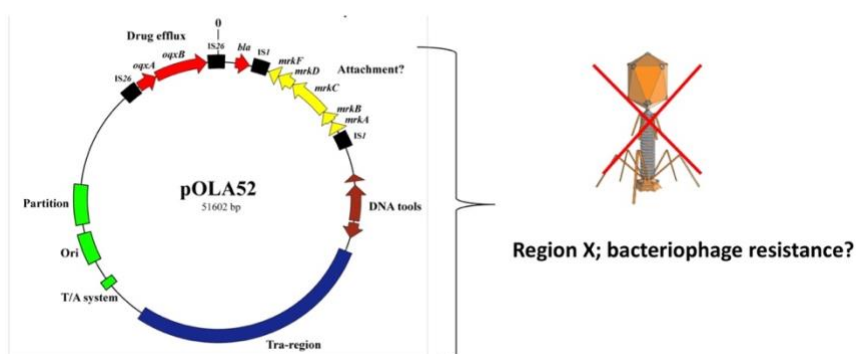


## Unraveling the secret behind plasmid-mediated resistance towards bacterial viruses.

**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, olaquinox, extensively used as a growth promoter in pigs. Interestingly, bacterial strains carrying pOLA52 also display an increased resistance towards some 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 find and characterize the 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](mailto:lhha@plen.ku.dk))  
Associate Prof. Leise Riber ([lriber@plen.ku.dk](mailto:lriber@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.

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

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

### Techniques

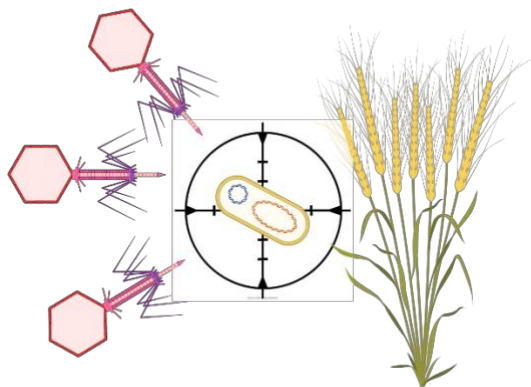
In this project, the candidate will isolate phages from the wheat phyllosphere, and use the double-agar overlay system to visualize potential phages against targeted bacteria. The isolates will be characterized through DNA sequencing and bioinformatics analyses. The dynamics between selected bacterial strains and phages isolated against them will finally be tested in plant models using actual wheat flag leaves.

### Importance

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

**Supervisors:** Prof. Lars Hestbjerg Hansen ([lhha@plen.ku.dk](mailto:lhha@plen.ku.dk))

Associate Prof. Leise Riber ([lriber@plen.ku.dk](mailto:lriber@plen.ku.dk))

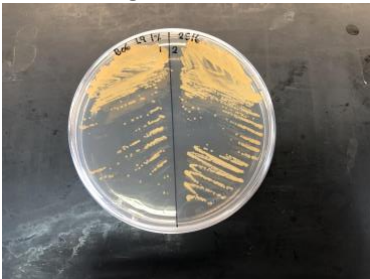


## Looking more closely at the major inhabitants of the wheat phyllosphere microbiome.

**Background.** Common wheat, *Triticum aestivum*, is one of the most important crops worldwide. In a world challenged by the prospects of feeding an increasing population while battling environmental changes, finding new sustainable ways to produce food becomes of significant importance. Today it is well-known that bacteria can benefit plant health in several ways; either by battling plant pathogens or as biofertilizers providing nutrients for the plant. The microbiome of the wheat phyllosphere consists of a multitude of different bacteria that all act together, each displaying a different role/function. In the initial part of this project, we have collected and sequenced several bacterial isolates from the surface of wheat leaves, and bacteria belonging to the genus *Sphingomonas* clearly revealed themselves as being one of the most dominant inhabitants of the wheat phyllosphere. The question is why exactly *Sphingomonas*? Which superpowers bring them to be the key players in this environment?

**Aim.** In this project we will try to find some answers to this question; which special abilities make *Sphingomonas* one of the major key players of the wheat phyllosphere? We currently have a huge collection of *Sphingomonas* isolates collected from wheat leaves from test fields in Taastrup, DK, and these will be characterized phenotypically in regards to their abilities to inhibit plant pathogenic fungi, their tolerance to various abiotic and biotic stresses, their airborne abilities, their production of certain metabolites and many others factors.

**The methods.** Depending on which traits you would like to investigate, you will work with molecular microbiology, various phenotypic assays, bacteria-fungi inhibition assays, and metabolite analyses. Interactions between selected *Sphingomonas* strains and plant pathogenic fungi will be tested both in simulated growth media and on actual wheat plants under controlled conditions.



Typical colors and morphology of *Sphingomonas* isolates when cultivated on L9 agar media

**The work environment.** This MSc project is part of a large, interdisciplinary project, the MATRIX, funded by the Novo Nordisk Foundation. You can read more about MATRIX at this link:

<https://plen.ku.dk/english/research/microbial-ecology-and-biotechnology/environmental-microbial-genomics/microbiome-assisted-triticum-resilience-in-x-dimensions-the-matrix/>.

During your MSc project, you will work with researchers involved with MATRIX as well as with researchers affiliated to Prof. Lars Hestbjerg Hansen's Environmental Microbial Genomics research group at the Section for Microbiology and Biotechnology at the Department of Plant and Environmental Sciences, UCPH.

If you are interested or would like to know more about the project, please contact the supervisors: Professor Lars Hestbjerg Hansen ([lhha@plen.ku.dk](mailto:lhha@plen.ku.dk)) and Associate Professor Leise Riber ([lriber@plen.ku.dk](mailto:lriber@plen.ku.dk)).

## Prophages: The helpful yet dangerous tenants.

**Background.** Inside bacteria, we find an even smaller world consisting of mobile genetic elements (MGEs), such as transposons and prophages. These MGEs are nearly ubiquitous in natural bacteria with prophages playing a crucial role in regulating bacterial populations. Prophages are formed when bacterial viruses known as temperate phages integrate their genome into that of their bacterial hosts. Subsequently, some prophages can undergo induction, bursting out of their host cell to infect and kill neighboring bacteria. Despite their importance, the study of prophages is complicated by the complex intracellular interactions between rival prophages and other MGEs. Bacteria generally harbor between 1 and 18 of these viruses in their genomes.

**The aim.** Two MSc projects offered aim to study the behavior of prophages in bacteria in an ongoing study that involves researchers from University of Copenhagen (us, Niels Bohr institute and San Diego state university). We aim to establish the ground rules governing both intra- and intercellular prophage dynamics by 1) rebuilding a MGE-free bacterial genome with increasing levels of prophage complexity, and 2) studying the behaviors of the lysogens compared to the “wild-type” using various assays.

**The methods.** Depending on which part of the project that interests you more, you will work with molecular microbiology, CRISPR-editing, 3rd generation sequencing (Nanopore), phenotypic and competition assays, and metabolite analysis. You will also work with various general microbiology techniques. If you have the interest or skills for bioinformatics, one project can evolve to have a strong bioinformatic component, phage gene annotation, phage defense and antidefense systems and insertion sites and induction kinetics of the prophages inserted.

From: Dougherty, P.E. *"Dude, what's in your SynCom."* <https://communities.springernature.com/posts/dude-what-s-in-your-syncom>

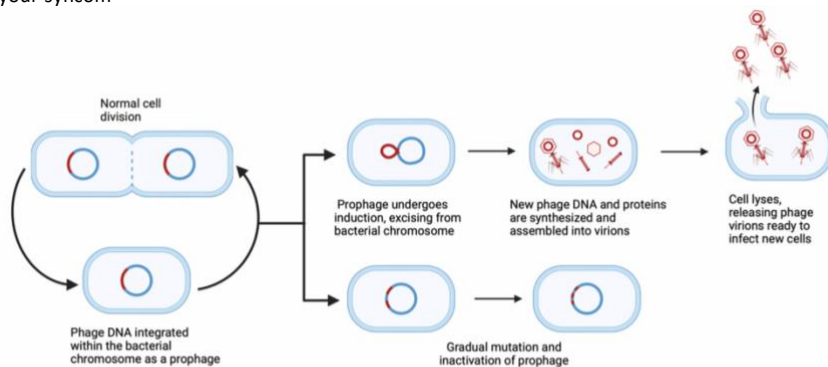


Figure 1. The possible fates of prophages. Created with BioRender.com

Literature related to this:  
Dougherty, P.E., Nielsen, T.K., Riber, L. .... Hansen LH. Widespread and largely unknown prophage activity, diversity, and function in two genera of wheat phyllosphere bacteria. ISME J 17, 2415–2425 (2023).

**The work environment.** The two MSc projects are connected to a larger ongoing project with researchers involved. You will be part of Prof. Lars Hestbjerg Hansen’s Environmental Microbial Genomics research group at the Section for Microbiology and Biotechnology at the Department of Plant and Environmental Sciences, UCPH.

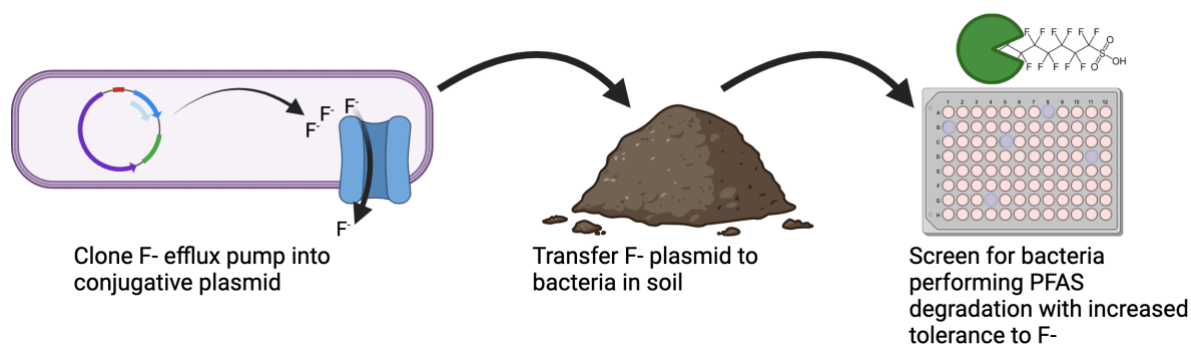
If you are interested or would like to know more about the project, please contact the supervisors: Professor Lars Hestbjerg Hansen ([lhha@plen.ku.dk](mailto:lhha@plen.ku.dk)); Associate Professor Leise Riber ([lriber@plen.ku.dk](mailto:lriber@plen.ku.dk)).

## Prepping environmental bacteria for the stress of degrading PFAS.

**Background.** PFAS is a large group of chemicals that is used for many industrial applications. Consequently, humans are constantly exposed to these chemicals, of which some are carcinogenic and hormone disruptors. They are termed “forever chemicals” as the strength of their characteristic C-F bonds make them resistant to degradation. Furthermore, upon breaking of these bonds, free F<sup>-</sup> minus is released which is toxic and at minimum stresses cells.

**The aim.** This MSc project aims construct a conjugative plasmid that expresses an F<sup>-</sup> efflux pump. This conjugative plasmid is then transferred to environmental samples (e.g.) soil for transfer of the plasmid to the microbial community. A high-throughput screening assay is then applied to screen for degradation of PFAS by members of the microbial community that have obtained the constructed plasmid and are thus more tolerant to F<sup>-</sup>.

**The methods.** You will work with molecular microbiology, including PCR, cloning and 3rd generation sequencing (Nanopore). You will also work with environmental samples and general microbiology techniques. Finally, you will apply chemistry in the high-throughput screening assay.



**The work environment.** This MSc project is connected to a larger ongoing project with many related activities. You will be part of Prof. Lars Hestbjerg Hansen’s Environmental Microbial Genomics research group at the Section for Microbiology and Biotechnology at the Department of Plant and Environmental Sciences, UCPH.

If you are interested or would like to know more about the project, please contact the supervisors: Tenure-track assistant professor Tue Kjærgaard Nielsen ([tkn@plen.ku.dk](mailto:tkn@plen.ku.dk)) and Postdoc Asal Forouzandeh ([asal@plen.ku.dk](mailto:asal@plen.ku.dk))

## Extremophile bacteria from the feathers of New World Vultures.

**Background.** Vulture plumage exposed to intense solar radiation constitutes one of the more extreme microbial environments found on or inside terrestrial and aquatic animals. Plumage temperatures recorded in sunning birds can reach more than 80°C at feather surfaces and the intense UV radiation and occasional dessication of the feathers poses a harsh environment for the microbiome that inhabits this ecological niche. We have isolated microbes from vulture feathers that seem to express extreme behaviours.

**The aim.** Two MSc projects offered aim to study the extreme microorganisms that were isolated from the feathers of New world Vultures in an ongoing study that involves researchers from University of Copenhagen and the Smithsonian Institution, Washington DC. We aim to do whole genome sequencing on numerous additional isolates followed by bioinformatic analysis. Then experiments on how well the bacteria survive extreme conditions such as UV radiation, heat and more will be done. Finally we aim to relate the genotype to the phenotypic findings.

**The methods.** Depending on which part of the project that interests you more, you will work with molecular microbiology, phenotypic and enzymatic assays, 3rd generation sequencing (Nanopore) and metabolite analysis. You will also work with real samples of Vulture feathers and general microbiology techniques. If you have the interest or skills for bioinformatics one project can evolve to have a strong bioinformatic component, with phylogenetic analysis, gene annotation and comparing isolate genomes from two flocks of birds.



Literature related to this:

Graves et al. *Animal Microbiome* (2020) 2:24. <https://doi.org/10.1186/s42523-020-00043-7>

Roggenbuck M, .....Graves GR. And Hansen LH. The microbiome of New World vultures. *Nat Commun.* 2014;5:5498. <https://doi.org/10.1038/ncomms6498>.

**The work environment.** The two MSc projects are connected to a larger ongoing project with researchers involved. You will be part of Prof. Lars Hestbjerg Hansen's Environmental Microbial Genomics research group at the Section for Microbiology and Biotechnology at the Department of Plant and Environmental Sciences, UCPH.

If you are interested or would like to know more about the project, please contact the supervisors: Professor Lars Hestbjerg Hansen ([lhha@plen.ku.dk](mailto:lhha@plen.ku.dk)) and Tenure-track assistant professor Tue Kjærsgaard Nielsen ([tkn@plen.ku.dk](mailto:tkn@plen.ku.dk)).



# Viral Host Interactions

## 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<sup>1</sup>.

**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<sup>2, 3</sup>.

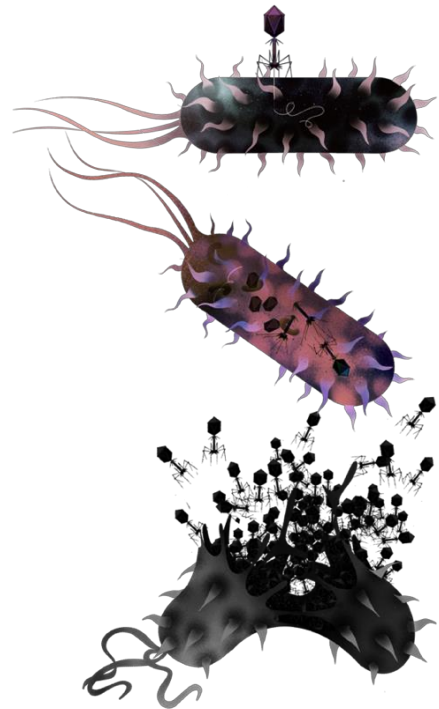
**The aim** of this project is to examine the potential roles and biological consequences of these phage gene products, successful results may lead to a scientific publication.

**Techniques:** DNA work, epigenetics, 3<sup>rd</sup> 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](mailto:lhha@plen.ku.dk) or Nikoline Olsen at [sno@plen.ku.dk](mailto: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](https://doi.org/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](https://doi.org/10.1128/JVI.01521-13).



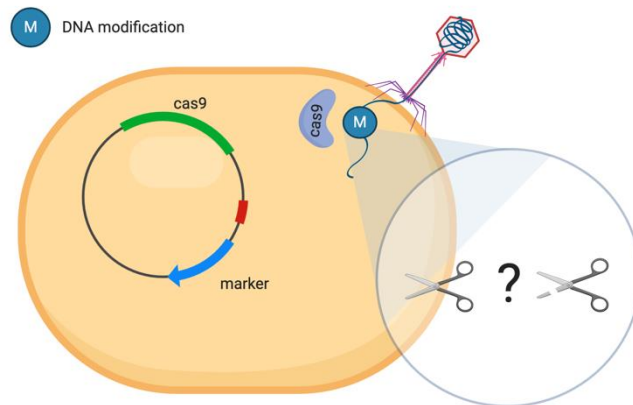
**Figure 1** Graphic illustration of a lytic phage infection cycle, by Andreas Y. Pilskog.

## 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](mailto:wk@plen.ku.dk))

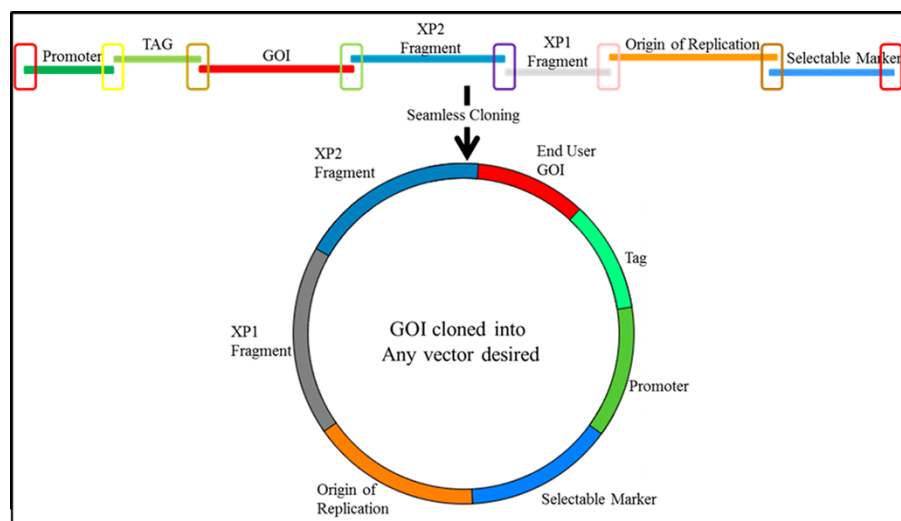
Relevant literature:

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<sub>0</sub>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



## 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).

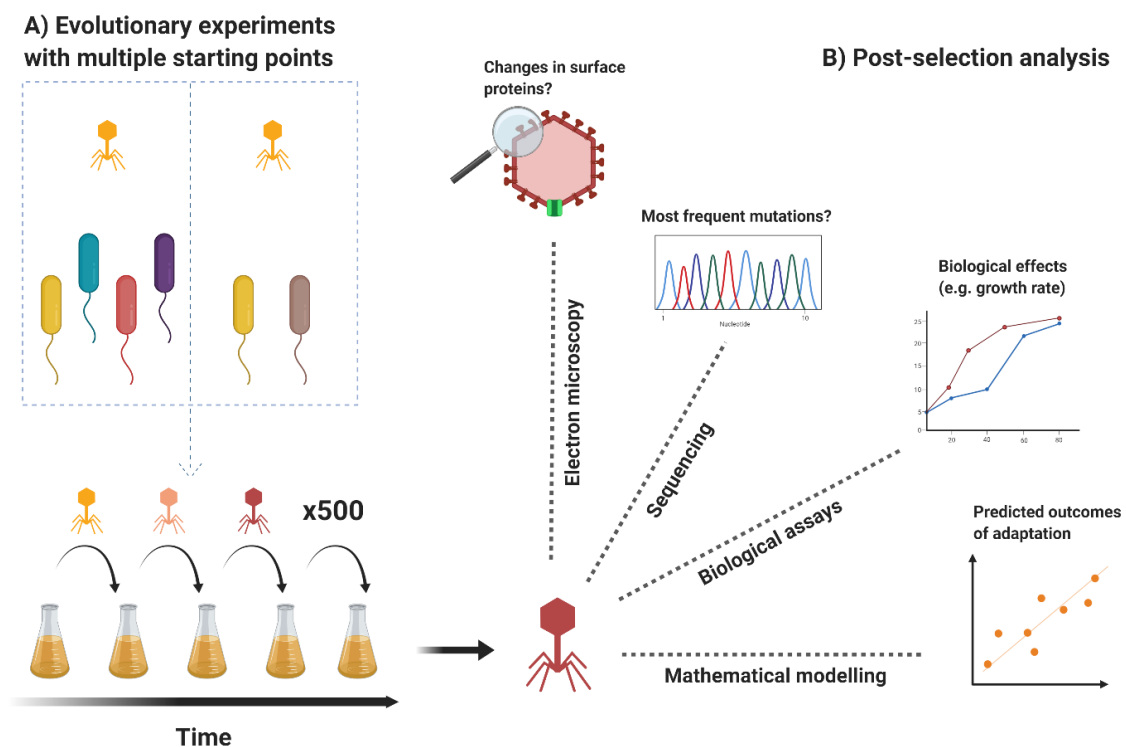
**Techniques:** DNA work, plasmid biology, bacterial genetics, molecular microbiology, bioinformatic analyses, DNA cloning, restriction enzymes, electroporation, plasmid stability assays, qPCR for copy number determination, gene editing.

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

## 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.



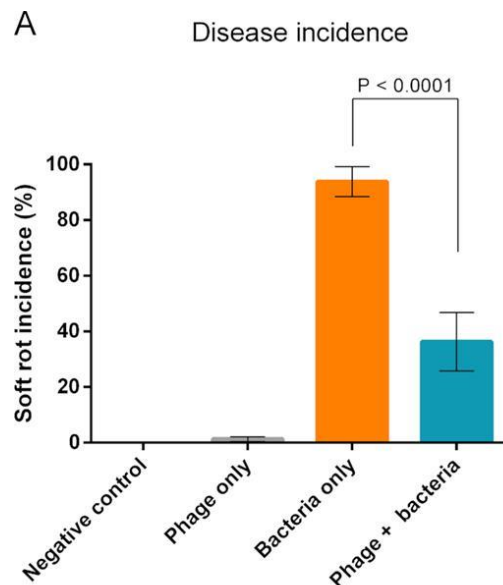
As a student in this project you will; set up experiments to study the evolution of viruses in the laboratory, perform DNA isolation and DNA sequencing of viruses, determine growth criteria of bacteriophages (host range, burst size, latent period). Although the overall goal of the project is decided 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](mailto:lhha@plen.ku.dk) or Alexander Byth Carstens at [alexander.carstens@plen.ku.dk](mailto:alexander.carstens@plen.ku.dk)

## Can phages replace pesticides?

(MSc and BSc)

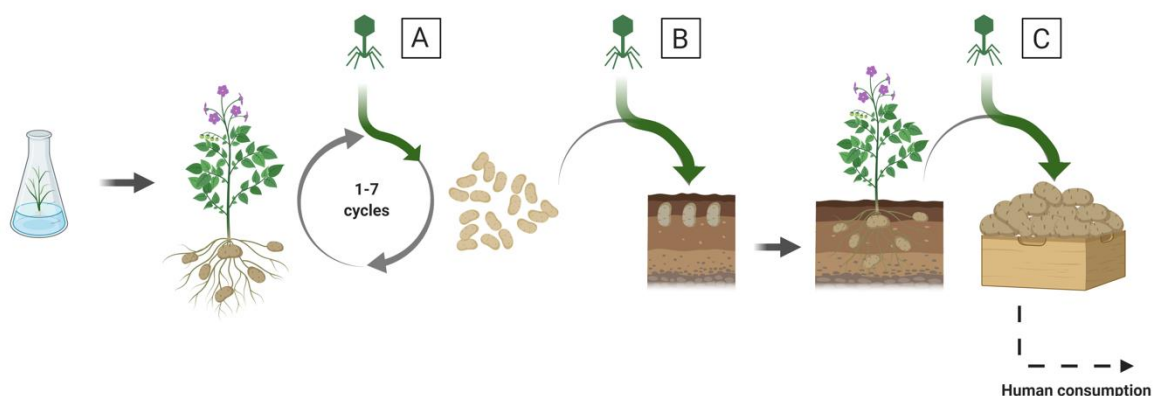
Bacterial plant diseases are a major problem in agriculture worldwide. To combat plant pathogenic bacteria we use pesticides and heavy metals, with detrimental effects on the environment and human health. In this project, we seek to develop bacteriophages (phages) as a green alternative to pesticides 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.



Laboratory growth

Seed potato production

In-field production and storage



We have several 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.

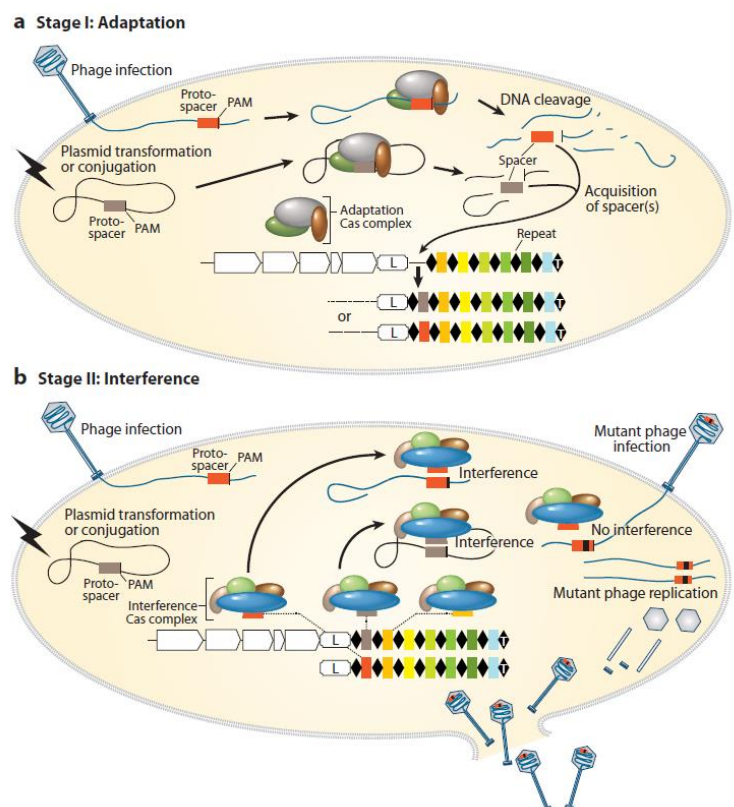
If you have any questions or want to hear more about the projects in the environmental microbial genomics group, you are more than welcome to contact Lars Hestbjerg Hansen at [lhha@plen.ku.dk](mailto:lhha@plen.ku.dk) or Alexander Carstens [alexander.carstens@plen.ku.dk](mailto:alexander.carstens@plen.ku.dk)

## 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.



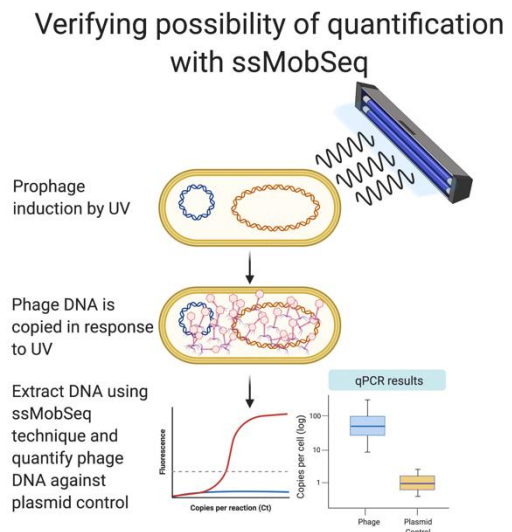
Deveau et al. 2010. Annual Review of Microbiology, doi: 0.1146/annurev.micro.112408.134123

**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](mailto:lhha@plen.ku.dk)), Assistant Prof. Witold Kot ([wk@plen.ku.dk](mailto:wk@plen.ku.dk)), PhD Student Amaru Djurhuus ([amaru@plen.ku.dk](mailto: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](mailto:tkn@plen.ku.dk) or Prof. Lars Hestbjerg Hansen [lhha@plen.ku.dk](mailto:lhha@plen.ku.dk)



## MSc project on characterisation of astrithrvirus, a novel group of tiny phages

**The aim** of this project is to perform a thorough characterization of a small group of so far undescribed *Salmonella* phages (virus of bacteria). Recently one of the isolates, *Salmonella* phage astrithr, gave rise to a new genera *Astrithrvirus Astrithr*. This genus has been posed by the International Committee on Taxonomy of Viruses (ICTV) to receive a higher taxonomic rank in future ratifications. But apart from the isolation host, isolation source and genomic sequence we know very little about these tiny viruses which can attack, take over and ultimately kill bacteria to produce their own progeny, all with just 15 genes.

**Background:** The group consists of three phages, Astrid, assan and astrithr, though Astrid and assan are considered strains of *Salmonella virus Astrithr*. They were all isolated from wastewater from Estbjerg and Skovlund using *Salmonella enterica* subsp. *enterica* serovar Enteritidis PT1 as propagation host. Phages with small genomes (16-20 kb with 20-29 CDS) are characterized by their special tail structure, inverted terminal repeats (ITR) and a rare protein primed polymerase (type B). The astrithrviruses have even smaller genomes (11.6-11.7 kb) and encode only 15 potential genes and even though they also have ITR and encode type B polymerases, they have very low DNA sequence similarity (<39%) with all other published phage genomes. In this project you will examine these phages both bioinformatically and through wet-lab assays and compile all the data into a thorough description of this new intriguing group of phages.

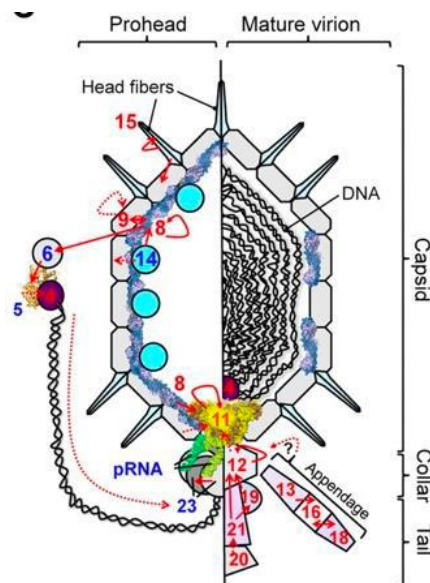


Figure: Schematic model of the virion of cp-1, one of the closest related phages. from Häuser et al., 2011.

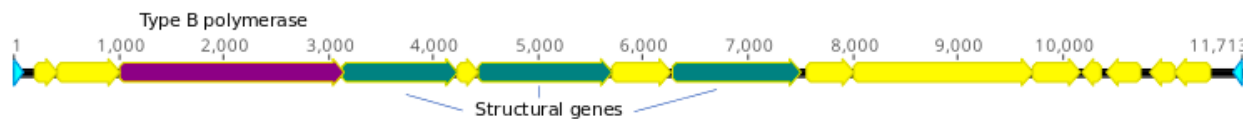


Figure: The genome of *Salmonella* phage Astrid, yellow arrows represent hypothetical genes, blue arrows are inverted terminal repeats (ITR).

**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 *Astrithrvirus* genus.

**Contact:** Lars Hestbjerg Hansen at [lhha@plen.ku.dk](mailto:lhha@plen.ku.dk) or Nikoline Olsen at [sno@plen.ku.dk](mailto:sno@plen.ku.dk)

### Relevant literature:

Häuser, R., M. Sabri & P. Uetz (2011). The Proteome and interactome of *Streptococcus pneumoniae* 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](https://doi.org/10.1128/aem.00031-12).



## 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

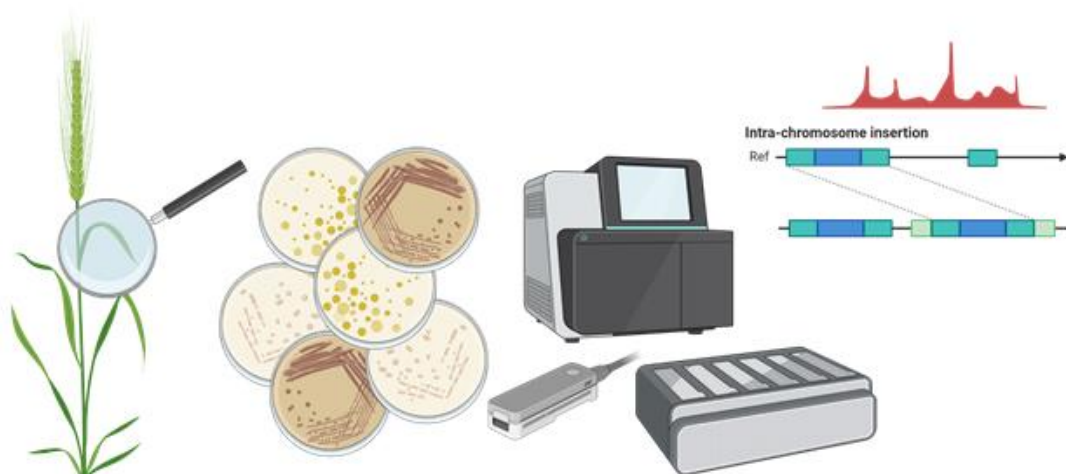
**Techniques:** In this project we will make an extensive use of classical microbiology isolation techniques, covering different growth media and conditions to obtain the largest diversity across the flag-leaf bacteriome. From selected isolates we will extract the DNA and prepare sequencing libraries 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)

Assistant Prof. Leise Riber (lriber@plen.ku.dk)

Contact us if you have any question and you want to know more about it

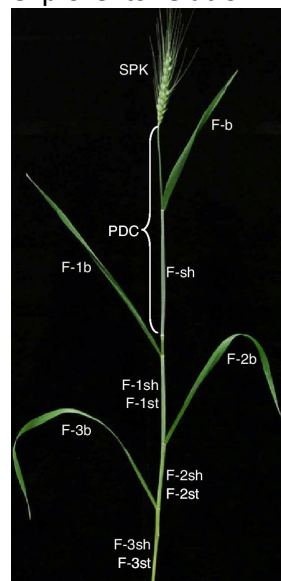


## Microbial shifts on wheat phyllosphere under double-fertilization regimes

**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](mailto:lhha@plen.ku.dk))

Assistant Prof. Leise Riber ([Iriber@plen.ku.dk](mailto:Iriber@plen.ku.dk))

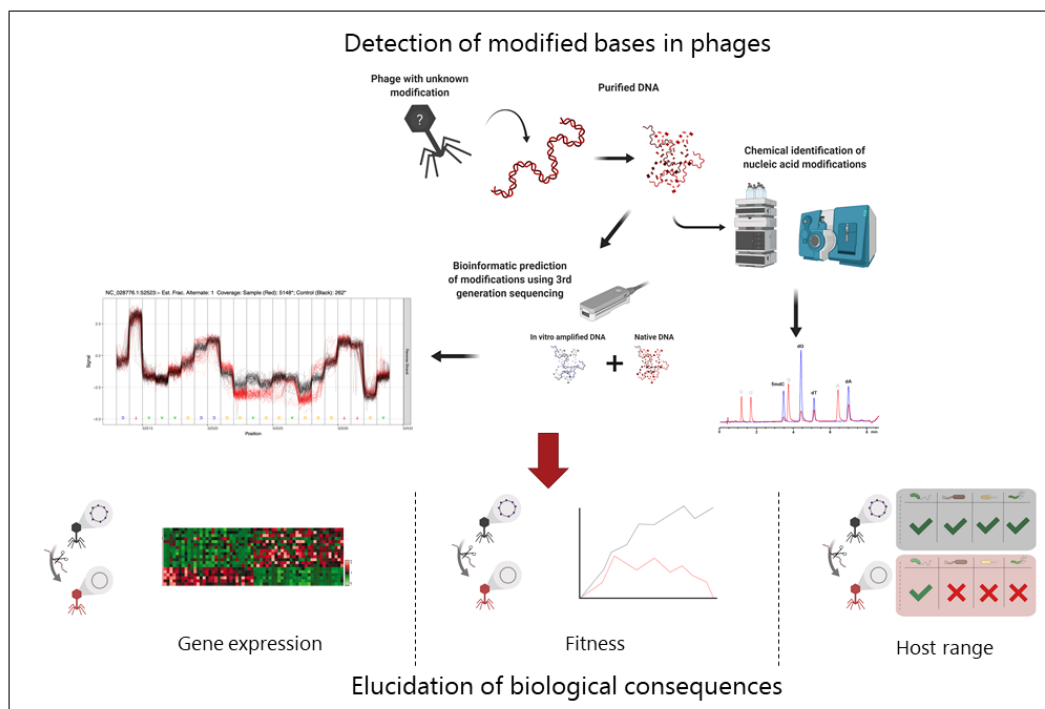
### References:

- 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

## 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 3<sup>rd</sup> 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.

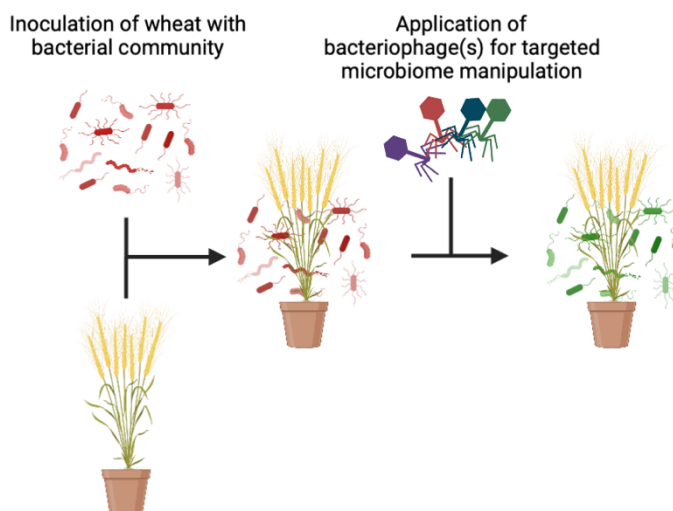
**Techniques:** DNA work, phage and bacterial genetics, 2<sup>nd</sup> and 3<sup>rd</sup> generation sequencing, molecular microbiology, phage characterization, CRISPR/Cas9 cloning, Gibson assembly.

**Supervisors:** Prof. Lars Hestbjerg Hansen ([lhha@plen.ku.dk](mailto:lhha@plen.ku.dk)), Assistant Prof. Witold Kot ([wk@plen.ku.dk](mailto:wk@plen.ku.dk))

# Manipulating the wheat microbiome with viruses

**Background:** More than a third of all crop yields are lost to stress factors such as drought and disease. Innovative agricultural methods are therefore necessary to feed a growing population. Recently, we have started to appreciate how the plant microbiome can protect their hosts and promote growth, but much research is needed on how to manipulate plant microbiomes. One possibility is to use bacteria-killing viruses (bacteriophages) to selectively suppress undesirable bacteria.

**Aim:** In this project, the student will attempt to kill targeted bacteria of the wheat leaf microbiome using bacteriophages. The student will grow wheat in a greenhouse and inoculate the wheat with synthetic bacterial communities with model bacterial pathogens. (S)he will then design bacteriophage cocktails to knock out the pathogens *in planta*. Treatment efficacy may be assessed using a cloned reporter gene or qPCR. Depending on the student's interests, this project may focus on basic research, or take on a more applied nature.



**Importance:** Plant microbiome manipulation is a promising and fast-moving field of research with huge potential and many unanswered questions. Are microbiome manipulations long-lasting? How effective are phages *in planta* and how are they best applied? What happens when plant microbiomes are transplanted? The student attempt to answer some of these questions in this project, providing a great opportunity to publish their results in a scientific journal.

**Techniques:** Greenhouse work, DNA sequencing, cloning, PCR, qPCR, bioinformatics analysis, bioassays investigating antagonism, host range, and stability.

**Contact:** Prof. Lars Hestbjerg Hansen ([lhha@plen.ku.dk](mailto:lhha@plen.ku.dk)), Peter Dougherty ([ped@plen.ku.dk](mailto:ped@plen.ku.dk))

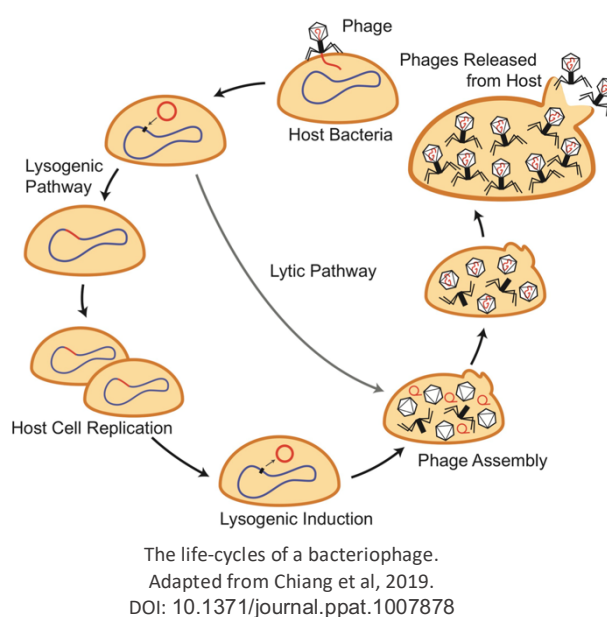
## Relevant literature

1. Buttimer, C., McAuliffe, O., Ross, R. P., Hill, C., O'Mahony, J., & Coffey, A. Bacteriophages and bacterial plant diseases. *Frontiers in Microbiology*, **8** (2017).
2. Forero-Junco, L. M., Alanin, K. W., Djurhuus, A. M., Kot, W., Gobbi, A., & Hansen, L. H. Preprint (2021). Viruses roam the wheat phyllosphere.

# Unravelling prophage dynamics; competition between microbial puppet-masters

**Background:** Around a third of bacteria worldwide are killed every day by viruses known as bacteriophages. In addition to this incredible turnover, many phages do not simply kill their bacterial hosts but instead integrate into bacterial genomes, lying dormant as prophages until they decide to break out and kill their hosts. As prophages, they may provide their hosts with benefits such as increased pathogenicity, and resistance to antibiotics and other phages. Despite the importance of prophages, there is much we don't know about how they interact with each other and compete for new hosts.

**Aim:** In this project, the student will investigate how prophages interact when their hosts are mixed with bacteria infected by rival prophages. (S)he will add and induce prophages to bacterial communities and monitor competition between prophages using different sequencing technologies. During this process, the student will likely discover novel phage species, and may sequence and characterize them. Depending on the student's interests, s(he) may investigate prophage ecology or focus in-depth on specific biological and molecular interactions. This project will also provide a great opportunity for publishing in a scientific journal.



**Importance:** Prophages are found ubiquitously in nature, and often increase bacterial virulence by providing dangerous toxin genes and spreading antibiotic resistance. Acting as both team-players and free agents with their own agendas, prophages are intriguing entities and understanding how they interact is an important basic research question in microbiology.

**Techniques:** Whole-genome and metagenomic sequencing with Illumina and Nanopore platforms, phage isolation and characterization, molecular microbiological techniques, and bioinformatic analysis.

**Contact:** Prof. Lars Hestbjerg Hansen ([lhha@plen.ku.dk](mailto:lhha@plen.ku.dk)), Peter Dougherty ([ped@plen.ku.dk](mailto:ped@plen.ku.dk)).

## Relevant literature

Howard-Varona, C., Hargreaves, K., Abedon, S. *et al.* Lysogeny in nature: mechanisms, impact and ecology of temperate phages. *ISME J* **11**, 1511–1520 (2017).

This Project is carried out at the Department of Environmental Science, AU-Roskilde and Environmental Microbial Genomics Group, MEB, PLEN, KU

# The microbiology of drinking water

## *- interactions with chemical pollutants*

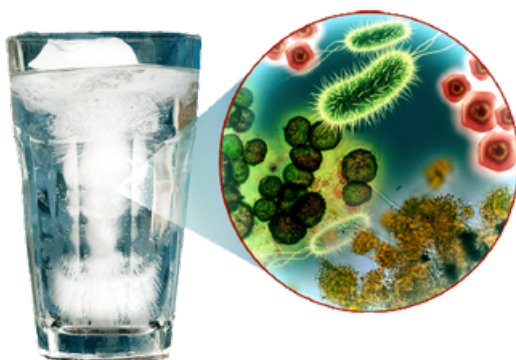
**Project type:** This project is available to Master students and is a part of the “SafeWater” project

**Background:** Clean drinking water is essential to all! In Denmark 100% of our drinking water comes from groundwater. It is usually of a good quality and requires simple treatment before distribution to consumers. Recent findings using holistic non-target analysis have shown that drinking water samples may contain many small molecules, such as pesticide residues and natural substances. We have also demonstrated that drinking water contain a community of diverse microorganisms. However little is known about the interactions between the chemical elements and the microbiology of drinking water.

### **Techniques & possible project elements:**

- Sampling of drinking water and sample preparation
- DNA extraction, library building and sequencing
- Bioinformatics
- Interactions between microbial community and chemical profile
- Bacterial communities of drinking water in different geographical regions

Other suggestions are welcome



**Supervisors:** Tenure-track researcher Lea Ellegaard-Jensen ([leael@envs.au.dk](mailto:leael@envs.au.dk)), assoc. prof. Martin Hansen ([martin.hansen@envs.au.dk](mailto:martin.hansen@envs.au.dk)) Prof. Lars Hestbjerg Hansen ([lhha@plen.ku.dk](mailto:lhha@plen.ku.dk)).

Contact us if you have any question and you want to know more about it