motifs for the Dcm-like modification patterns. (**F**) Restriction digest with enzymes RsaI (GT/AC) and HinfI (G/ANTC) of phage ukendt genomic DNA and a control plasmid, phage Ukendt DNA is undigested by RsaI suggesting protection by the unknown modification of GTAC.

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References: ***1.*** *Vlot M., et al., Bacteriophage DNA glucosylation impairs target DNA binding by type I and II but not type V CRISPR-Cas effector complexes. Nucleic Acids Research* 46, 2, (2018) doi: 10.1093/nar/gkx1264. ***2.*** Thomas JA, Orwenyo J, Wang LX, Black LW. The Odd "RB" Phage-Identification of Arabinosylation as a New Epigenetic Modification of DNA in T4-Like Phage RB69. Viruses. 2018 Jun 8;10(6):313. doi: 10.3390/v10060313. PMID: 29890699; PMCID: PMC6024577. ***3.*** Kot W., Olsen S.N., Nielsen T.K. et al. Detection of $preQ_0$deazaguanine modifications in bacteriophage CAjan DNA using Nanopore sequencing reveals same hypermodification at two distinct DNA motifs. *Nucleic Acids Research* 48, 18, (2020) doi: 10.1093/nar/gkaa735