

Temperate bacteriophages in *Hydra* and their role in metaorganism maintenance

Dissertation

zur Erlangung des Doktorgrades der Mathematisch-
Naturwissenschaftlichen Fakultät der Christian-Albrechts-
Universität zu Kiel

vorgelegt von

Janina Lange

Kiel, im Februar 2020

Erster Gutachter: Prof. Dr. Dr. h.c. Thomas C. G. Bosch

Zweiter Gutachter: Prof. Dr. Thomas Roeder

Datum der mündlichen Prüfung: 15.04.2020

“A strong feeling of adventure is animating those who are working on bacterial viruses, a feeling that they have a small part in the great drive towards a fundamental problem in biology.”

- Max Ludwig Henning Delbrück

Table of Contents

Abstract	1
Zusammenfassung	3
General introduction	5
The metaorganism concept	5
The role of bacteria in host fitness	6
Maintenance of bacterial communities	8
Bacteriophages are pulling the strings	9
Bacteriophage lifestyles and host range.....	9
The role of bacteriophages in microbial communities.....	11
Bacteria-phage coevolution.....	12
Phage resistance and phage receptors.....	13
Advantages and costs of phage resistance.....	15
From correlation to causation.....	16
The model organism <i>Hydra</i>	17
The metaorganism <i>Hydra</i>	19
Aims of this study	22
References	24
Chapter I: The neglected part of the metaorganism: Prophages in <i>Hydra</i>-associated bacteria	35
Abstract	35
Introduction	36
Material and Methods	38
Bacterial strains and culture conditions.....	38
Identification and purification of prophages	38
Transmission electron microscopy (TEM)	38
Environmental stressors.....	39
Phage quantification	39
Crossinfection experiments.....	39
Data and statistical analysis.....	40
Results	40
Identification of bacteriophages from <i>Hydra</i> -associated bacteria.....	40
Environmental influence on prophages of <i>Hydra</i> -associated bacteria	44
Cross infectivity of temperate bacteriophages of <i>Hydra</i> -associated bacteria.....	47
Discussion	49
Supplementary	54

| Table of Contents |

References.....	57
Chapter II – Prophage TJ1 acts as an internal regulator of the <i>Hydra</i> microbiome.....	62
Abstract.....	62
Introduction	63
Results	64
<i>Curvibacter</i> sp. (strain AEP1.3) harbours a prophage that can be induced in culture and on <i>Hydra</i>	64
<i>Curvibacter</i> phage TJ1 can infect a broad range of <i>Hydra</i> -associated bacteria.....	68
Impact of <i>Curvibacter</i> phage TJ1 on Microbial community composition on <i>Hydra</i>	72
<i>Curvibacter</i> can use phage TJ1 to shape the <i>Hydra</i> -associated microbiota	72
Discussion	73
Material and Methods.....	76
Identification and induction of <i>Curvibacter</i> prophage.....	76
Phage DNA extraction and Sequencing	77
Phage annotation and comparison.....	77
Phage quantification	78
Environmental stress induction of phage TJ1 <i>in vitro</i>	79
Recolonization of <i>Hydra</i>	79
Environmental stress induction of phage TJ1 <i>in vivo</i>	80
Cross-infection and host range assay <i>in vitro</i>	80
Cross-infection assay <i>in vivo</i>	80
Impact of phage TJ1 on microbial community	81
Phage induction by different bacterial colonizers <i>in vivo</i>	81
Statistical data analysis.....	82
Supplementary	83
References.....	88
Chapter III – Evolution of temperate phage resistance interferes with bacterial colonization on <i>Hydra</i>.....	92
Abstract.....	92
Introduction	93
Material and Methods.....	94
Bacteria culture conditions, phage induction and concentration	94
Phage quantification	95
Coevolution experiment	95
DNA Extraction, Library prep and Sequencing	96
Genome Assembly and Annotation	96
Genomic mutation analysis	96

| Table of Contents |

Resistance assay.....	97
Germfree <i>Hydra</i>	97
Recolonization capacity of (co-)evolved bacteria.....	97
Biofilm quantification	98
Swimming assay	98
Statistics and data analysis	98
Results	99
Dynamics of <i>Hydra</i> -associated bacteria and Phage TJ1	99
Phage resistance and growth <i>in vitro</i>	101
Phage resistance led to an altered <i>Hydra</i> colonization pattern.....	102
Genomic changes	104
Causes of decreased recolonization success of TJ1 resistant bacteria	107
Discussion	108
Supplementary	112
References	124
General discussion	130
Bacteriophages - the most abundant entity in the world.....	130
Environmental stressors and their potential in bacterial community alteration	130
Bacteriophage host range	132
The functional role of temperate phages within metaorganisms.....	133
Bacteria-phage coevolution and the influence on metaorganisms	135
Phage-bacteria coevolution dynamics.....	135
Phage-bacteria interactions as a possible explanation for colonization pattern	137
References	139
Conclusion.....	144
List of publications	145
Acknowledgements.....	146
Erklärung	148

Abstract

All eukaryotic organisms are associated with a complex set of microbes, including bacteria, fungi and viruses. This association is termed metaorganism or holobiont. Bacteria are well-known key players within metaorganisms and play an important role in health and disease of their eukaryotic host. Shifts in the bacterial community composition are associated with diseases like inflammatory bowel disease, diabetes, obesity and even depression. Therefore, it is important to maintain a stable microbiome. Considering that bacteriophages (bacterial viruses, short: phages) are often obligate killers to their bacterial hosts, they can shape whole bacterial communities. Consequently, researching and understanding phage-bacteria interactions is of enormous importance. While several correlative virome studies have been conducted, little is known about the functional role of bacteriophages within metaorganisms. Hidden in the genome of their bacterial host, especially temperate phages are often neglected and overlooked.

A perfect organism for studying bacteria-phage-host interactions is the freshwater polyp *Hydra*, because it features a host specific bacterial and viral community. To shed light on the role of temperate phages within metaorganisms, I first identified prophages that are integrated in *Hydra*-associated bacteria. Therefore, I screened the genomes of the bacterial culture collection of *Hydra* for prophage signatures, of which six phages are inducible by antibiotics and environmental stressors. With cross infection experiments I analyzed the host range of these phages, of which three featured a broad host range. One of them was the phage of *Hydras* main colonizer *Curvibacter* sp. (phage TJ1).

To evaluate the role of this dominant phage TJ1 in homeostasis of *Hydra*, I analyzed the activity and the infectivity of this phage *in vitro*. I could demonstrate that phage TJ1 is active on the eukaryotic host and negatively affects the growth of the next most abundant bacterial colonizers: *Duganella* sp., *Undibacterium* sp. and *Pelomonas* sp.. Furthermore, I could show that the phage is not only inducible by environmental stressors but also by competing bacteria *in vivo*. This indicates that TJ1 is active on *Hydra* without additional, artificial stimulation.

Although exposed to TJ1, susceptible bacterial isolates seem to be unable to evolve resistance on *Hydra*. To evaluate the mechanisms behind this observation, I

| Abstract |

conducted an *in vitro* coevolution experiment with phage TJ1 and the susceptible bacterial colonizers *Duganella* sp., *Undibacterium* sp. and *Pelomonas* sp.. I could show that after gaining resistance to the phage, the bacteria lose their ability to colonize their host, probably caused by mutations that resulted in reduced biofilm formation and motility.

In conclusion, my results are an indication for the importance of temperate phages within metaorganisms and give insights into the functional role of the dominant *Curvibacter* phage TJ1. This phage contributes to the maintenance of *Hydras* species-specific bacterial community by down-regulating other highly abundant bacterial colonizers. Moreover, my data gives a hint to how temperate bacteriophages and bacteria can coexist on a eukaryotic host: High fitness costs in resistant strains result in favouring phage susceptibility over resistance on *Hydra*.

Zusammenfassung

Alle Pflanzen, Tiere und Menschen sind mit einer Vielzahl von Mikroben, einschließlich Bakterien, Pilzen und Viren, assoziiert. Dieser Zusammenschluss wird Metaorganismus oder Holobiont genannt. Besonders Bakterien sind im Metaorganismus von besonderer Bedeutung, denn sie spielen eine wichtige Rolle in der Gesundheit des eukaryotischen Wirts. Verändert sich die Zusammensetzung der bakteriellen Gemeinschaft drastisch, kann dies zu Krankheiten, wie entzündlichen Darmerkrankungen, Diabetes, Fettleibigkeit und sogar Depressionen führen. Daher ist es wichtig ein gesundes, stabiles Mikrobiom aufrechtzuerhalten. In Anbetracht der Tatsache, dass Bakteriophagen (Viren für Bakterien, kurz: Phagen) Bakterien infizieren und damit töten können, sind diese potentiell in der Lage die Zusammensetzung von Bakteriengemeinschaften zu beeinflussen und zu formen. Daher ist die Erforschung von Phagen-Bakterien Interaktionen von besonderer Bedeutung. Obwohl bereits korrelative Studien über das Virom verschiedener Metaorganismen durchgeführt wurden, ist über die funktionelle Rolle der Bakteriophagen nur sehr wenig bekannt. Besonders temperate Phagen werden oft vernachlässigt und übersehen, da sie in dem Genom ihres Bakterien-Wirts integriert sind.

Ein perfekter Organismus für die Untersuchung von Bakterien-Phagen-Wirt-Wechselwirkungen ist der Süßwasserpolyt *Hydra*, da dieser eine stabile, wirtsspezifische bakterielle und virale Gemeinschaft aufweist. Um die Rolle von temperaten Phagen in Metaorganismen zu untersuchen, habe ich die Genome der Bakterienkultur-Sammlung von *Hydra* auf Prophagen-Sequenzen durchsucht. Von den identifizierten Prophagen konnten sechs Phagen durch Antibiotika und Umweltstressoren induziert werden. Mit Hilfe von Infektions-Experimenten wurde das Wirtsspektrum dieser Phagen analysiert. Drei Phagen wiesen ein besonders breites Spektrum auf, einschließlich der Phage von *Hydras* dominantestem Bakterium *Curvibacter* sp. (Phage TJ1).

Um die Rolle dieser dominanten Phage zu evaluieren, habe ich die Aktivität und Infektiosität dieser Phage *in vitro* getestet. Ich konnte zeigen, dass die Phage TJ1 auf dem eukaryotischen Wirt aktiv ist und das Wachstum anderer Bakterien (*Duganella* sp., *Undibacterium* sp. and *Pelomonas* sp.) *in vivo* verringert. Darüber hinaus konnte

| Zusammenfassung |

ich zeigen, dass die Phage nicht nur durch Umweltstressoren, sondern auch durch konkurrierende Bakterien induziert werden konnte. Dies deutet darauf hin, dass die Phage TJ1 ohne zusätzliche, künstliche Stimulation auf *Hydra* induzierbar und aktiv ist.

Obwohl die sensitiven Bakterien der Phage TJ1 ausgesetzt sind, konnten keine resistenten Isolate dieser Bakterien auf *Hydra* gefunden werden. Um der Ursache dieser Beobachtung auf den Grund zu gehen, habe ich ein *in vitro* Ko-evolutionsexperiment mit der Phage TJ1 und den sensitiven Bakterien *Duganella* sp., *Undibacterium* sp. und *Pelomonas* sp. durchgeführt. Hier habe ich gezeigt, dass nachdem die Bakterien Resistenzen gegen TJ1 ausgebildet haben, diese resistenten Stämme ihre Fähigkeit verlieren, *Hydra* Polypen zu besiedeln. Dies wird wahrscheinlich durch Mutationen verursacht, die die Fähigkeit zur Biofilm-Bildung oder die Mobilität resistenter Bakterien verringern.

Zusammenfassend verdeutlichen meine Ergebnisse, wie wichtig temperate Phagen innerhalb des Metaorganismus sind. Die *Curvibacter* Phage TJ1 ist in der Lage andere *Hydra*-assoziierte Bakterien zu infizieren und zu regulieren und damit die Art-spezifische Bakteriengemeinschaft zu beeinflussen und aufrechtzuerhalten. Darüber hinaus geben meine Daten einen Hinweis darauf, wie temperate Bakteriophagen und Bakterien auf dem eukaryotischen Wirt koexistieren können: Aufgrund von Resistenzbedingten Fitnesskosten, wird eine Phagen-Sensitivität gegenüber einer Phagen-Resistenz auf *Hydra* bevorzugt.

General introduction

The metaorganism concept

For a long time animals and other eukaryotes were considered as independent entities within their own environment (1). However, when eukaryotes first emerged, bacteria were already colonizing the earth for 2 billion years, which indicates that eukaryotic organisms constantly developed in the presence of microbes (2). From this point of view, it comes as no surprise that a strong interaction evolved between bacteria and multicellular eukaryotes. Those interactions take place at eukaryotic surfaces that are constantly exposed to microbes, such as skin epitheliums and/or mucus layers present in several parts of the organism, e.g. in the gut or reproductive organs (1–5). Those surfaces represent an ideal environment for the colonizing bacteria, providing space and essential nutrients (1, 6, 7). For a long time, the prevalent opinion was that these benefits are only exploited by pathogens. Nowadays it is well recognized that the evolved bacteria-host interactions go beyond the typical assumed parasitism or pathogenicity and also include mutualism and commensalism (5, 8). All eukaryotic organisms are living in an interdependent and mutualistic relationship with several microbes, such as bacteria, viruses, fungi and protists (2, 5). This close association is termed metaorganism or holobiont (2, 5) (Fig. 1). All microbes colonizing the eukaryotic host are summarized as microbiota, while the sum of their genetic material is termed microbiome (9). It has been suggested, that the host genome and the microbiome form the hologenome, a unit of selection in evolution (10, 11).

Different bacteria evolved to colonize different body parts (12), including oral cavities (13), specialized organs like the light organ of the squid *Euprymna scolopes* (14) or the reproductive parts of several female animals (15) and the gastrointestinal tract (16). Those bacteria can even be shared between individuals, which are either genetically related (17) or share the same habitat (17, 18). Considering this specialization and the fact that for example humans consist of at least as much bacterial cells as own host cells (16), it is unsurprising that bacteria play an important role in health and disease of the host (1, 19).

The role of bacteria in host fitness

During the last decades, several studies have shown the importance of microbiota for host fitness and physiology (1, 20). Bacteria are known to play an important part in several fundamental processes, for example behaviour, development and metabolism (1) (Fig. 1).

Intracellular bacteria in corals are hypothesized to provide nutrients and metabolites to their host and facilitate the host-dinoflagellate endosymbiosis (21). This implies a major role of intracellular bacteria in functional and coevolutionary dynamics within the coral metaorganism (21). Similar strong associations have been identified in photosynthetic eukaryotes. Several intracellular bacteria have been shown to supply their host with fixed nitrogen from the environment. For instance, the planktonic nitrogen-fixing cyanobacterium (UCYN-A) is living in a mutualistic relationship with single-celled eukaryotic algae. The algae receives nitrogen from UCYN-A in exchange for carbon (22). Similar observations could be detected in several *Rhizobiales* strains (23), which additionally promote root-growth in *Arabidopsis thaliana* (24–26).

In invertebrates, such as *Caenorhabditis elegans* or *Buchnera*, the microbiota produces essential nutrients or amino acids (27, 28). Similar observations have been made in mammalian guts, where bacteria play an important role in extracting and processing nutrients and energy from food (29–31). Moreover, gut-associated bacteria are involved in several fundamental processes (20). They can influence intestinal functions like gut motility (20) and permeability (32, 33), the establishment and maturation of a normal functioning immune system (34–36) and even behaviour (37–39).

Several microbes, including gastrointestinal bacteria, have also been shown to advance host organ morphogenesis and development (20, 38, 40). Germfree *Drosophila* larvae showed a higher mortality rate, smaller body size and a lengthened time to develop into puparium, compared to bacteria-associated individuals (41), while axenic (germfree) mosquitos (*Culcidae*) larvae failed completely to develop beyond the first instar stage (42). In the squid *Eupreymna scolopes*, the development of the light organ is triggered by bacterial Lipopolysaccharides of the bacterial symbiont *Vibrio fischerii* (43). *V. fischerii* promotes differentiation of the epithelial cells, which defines size and shape of the light organ (44), and induces and ensures host organ morphogenesis (40).

| General introduction |

Not only bacteria within the body are important for the metaorganism health. Studies demonstrate that the skin microbiota also harbours a great diversity and variability (3) and is surprisingly stable in the respective individual (45). Those skin bacteria have also been shown to play important roles during inflammation (46) and immune responses (47).

Given the crucial role of microbes within metaorganisms (1), it is not surprising that an overall healthy microbiome can protect its host from fungal (48–50) and bacterial infections (51) and also aid in host defence against a neurotropic virus (52).

Considering the health benefits of host-associated microbes, it is crucial for all metaorganisms to maintain a natural balance in the structural and functional microbial composition (20). An imbalance in the microbial structure is called dysbiosis and refers to a change in bacterial composition, bacterial metabolic activities or in bacterial distribution within the host (53). Dysbiosis can be a result of antibiotic treatments (54–56), diets that are high in sugar and saturated fat (western diet) (57, 58), pathogenic infections or a combination of those (20, 37) (Fig. 1). Dysbiosis is often associated with several diseases (Fig. 1). For instance, a disturbed ratio of obligate and facultative anaerobes in the intestine results in diabetes and can lead to the leaky gut syndrome (59), while a low level and a disturbed ratio of the gut commensals *Bacteroides* and *Firmicutes* is linked to an obese phenotype (29, 58, 60) and IBD (61, 62). Symptoms of anxiety are often connected to IBD (63). Therefore, it is not surprising that an infection with *Citrobacter rodentium*, a bacterium that induces IBD-like symptoms, leads to anxiety-like symptoms in mice (37). Coherent, a probiotic treatment with a *Lactobacillus* strain (64) or even the depletion of all microbes (39, 65) reduces this anxiety- and depressive-like phenotype in the mouse model (Fig. 1).

Additionally, bacterial communities change constantly throughout life and play an important role in development and age related diseases (1, 66). The development of the human infant intestinal microbiota follows a robust pattern (67), diversifies during childhood and stabilizes in adulthood (1, 68, 69). However, in elderly, the microbiome diversity decreases drastically (68, 69), which is often associated with typical aging-associated diseases, like basal inflammation (70).

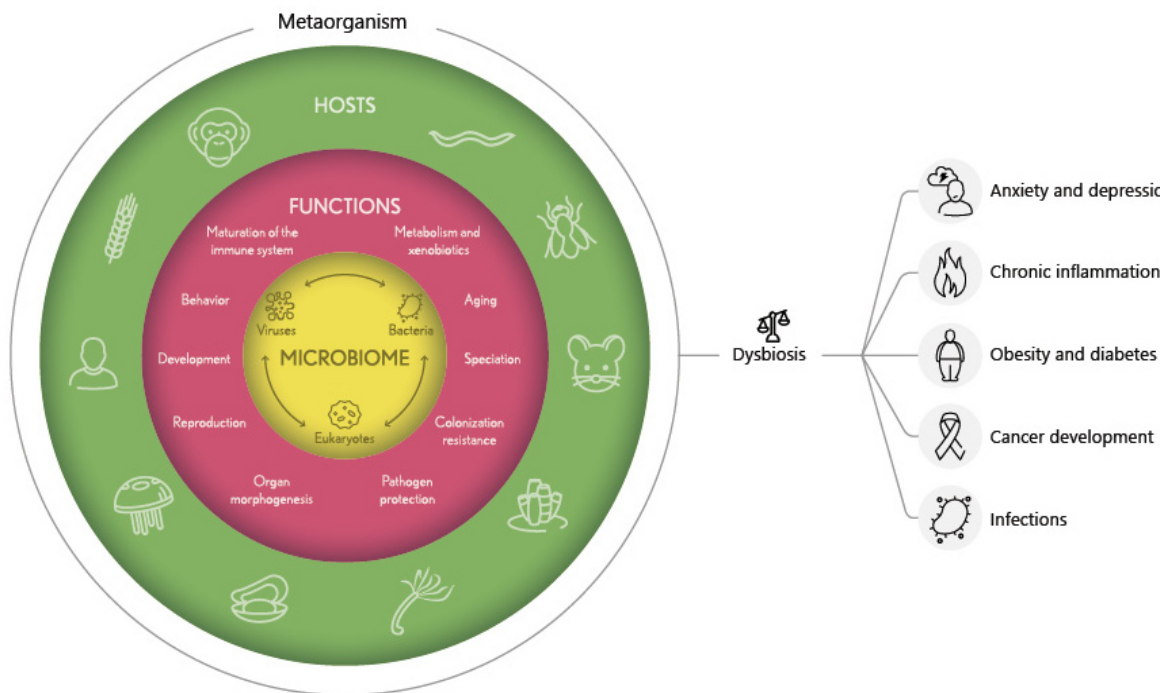


Figure 1: Functional interactions within metaorganisms between host and its microbiome, which plays an important role in e.g. the normal development of organs, metabolism and immune system and pathogen protection. A dysbiosis results in various diseases, including anxiety, obesity and diabetes and chronic inflammation. Figure from Esser, Lange *et al.* 2018 (1).

Maintenance of bacterial communities

Given the beneficial effects of microbes on the eukaryotic host, but also the disease phenotypes of a dysbiosis, it is important to understand how bacterial communities are established and controlled. Common signal molecules that control the microbial community composition are antimicrobial peptides (AMPs) (71). AMPs are secreted by epithelial cells and can maintain the natural microbiota (71). This secretion can be elevated when the host is exposed to pathogens and inflammation (72). AMPs are able to inhibit the growth of several pathogenic bacteria (73, 74), while the beneficial host-associated bacteria are often resistant against host-specific AMPs (72, 75). Other microbiome shaping host factors are, for example, secreted antimicrobial lectins (72, 76) and diffusible metabolites (77). In addition to host derived molecules and processes, the microbiome is shaped by non-host derived factors, such as diet (72, 78–80) or bacteria-bacteria interactions (81, 82). Every bacterium produces and excretes large amounts of several molecules, e.g. quorum sensing or metabolic

derived molecules, which can be used by and exchanged with other bacteria, colonizing the same niche (72).

While the role of bacteria in health and disease has been intensively studied and characterized, much less is known about the function of bacteriophages within metaorganisms.

Bacteriophages are pulling the strings

Bacteriophages (short: phages) are viruses that solely infect and kill bacteria and are the most abundant entity in the world (83). They outnumber bacteria by at least 10-fold in the oceans (83) and are present in a 1:1 ratio in the mammalian gut (84). Bacteriophages can be found in any environment, where the majority of their potential bacterial hosts are present (85). Several virome studies have proven that bacteriophages are highly abundant and diverse in metaorganisms, like sponges (86), *Aiptasia* (87), *Hydra* (88) and also humans (89). However, their functional role remains mostly unclear.

Bacteriophage lifestyles and host range

Phages can follow either of two lifecycles: lytic or lysogenic. Lytic phages attach to the bacterium, get replicated within its bacterial host and kill the bacterial cell by lysing the host cell and releasing all assembled virions to the environment (90) (Fig. 2). Lysogenic or temperate phages infect the host cell but are able to form long-lasting associations with their bacterial hosts. They integrate their genome into the host genome (prophage), get transferred vertically and remain dormant (91, 92). However, temperate phages can switch to the lytic cycle, a process called prophage induction (93, 94). It results in phage replication, death of the bacterial cell and release of phages (95, 96) (Fig. 2).

This prophage induction is triggered by an activated SOS response in the bacterial host (94, 97). The SOS signal cascade is activated, when the bacterium is exposed to agents that result in DNA damage (98, 99). Depending on the phage, the sensitivity to stressors varies. For instance, UV light is known to cause severe DNA damage (100) and is considered a great factor in phage induction (94, 101). A shift towards an acidic pH is also well-known to result in prophage induction (101, 102), as well as heat and

Reactive Oxygen Species (ROS) (94). Temperate phages can also be forced to excise their genome out of the host genome via the antibiotic Mitomycin C. This is a well-established method to induce prophages for isolation (103, 104). Recently another trigger for lysis was described: an internal phage communication system, termed arbitrium system (105). Here, phages produce peptides to communicate with each other and guide lysis-lysogeny decisions: the more peptide is produced the more likely phages decide for lysogeny to prevent host extinction (105).

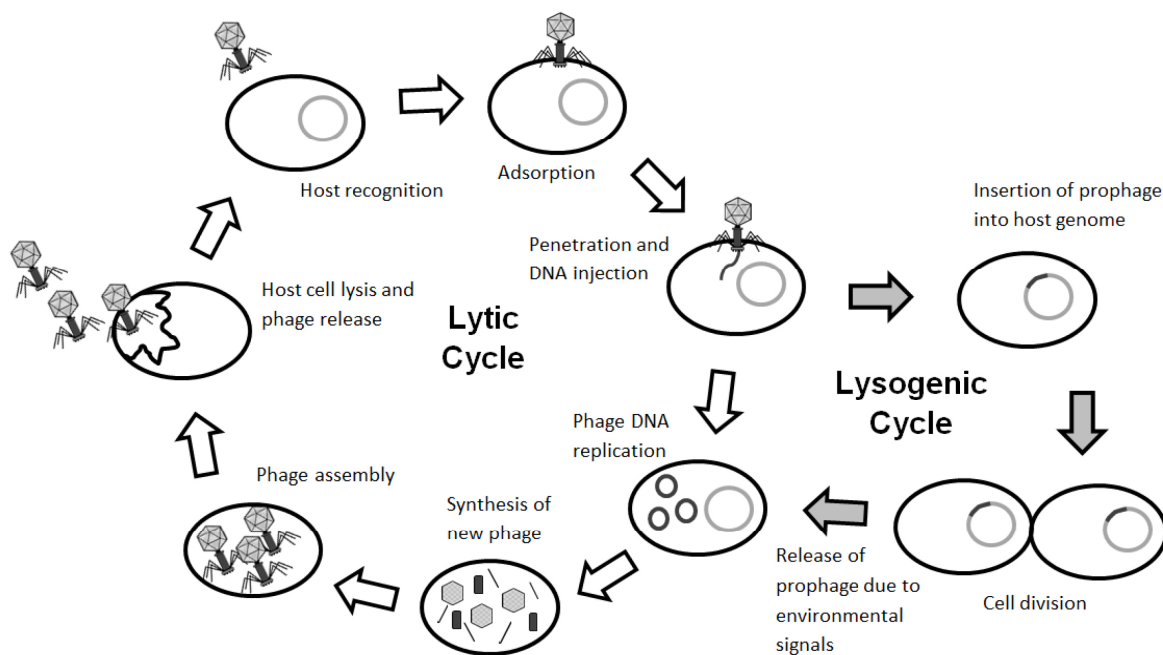


Figure 2: Life cycle of lytic and lysogenic bacteriophages: Lytic phages attach to the bacterial host cell, get replicated, synthesized and assembled using the bacteria host machinery and by lysing the bacteria cell, released to the environment. Lysogenic phages integrate their genome into the host genome, stay dormant in bacterium (prophage) and get transferred vertically. However, lysogenic phages can switch to the lytic cycle (prophage induction) via environmental stressors. Figure from Doss *et al.* 2017 (217).

No matter their initial lifestyle, eventually all phages get released to the environment. There, they can spread through the bacterial community and infect different bacteria, depending on their respective host range. Host range describes the taxonomic diversity of bacteria a respective phage can successfully infect (106). This depends on a diverse range of factors that are crucial for successful phage infection (107), including phage binding proteins, biochemical interactions during and after adsorption and bacteria-phage resistance mechanisms (107–109).

To date, phages are still considered species specific, meaning they feature a rather narrow host range. Those “specialists” only target a single bacterial species or even only a few strains within that species (109, 110). For instance, cyanophages (*Podoviridae* and *Siphoviridae*) that infect estuarine *Synechococcus* strains are exclusively host specific and can only lyse their original hosts (111). Similarly, bacteriophages isolated from fecal samples or sewage show a high specificity to either the *Escherichia coli* O157 antigen (112) or the K1 polysaccharide antigen from *E.coli* (113). However, there is increasing evidence that phages are not as specific. “Generalist” phages with broader host ranges (polyvalent) have been characterized to infect several bacteria strains, including *Staphylococcus* (114). Phage Stau2 lyses a wide range of different *Staphylococcus aureus* strains (114), while the polyvalent phage K inhibits nine different species of *Staphylococcus*, including *S. aureus*, *S. epidermidis*, *S. saprophyticus*, *S. chromogenes*, *S. capitis*, *S. hominis*, *S. haemolyticus*, *S. caprae*, and *S. hyicus* (115). Even broader host ranges have been observed in freshwater phages isolated from lake Michigan, which demonstrate a host range spanning several phyla of bacteria (116).

The ability to infect and kill and therewith control the growth of bacteria already hint to the important function they may undertake in microbial communities.

The role of bacteriophages in microbial communities

Given the fact that phages are often obligate killers to their host bacteria and possess specific host ranges, they play an important role in microbial communities (110). Since prophages are integrated in the bacterial host cell genome, they are especially important agents of lateral gene transfer (117). Mostly known for transferring virulence genes (118–121), phages can also transfer beneficial genes to their host and thereby alter bacteria geno- and phenotype (122, 123). For instance, prophages of the human pathogen *Staphylococcus aureus* enable their host bacterium to acquire antibiotic resistance genes from other competing, susceptible bacteria (124). Even cryptic (non-inducible) prophages can benefit the bacterial host. Remnants of former phage genomes are responsible for an enhanced growth, enhanced nutrient utilization and withstand to environmental stress and antibiotic exposure in *Escherichia coli* (125). This gives the prophage-carrying bacteria an advantage over other competing bacteria (122, 123, 125). Temperate phages usually provide super-infection exclusion genes to

their bacterial hosts, which immunizes lysogenized bacteria against infections of the same or closely related phages (123, 126–129). Those induced phages can also function as self-replicating weapons against other competing bacteria. This can alter competition among bacterial strains and species (123, 130). The coli bacteriophage λ acts as a replicating weapon and benefit their host during competition over non-lysogenized *E. coli* cells (131). Similarly, lysogenized *Pseudomonas aeruginosa* can invade phage-susceptible *P. aeruginosa* populations and outcompete non-lysogens in rat lungs (132).

However, if a prophage is beneficial highly depends on the induction rate: the lower the induction rate, the higher the advantage for the bacterium (133). For example, prophage λ induction is two orders of magnitude higher in the mouse intestine than under liquid culture conditions (133). On the one hand, those high induction rates *in vivo* reduce the number of lysogens in the population and benefit non-lysogenic bacteria (133). On the other hand, high induction rates benefit temperate phages. Released to the environment, they are able to infect other bacteria and can spread through the whole bacterial population (134). Predation by phages is predicted to follow the “Killing-the-winner” (KtW) theory. KtW underlies negative frequency dependent selection, since bacterial fitness is linked to bacterial abundance in this context (110, 135). Here, phages prey on the most abundant and/or most active bacteria population, for example those with the highest metabolic rate (136) and keep their abundance on a constant level (136, 137). Those complex interactions result in a higher species diversity within bacterial populations (136, 138), genetic divergence and drives coevolution (139).

Bacteria-phage coevolution

Coevolution between bacteria and their bacteriophages are central to ecology and evolution of microbial communities (140). Bacteria-phage interactions are suggested to be antagonistic, resulting in a process of reciprocal host defence and parasitic counter-defence (141). Exposed to phages, bacteria hosts evolve resistance (142–144), while phages respond with counter-adaptation by increasing phage infectivity (145). This is called coevolution. Coevolution is implicated for a number of ecological and evolutionary processes, including host-parasite population dynamics, the evolution of diversity and speciation and pathogen virulence (146–148). The pressure

for innovation during coevolution results in selection for mechanisms that permit higher mutation rates (149) and therewith increase evolvability (150). Consistently, lab strain *Pseudomonas fluorescens* that coevolves with phage SBW25 Φ 2 and phage Phi2 has an elevated mutation rate compared to control treatments in the absence of phages (139, 149). Phage SBW25 Φ 2 also shows a higher molecular mutation rate when coevolving with its host *Pseudomonas fluorescens* SBW25 in comparison to the scenario where the phage evolves against a constant, ancestral SBW25 host (151). Coevolution of the marine bacterium *Synechococcus* sp. WH7803 with the virus RIM8 results in rapid diversification of both, host and virus. This may have an impact on whole bacteria-virus dynamics in the marine environment (152).

Two main concepts are known to explain antagonistic coevolution: Arms race dynamics (ARD) and fluctuating selection dynamics (FSD) (153–156). In case of ARD, coevolution is largely driven by directional selection, meaning a shift of allele frequency to a favoured extreme phenotype (154). In consequence, novel host resistances and parasite infectivity mutations accumulate over time (154). The concept of FSD underlies frequency dependent selection. Changes in allele frequencies in host and parasite populations result in parasite-mediated selection against common host resistance alleles (154) and fluctuation of genotypes (156). Therefore, FSD underlying coevolution does not result in broadening neither parasite infectivity, nor host resistance (154).

It is suggested that bacteria-phage coevolution is mostly driven by ARD rather than FSD dynamics (141, 154). However, in contrast to laboratory studies (154), coevolution between *Pseudomonas fluorescens* and SBW25 Φ 2 in soil microcosms followed FSD dynamics during the later timepoints of coevolution (146). It is suggested that only over short-term coevolution, ARD will be prevalent. Due to high costs of resistance, which negatively affect the bacteria (157, 158), it gives way to FSD at later timepoints (146).

Phage resistance and phage receptors

As mentioned above, phage infection often leads to the death of their bacterial host cell. Logically, bacteria are in need of tools that help them to avoid or resist phage infections. Phage resistance can evolve in several different ways, including molecular mechanisms and/or *de novo* mutations (140). Molecular mechanisms, such as restriction-modification (R-M) systems, the CRISPR-Cas system or the abortive

infection (Abi) system, ensure the recognition and degradation of foreign phage DNA after adsorption (159). This either happens via restriction enzymes (R-M system) (159, 160), interspaced short palindromic repeats, called spacer (CRISPR-Cas) (161) or a wide range of heterologous proteins that stop cell multiplication and result in phage abortion (Abi-system) (162–164).

Another mechanism is blocking the phage adsorption by mutating and changing receptors that are used by phages to enter the host cell (159, 165) (Fig. 3). The type and location of host cell receptors for adsorption highly depends on the bacteriophage and host (165) (Fig. 3).

Due to the structure of the cell wall of gram-positive bacteria, peptidoglycan (murein) (166–168) and teichoid structures (polysaccharides) (168–170) are the most important components involved in phage adsorption (165). Nevertheless, most of the studies on phage receptor recognition and adsorption are done in gram-negative bacteria, probably by reason of the challenge to classify phage receptors in such a dense and complex cell wall (165).

In gram-negative bacteria most phages rely on protein- and LPS-associated receptors (165), as the thin cell wall consists of a lean peptidoglycan layer and an outer membrane, including the Lipopolyaccharide (LPS) layer (171). For example, major outer membrane proteins (Omp) from *E. coli* function as bacteriophage receptors (172). Membrane porine OmpC and membrane domain OmpA are targets for phages, such as phage 434, Me1, M1 or Ox2, to name just a few (173–176). Beside several other outer membrane proteins, Lipopolysaccharides also play an important role in phage adsorption (125,142,143). *Pseudomonas aeruginosa* phage JG004 (179), *E. coli* phage T7 (177) and several *Podoviridae* phages of *Salmonella* (180) use LPS or LPS-related receptors to enter the bacteria host cell. Some phages, such as Tula, Tulb and Tull, which infect *E. coli*, even rely on both, outer membrane proteins (OmpA, OmpC, OmpF) and LPS for successful infection (181) (Fig. 3).

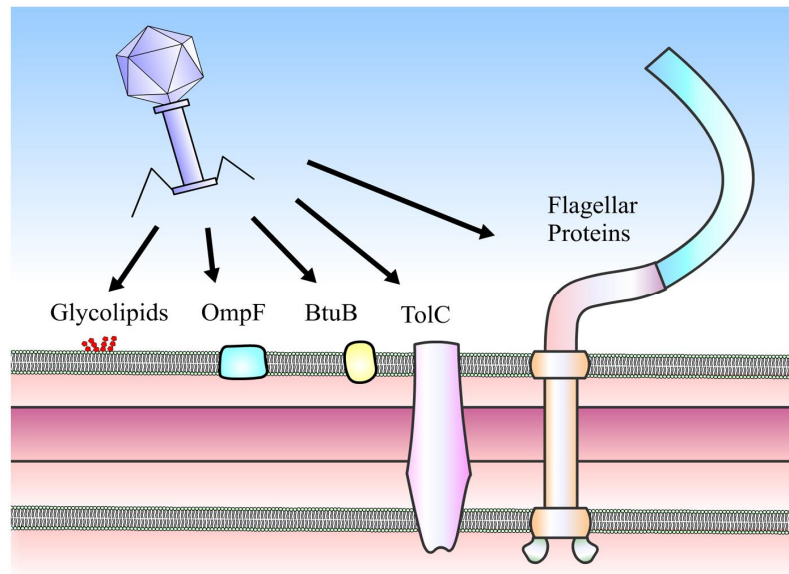


Figure 3: Phages can use a variety of cell surface moieties as receptor to initiate phage adsorption, such as Lipopolysaccharides, outer membrane proteins (e.g. OmpF, OmpA) and several parts of the bacterial flagella. Figure adapted from Chaturongakul *et al.* 2014 (182).

Apart from cell wall components, phages can also use other bacterial structures, such as flagella, for infection (165) (Fig. 3). Using protein–protein interactions between phage tail fiber proteins and bacterial flagella filaments, flagellatropic phages attach to the bacterial hosts via flagellin proteins (165, 182). One of those proteins is *FliK*, which is crucial for the infection of *Salmonella* by *Siphoviridae* phage iEPS5 (183). In the absence of polar flagella rotation, the phage infection of *Vibrio parahaemolyticus* is drastically inhibited (184), which suggests that not only the presence but also the rotation of a flagellum plays a crucial role in phage susceptibility and resistance (183, 184).

Usually, phages are considered species and receptor specific (110, 172), however, phages with broad host ranges are in need of a relaxed receptor specificity (185). Therefore, some phages are able to enter through different receptors (175), like the *Escherichia coli* phage AR1, which may use Lipopolysaccharides (LPS) and an outer membrane porine (OmpC) as host receptors (186–188).

Advantages and costs of phage resistance

Lytic phages are often harmful for their bacterial host. Therefore, the logic consequence would be a selection for and fixation of resistance mechanisms and mutations (see above).

However, resistances are not always fixed within populations (140). One explanation are disadvantageous fitness costs that are accompanied by phage resistance (140). Carrying the resistance benefits the bacteria initially, but as soon as the number of phages drop, due to less available hosts, the resistance becomes less urgent and the advantage of resistance gets lost (140). This results in less offspring of the resistant bacteria and favours the susceptible cells that have an advantage in the absence of phages. Consistently, it has been shown that phage resistance (acquired under broth conditions) is more costly in natural environments than under laboratory conditions. Bacteriophage SBW25F2 resistant *Pseudomonas fluorescens* SBW25 and phage-resistant *Pseudomonas syringae* pathovar *tomato* showed a reduction in growth in natural environments, while there were no competitive cost associated with resistance in broth environments (146, 189).

Another reason for the absence of resistance could be an ongoing arms race between bacteria and phages (145). Phages are able to overcome evolved bacterial resistances by several mechanisms: They can acquire anti-CRISPR genes, mutate spacer regions, methylate their DNA, mimic host DNA to avoid restriction and interfere with the host machinery to avoid abortion (145). Therefore, some resistances lose their benefits and get lost over coevolution time.

From correlation to causation

Today it is well recognized that bacteria play an important role in the health of all multicellular living organisms, from simple organisms like corals right up to humans (2, 5). Therefore, it is important to understand how bacterial communities are structured, established and, especially, maintained. Several host factors have been identified to influence the microbiome (190–193), however how the bacterial communities are maintained is still not fully understood yet. Phages can influence microbial communities (110, 146, 147, 149). Especially temperate phages seem to be important in microbiomes since they often transfer beneficial traits to their bacterial hosts (122, 123, 194). Those traits often provide the prophage carrying bacteria with an advantage over other competing bacteria (123). Nevertheless, prophages or temperate phages are often overlooked because they are hidden within their bacterial hosts and the functional role of temperate phages within metaorganism microbiomes remains mostly unclear.

So far, most metaorganism studies focus on correlation (88, 89, 195) instead of causation and the comprehension of phage-bacteria-host interactions.

To disentangle those complex interactions and to unravel the functional role of phages, it requires a simple model organism. This organism needs to be well-established, colonized by a limited number of microbes, and give the possibility to generate germfree animals, to analyse single bacteria-phage interactions *in vivo*. All these criteria are fulfilled by the freshwater polyp *Hydra*.

The model organism *Hydra*

Introduced as a model organism in 1744 for developmental biology (196), *Hydra* became an important model for host-microbe interactions in recent years (2, 5). *Hydra* belongs to the *Cnidaria*, a sister group of the Bilateria and is a highly diverse group within the eumetazoans (197) (Fig. 4).

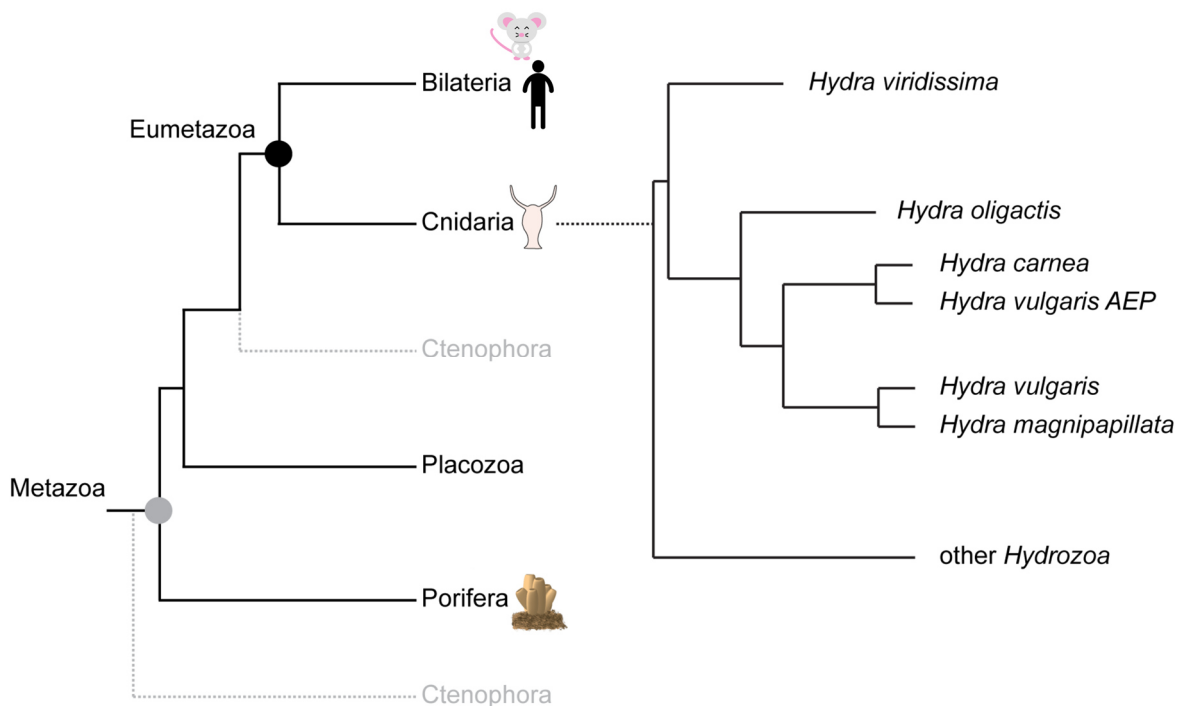


Figure 4: Schematic phylogenetic tree, showing the Cnidaria as a sister group of the Bilateria and of the different *Hydra* species within the Hydrozoa. Adapted from Hemmrich *et al.* 2012 (218).

Analysis of morphology and mitochondrial and nuclear marker classifies *Hydra* within the genus Hydrozoa into different species: *Hydra viridissima*, *H. oligactis*, *H. carnea*, *H. vulgaris* and *H. magnipapillata* (198–200). At the very base of the phylogenetic tree is *Hydra viridissima*, followed by *H. oligactis* and *H. carnea*. The group of *Hydra vulgaris* and *Hydra magnipapillata* was identified to be the most derived but also most diverse sub group within the Hydrozoans (201). The well-established lab strain *Hydra vulgaris* AEP, which is utilized for transgenesis (202), sterility and gnotobiosis (71), is phylogenetically classified within the *H. carnea* species (199) (Fig. 4). All *Hydra* species display a simple body plan, consisting of a head, with tentacles, a body column and a foot region (200).

The gastric cavity is surrounded by two monolayered epithelia: the ectoderm and the endoderm. Those layers are connected by an extracellular matrix, termed mesoglea (200). The glycocalyx is an extracellular, at least 1.5 μm thick, multi-layered mucus that covers the outside of *Hydra* polyps (203). It builds a physical barrier toward the environment and also functions as habitat for the *Hydra*-associated, surface microbiome (204) (Fig. 5).

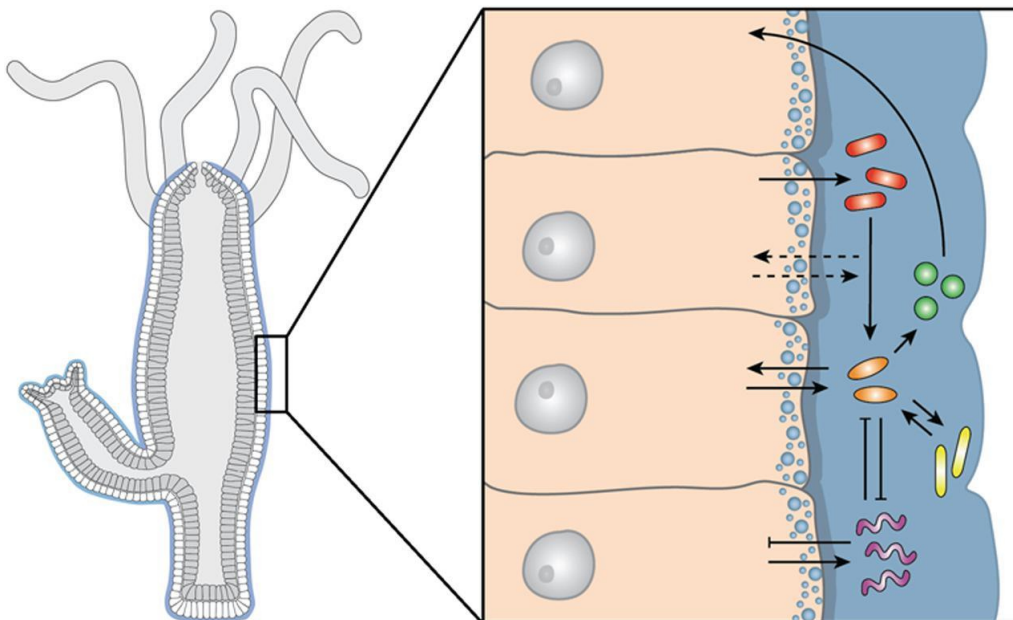


Figure 5: Schematic depiction of the metaorganism *Hydra* and its outer ectodermal cell layer with the overlying glycocalyx. The glycocalyx is the habitat for *Hydra*-associated bacteria, interacting with each other but also with the host (displayed by arrows). Adapted from Deines & Bosch 2016 (81).

The metaorganism *Hydra*

Compared to humans, the freshwater polyp *Hydra* displays a rather simple bacterial community composition. Those microbial populations are dominated by gram-negative bacteria, mostly belonging to the class of either β -Proteobacteria or Bacteroides (205). Most strikingly, even after over 20 years of lab culturing, bacteria, colonizing *Hydra* lab strains, are still similar to polyps taken from the wild (205). Investigation of different *Hydra* species revealed that even closely related species feature a very distinct bacterial population, reflecting the phylogenetic relationship of their hosts (71), a phenomena termed phylosymbiosis. (206) (Fig. 6).

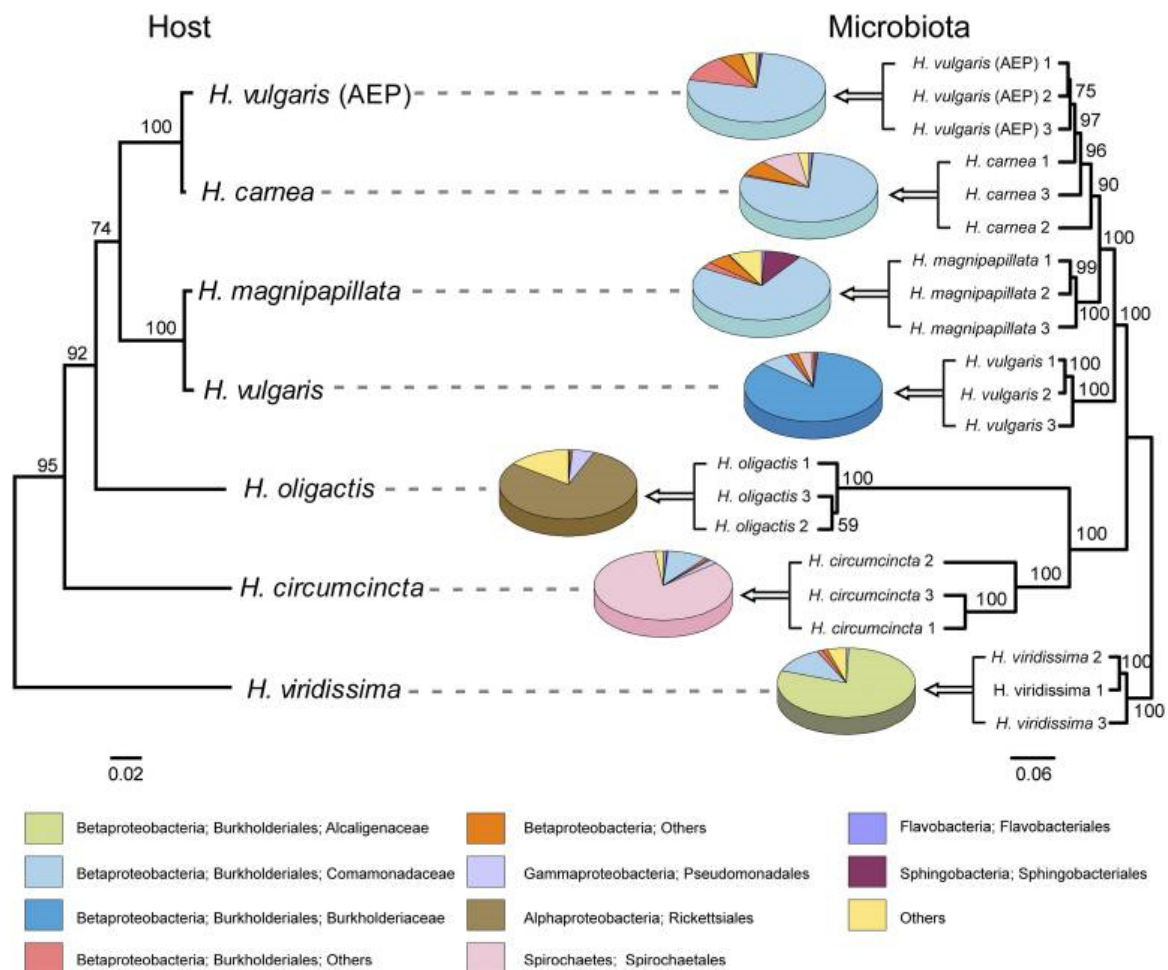


Figure 6: Comparison of phylogenetic trees of *Hydra* host strains (left) and their corresponding bacterial communities (right). The phylogeny of bacterial communities reflects the phylogeny of their corresponding hosts. Pie charts display the mean relative abundance of bacterial orders, present on the distinct *Hydra* strains. Adapted from Franzenburg *et al.* 2013 (71).

Hydras bacterial community composition is remarkably stable and species-specific, even during co-cultivation with other *Hydra* strains (71, 205). The host and its commensal microbes are living in an interdependent relationship with each other. While the glycocalyx provides a habitat for the bacteria (204), the bacteria provide beneficial traits to their eukaryotic host in return. For instance, germfree (gf) *Hydra* polyps show a reduced contractile spontaneous behaviour (207), a phenotype associated with diseases like IBD in the mammalian intestine (208). However, by reintroducing bacteria to gf *Hydra*, the frequency can be restored to a nearly normal level, suggesting that bacteria modulate those contractions and directly interact with the neuronal nerve net of *Hydra* (207). Moreover, the microbiome provides protection against potential pathogens, so called colonization resistance (209). In *Hydra*, germfree polyps are prone to lethal infections with the fungi *Fusarium* sp.. To rescue the disease phenotype, mono-association is not enough. At least two colonizers are needed to save the polyp from fungal infection (49).

The bacterial community of the lab strain *Hydra vulgaris* AEP is dominated by only five important main colonizers, which notably can all be cultivated (49, 66): *Curvibacter* sp. makes up 75 % of the whole bacterial community followed by *Duganella* sp (11 %), *Undibacterium* sp. (2 %), *Acidovorax* sp. (0.7 %) and *Pelomonas* sp. (0.2 %) (49) (Fig. 6). The most efficient anti-fungal protection was transferred by the two main colonizer of *Hydra vulgaris* AEP: *Curvibacter* and *Duganella* (49). This observation suggests that the interaction between bacteria plays an important role for the internal balance of the *Hydra* metaorganism. Mathematical models suggest that cooperation between microbes can be beneficial over a short coevolutionary scale but inevitably result in the collapse of the whole community (81, 210). Therefore, competing forces are, with at least 85 %, the majority of interactions in microbial communities (82, 211, 212). By looking deeper into the *Curvibacter-Duganella* interaction, it was shown that their interaction indeed seems to be competitive. When growing alone *in vitro*, *Duganella* displayed a growth rate that was more than twice as high as *Curvibacter's* (213). However, in co-culture, the growth rate of *Duganella* was significantly decreased, especially at low and high frequencies of *Curvibacter*, and never reached its original value. The presence of *Duganella* does not negatively affect the growth rate of *Curvibacter* (213). Mathematical modelling of the direct pairwise interactions between those two bacteria cannot explain the observed dynamics (213). This suggests that

bacteria are involved in “multiplayer” interactions (213), which also involve a bacteriophage, integrated in the genome of *Curvibacter* (130).

Furthermore, *Hydra* contains several other viruses and features a species-specific virome (88). This is dominated by the *Herpesviridae* family within the eukaryotic viruses, but overall by bacteriophages. The possible hosts that are predicted for the identified phages show a high similarity to bacteria that are actually colonizing the host. Hence, it is suggested that the phageome of *Hydra* mirrors *Hydras* species specific bacterial community and that those phages can influence *Hydras* microbial community (88) (Fig. 7).

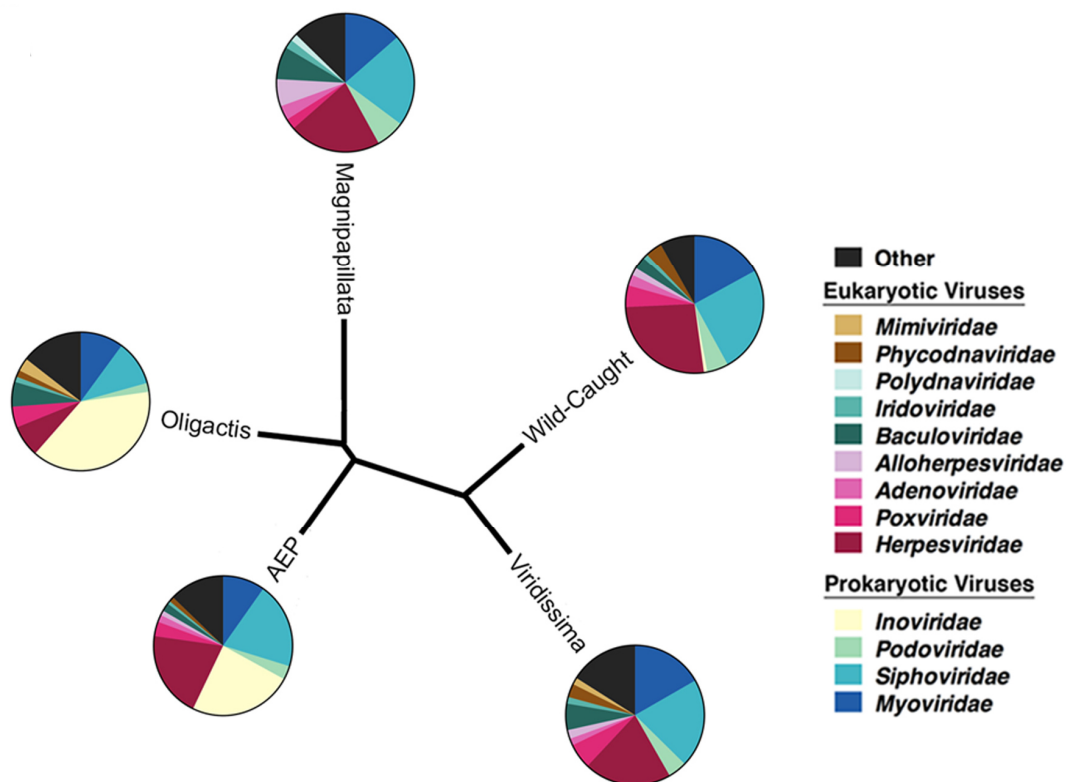


Figure 7: Species-specific virome of *Hydra oligactis*, *Hydra magnipapillata*, *Hydra viridissima*, *Hydra vulgaris* AEP and wild-caught *Hydra*, including eukaryotic and prokaryotic viruses. Adapted from Grasis *et al.* 2014 (88).

While the study of Grasis *et al.* (88) provides insights into the *Hydra* virome, the function of the highly abundant phages remains unclear. Considering that phages are crucial drivers within microbial communities (92), it is likely that phages play an important role in shaping and maintaining *Hydras* bacterial community from inside out.

Aims of this study

Bacteriophages are well-known to be important drivers of diversification and evolution within bacterial populations (92). Some studies already described the relative abundances of viruses and bacteriophages in metaorganisms (89, 195, 214–216), including the freshwater polyp *Hydra* (88). However, evaluating the recent literature, an important question still remains: What is the functional role of temperate bacteriophages within metaorganisms?

Considering that phages can infect bacterial cells, I hypothesize that temperate phages function as essential, non-host derived factors that shape and maintain the bacterial community of the metaorganism *Hydra*. To investigate this overall hypothesis, three important questions are addressed in the following three chapters:

- I. Are prophages present in *Hydra*-associated bacteria and what are their characteristics?

In chapter I and the first part of chapter II, I analyzed the genomes of *Hydra*-associated bacteria and could identify six active prophages. These inducible phages were characterized in host range and environmental sensitivity, to identify potential benefits or disadvantages those prophages can offer their *Hydra*-associated bacterial hosts and *Hydra*.

- II. Are temperate phages active on the *Hydra* host? Can they regulate other competing bacteria and shape the microbial community *in vitro*?

In chapter II, I hypothesize that temperate *Curvibacter* phage TJ1 is able to regulate the frequency of other bacterial colonizers *in vitro* and *in vivo* and influence the overall bacterial community composition on *Hydra*. To address this hypothesis, I analyzed the phage TJ1 genome, the inducibility and host range of this dominant phage *in vitro*. Moreover, I examined the cross-infectivity and ability to influence the recolonization pattern of phage TJ1 *in vivo*. The fact that susceptible bacteria seem to not evolve phage resistance on *Hydra* polyps, lead to the third question:

- III. Are bacteria able to evolve resistance against phage TJ1? If so, how is phage resistance interfering with bacteria-*Hydra* interactions?

To address these questions in chapter III, I analyzed the dynamics between three susceptible bacterial colonizers (*Duganella*, *Pelomonas*, *Undibacterium*) and phage TJ1 *in vitro*. Afterwards I investigated how mutation-based resistance influences the colonization ability of the respective resistant bacteria. This provides understandings, how phage-bacteria dynamics interfere with *Hydra*-bacteria interactions.

All together this study is designed to give insights into the functional role of temperate bacteriophages, not only within isolated microbial communities, but in the context of the whole metaorganism.

References

1. Esser D, et al. (2018) Functions of the Microbiota for the Physiology of Animal Metaorganisms. *J Innate Immun.* 11:393-404
2. Mcfall-ngai M, et al. (2013) Animals in a bacterial world , a new imperative for the life sciences. 110(9):3229–3236.
3. Grice EA, Segre JA (2011) The skin microbiome. *Nat Rev Microbiol* 9:244–253.
4. Moreno I, Simon C (2018) Deciphering the effect of reproductive tract microbiota on human reproduction. *Reprod Med Biol* 18(1):40–50.
5. Bosch TCG, McFall-Ngai MJ (2011) Metaorganisms as the new frontier. *Zoology* 114(4):185–190.
6. Li H, et al. (2015) The outer mucus layer hosts a distinct intestinal microbial niche. *Nat Commun* 6:1–13.
7. Kamphuis JBJ, Mercier-Bonin M, Eutamène H, Theodorou V (2017) Mucus organisation is shaped by colonic content; a new view. *Sci Rep* 7(1):8527.
8. Fraune S, Bosch TCG (2010) Why bacteria matter in animal development and evolution. *BioEssays* 32(7):571–580.
9. Lederberg BJ, Mccray AT (2001) ' Ome Sweet ' Omics-- A Genealogical Treasury of Words. *Sci* 15(7).
10. Zilber-rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants : the hologenome theory of evolution. *FEMS Microbiol Rev* 32:723–735.
11. Gilbert SF, et al. (2016) Getting the Hologenome Concept Right : an Eco-Evolutionary Framework for Hosts and Their Microbiomes. *mSystems* 1(2):1–6.
12. Silverman JD, Washburne AD, Mukherjee S, David LA (2017) A phylogenetic transform enhances analysis of compositional microbiota data. *Elife* 6:1–20.
13. Koskinen K, et al. (2017) First Insights into the Diverse Human Archaeome: Specific Detection of Archaea in the Gastrointestinal Tract, Lung, and Nose and on Skin. *MBio* 8(6):1–17.
14. Mcfall-ngai MJ (2014) The Importance of Microbes in Animal Development : Lessons from the Squid-Vibrio Symbiosis. *Annu Rev Microbiol* 68:177–194.
15. Funkhouser LJ, Bordenstein SR (2013) Mom Knows Best : The Universality of Maternal Microbial Transmission. *PLoS Biol* 11(8):1–9.
16. Sender R, Fuchs S, Milo R (2016) Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol* 14(8):e1002533.
17. Yatsunencko T, et al. (2012) Human gut microbiome viewed across age and geography. *Nature* 486(7402):222–227.
18. Song SJ, et al. (2013) Cohabiting family members share microbiota with one another and with their dogs. *Elife* 2013(2):1–22.
19. Wlodarska M, et al. (2017) Indoleacrylic Acid Produced by Commensal Peptostreptococcus Species Suppresses Inflammation. *Cell Host Microbe* 22(1):25–37.e6.
20. Sommer F, Backhed F (2013) The gut microbiota--masters of host development and physiology. *Nat Rev Microbiol* 11(4):227–238.
21. Ainsworth TD, et al. (2015) The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *ISME J* 9(10):2261–2274.

| General introduction |

22. Thomspon AW, et al. (2012) Unicellular Cyanobacterium Symbiotic with a Single-Celled Eukaryotic Alga. *Science* (80-) 337(September):1546–1550.
23. Santi C, Bogusz D, Franche C (2013) Biological nitrogen fixation in non-legume plants. *Ann Bot* 111(5):743–767.
24. Yeoh YK, et al. (2017) Evolutionary conservation of a core root microbiome across plant phyla along a tropical soil chronosequence. *Nat Commun* 8(1):1–9.
25. Hassani MA, Özkurt E, Seybold H, Dagan T, Stukenbrock EH (2019) Interactions and Coadaptation in Plant Metaorganisms. *Annu Rev Phytopathol* 57(1):483–503.
26. Schlaeppli K, Dombrowski N, Oter RG, Ver Loren Van Themaat E, Schulze-Lefert P (2014) Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives. *Proc Natl Acad Sci U S A* 111(2):585–592.
27. Lenaerts I, Walker GA, Hoorebeke L Van, Gems D, Vanfleteren JR (2008) Dietary Restriction of *Caenorhabditis elegans* by Axenic Culture Reflects Nutritional Requirement for Constituents Provided by Metabolically Active Microbes. *J Gerontol Biol Sci* 63(3):242–252.
28. Baumann P (2005) Biology of Endosymbionts of Plant Sap-Sucking Insects. *Annu Rev Microbiol* 59:155–189.
29. Turnbaugh PJ, et al. (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444(December):1027–1031.
30. Turnbaugh PJ, et al. (2009) A core gut microbiom in obese and lean twins. *Nature* 457(32089):480–484.
31. Ding H, et al. (2004) The gut microbiota as an environmental factor that regulates fat storage. *PNAS* 101(44):15718–15723.
32. Rakoff-nahoum S, Paglino J, Eslami-varzaneh F, Edberg S, Medzhitov R (2004) Recognition of Commensal Microflora by Toll-Like Receptors Is Required for Intestinal Homeostasis. *Cell* 118:229–241.
33. Baillie GS, et al. (2001) Molecular Analysis of Commensal Host-Microbial Relationships in the Intestine. *Science* (80-) 291(February):881–885.
34. Brestoff JR, Artis D (2013) Commensal bacteria at the interface of host metabolism and the immune system. *Nat Immunol* 14(7):676–684.
35. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL (2005) of Symbiotic Bacteria Directs Maturation of the Host Immune System. *Cell* 122:107–118.
36. Kamada N, Chen GY, Inohara N, Núñez G (2013) Control of pathogens and pathobionts by the gut microbiota. *Nat Immunol* 14(7):685–690.
37. Lyte M, Li W, Opitz N, Gaykema RPA, Goehler LE (2006) Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia *Citrobacter rodentium*. *Physiol Behav* 89:350–357.
38. Diaz R, et al. (2011) Normal gut microbiota modulates brain development and behavior. *PNAS* 108(7):1–6.
39. Neufeld KM, Kang N, Bienenstock J (2011) Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil* 23:255–265.
40. Montgomery MK, Mcfall-ngai M (1994) Bacterial symbionts induce host organ morphogenesis during early postembryonic development of the squid *Euprymna scolopes*. *Development* 120:1719–1729.
41. Dally JM, et al. (2011) *Drosophila* Microbiome Modulates Host Developmental and Metabolic Homeostasis via Insulin Signaling. *Science* (80-) 334(November):670–675.
42. Street C, Sciences B (2014) Mosquitoes rely on their gut microbiota for development.

| General introduction |

Mol Ecol 23:2727–2739.

43. Foster JS, Apicella MA (2000) *Vibrio fischeri* Lipopolysaccharide Induces Developmental Apoptosis , but Not Complete Morphogenesis , of the Euprymna scolopes Symbiotic Light Organ. *Dev Biol* 226:242–254.
44. Lamarq LH, Fall-ngai MJMC (1998) Induction of a Gradual , Reversible Morphogenesis of Its Host ' s Epithelial Brush Border by *Vibrio fischeri*. *Infect Immun* 66(2):777–785.
45. Oh J, Byrd AL, Park M, Kong HH, Segre JA (2016) Temporal Stability of the Human Skin Microbiome. *Cell* 165(4):854–866.
46. Lai Y, et al. (2009) Commensal bacteria regulate toll-like receptor 3-dependent inflammation after skin injury. *Nat Med* 15(12):1377–1382.
47. Belkaid Y, Tamoutounour S (2016) The influence of skin microorganisms on cutaneous immunity. *Nat Rev Immunol* 16(6):353–366.
48. Harris RN, James TY, Lauer A, Simon MA, Patel A (2006) Short Communication Amphibian Pathogen *Batrachochytrium dendrobatidis* Is Inhibited by the Cutaneous Bacteria of Amphibian Species. *Ecohealth* 53:53–56.
49. Fraune S, et al. (2015) Bacteria-bacteria interactions within the microbiota of the ancestral metazoan Hydra contribute to fungal resistance. *ISME J* 9:1543–1556.
50. Harris RN, Lauer A, Simon MA, Banning JL, Alford RA (2009) Addition of antifungal skin bacteria to salamanders ameliorates the effects of chytridiomycosis. *Dis Aquat Organ* 83(1):11–16.
51. Kissoyan KAB, et al. (2019) Natural C . elegans Microbiota Protects against Infection via Production of a Cyclic Lipopeptide of the Viscosin Group Report Natural C . elegans Microbiota Protects against Infection via Production of a Cyclic Lipopeptide of the Viscosin Group. *Curr Biol* 29(6):1030-1037.e5.
52. Brown DG, et al. (2019) The microbiota protects from viral- induced neurologic damage through microglia-intrinsic TLR signaling. *Elife*:1–22.
53. Degruittola AK, Low D, Mizoguchi A, Mizoguchi E (2016) Current understanding of dysbiosis in disease in human and animal models. *Inflamm Bowel Dis* 22(5):1137–1150.
54. Lange K, Stallmach A (2016) Effects of Antibiotics on Gut Microbiota. *Dig Dis*:260–268.
55. Fröhlich EE, et al. (2016) Brain , Behavior , and Immunity Cognitive impairment by antibiotic-induced gut dysbiosis : Analysis of gut microbiota-brain communication. *Brain Behav Immun* 56:140–155.
56. Petersen C, Round JL (2014) Microreview Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol* 16(June):1024–1033.
57. Noble EE, Hsu TM, Kanoski SE (2017) Gut to Brain Dysbiosis : Mechanisms Linking Western Diet Consumption , the Microbiome , and Cognitive Impairment. *Front Behav Neurosci* 11(January):1–10.
58. Brown K, Decoffe D, Molcan E, Gibson DL (2012) Diet-Induced Dysbiosis of the Intestinal Microbiota and the Effects on Immunity and Disease. *Nutrients* 4:1095–1119.
59. Ikeda F, Yoshihara T, Komiya K, Kawaguchi M (2014) Gut Dysbiosis and Detection of “ Live Gut Bacteria ” in Blood of Japanese Patients With Type 2 Diabetes. *Diabetes Care* 37(August):2343–2350.
60. Karlsson CLJ, et al. (2012) The Microbiota of the Gut in Preschool Children With Normal and Excessive Body Weight. *Obesity* 20(11):2257–2261.
61. Zhou Y, Zhi F (2016) Lower Level of Bacteroides in the Gut Microbiota Is Associated with Inflammatory Bowel Disease: A Meta-Analysis. *Biomed Res Int* 2016:5828959.
62. Wang W, et al. (2014) Increased proportions of Bifidobacterium and the Lactobacillus

| General introduction |

- group and loss of butyrate-producing bacteria in inflammatory bowel disease. *J Clin Microbiol* 52(2):398–406.
63. Kurina LM, Goldacre MJ, Yeates D, Gill LE (2001) Depression and anxiety in people with inflammatory bowel disease. *J Epidemiol Community Health* 55:716–720.
 64. Bravo JA, Forsythe P, Chew M V, Escaravage E, Savignac HM (2011) Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *PNAS* 108(38):16050–16055.
 65. Foster JA, Neufeld KM (2013) Gut – brain axis : how the microbiome influences anxiety and depression. *Trends Neurosci* 36(5):305–312.
 66. Franzenburg S, et al. (2013) Bacterial colonization of Hydra hatchlings follows a robust temporal pattern. *ISME J* 7(4):781–90.
 67. Palmer C, Bik EM, Digiulio DB, Relman DA, Brown PO (2007) Development of the Human Infant Intestinal Microbiota. *PLoS Biol* 5(7):1556–1573.
 68. Odamaki T, et al. (2016) Age-related changes in gut microbiota composition from newborn to centenarian: A cross-sectional study. *BMC Microbiol* 16(1):1–12.
 69. Langille MG, et al. (2014) Microbial shifts in the aging mouse gut. *Microbiome* 2(1):1–12.
 70. Fransen F, et al. (2017) Aged Gut Microbiota Contributes to Systemical Inflammation after Transfer to Germ-Free Mice. *Front Immunol* 8:1385.
 71. Franzenburg S, et al. (2013) Distinct antimicrobial peptide expression determines host species-specific bacterial associations. *Proc Natl Acad Sci U S A* 110:E3730-8.
 72. Fischbach MA, Segre JA (2016) Signaling in Host-Associated Microbial Communities. *Cell* 164(6):1288–1300.
 73. Jung S, et al. (2009) Hydracin-1 , Structure and Antibacterial Activity of a Protein from the Basal Metazoan Hydra *. *J Biol Chem* 284(3):1896–1905.
 74. Singh PK, Parsek MR, Greenberg EP, Welsh MJ (2002) A component of innate immunity prevents bacterial biofilm development. *Nature* 417(May):4–7.
 75. Cullen TW, et al. (2015) Antimicrobial peptide resistance mediates resilience of prominent gut commensals during inflammation. *Science (80-)* 347(6218):170–175.
 76. Vaishnava S, et al. (2011) The Antibacterial Lectin RegIIIg Promotes the Spatial Segregation of Microbiota and Host in the Intestine. *Science (80-)* 334:255–259.
 77. Lebeis SL, Paredes SH, Lundberg DS, Glavina T, Jones CD (2015) Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science (80-)* 349(6250):1678–1681.
 78. Carmody RN, et al. (2015) Diet Dominates Host Genotype in Shaping the Murine Gut Microbiota. *Cell Host Microbe* 17(1):72–84.
 79. David LA, et al. (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505(7484):559–563.
 80. Staubach F, Baines JF, Künzel S, Bik EM, Petrov DA (2013) Host Species and Environmental Effects on Bacterial Communities Associated with Drosophila in the Laboratory and in the Natural Environment. *PLoS One* 8(8):e70749.
 81. Deines P, Bosch TCG (2016) Transitioning from microbiome composition to microbial community interactions: The potential of the metaorganism hydra as an experimental model. *Front Microbiol* 7(OCT):1–7.
 82. Deines P, Lachnit T, Bosch TCG (2017) Competing forces maintain the Hydra metaorganism. *Immunol Rev* 279(1):123–136.
 83. Suttle C a (2007) Marine viruses--major players in the global ecosystem. *Nat Rev*

- Microbiol* 5(10):801–812.
84. Mirzaei MK, Maurice CF (2017) Ménage à trois in the human gut : interactions between host , bacteria and phages. *Nat Rev Microbiol* 15(7):397–408.
 85. Clokie MRJ, Millard AD, Letarov A V., Heaphy S (2011) Phages in nature. *Bacteriophage* 1(1):31–45.
 86. Jahn MT, et al. (2019) A Phage Protein Aids Bacterial Symbionts in Eukaryote Immune Evasion. *Cell Host Microbe* 26(4):542–550.
 87. Brüwer JD, Voolstra CR (2018) First insight into the viral community of the cnidarian model metaorganism *Aiptasia* using RNA-Seq data. *PeerJ* 6:e4449.
 88. Grasis JA, et al. (2014) Species-specific viromes in the ancestral holobiont hydra. *PLoS One* 9(10):e109952.
 89. Minot S, et al. (2011) The human gut virome: Inter-individual variation and dynamic response to diet. *Genome Res* 21:1616–1625.
 90. Lenski RE (1988) Dynamics of Interactions between Bacteria and Virulent Bacteriophage. *Advances in Microbial Ecology*, ed Marshall KC (Springer US, Boston, MA), pp 1–44.
 91. De Paepe M, Leclerc M, Tinsley CR, Petit M-A (2014) Bacteriophages: an underestimated role in human and animal health? *Front Cell Infect Microbiol* 4(March):1–11.
 92. Koskella B, Brockhurst MA (2014) Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS Microbiol Rev* 38(5):916–931.
 93. Ranquet C, Toussaint A, De Jong H, Maenhaut-Michel G, Geiselmann J (2005) Control of bacteriophage μ lysogenic repression. *J Mol Biol* 353(1):186–195.
 94. Nanda AM, Thormann K, Frunzke J (2015) Impact of spontaneous prophage induction on the fitness of bacterial populations and host-microbe interactions. *J Bacteriol* 197(3):410–419.
 95. Livny J, Friedman DI (2004) Characterizing spontaneous induction of Stx encoding phages using a selectable reporter system. *Mol Microbiol* 51(6):1691–1704.
 96. Refardt D, Rainey PB (2010) Tuning a genetic switch: Experimental evolution and natural variation of prophage induction. *Evolution (N Y)* 64(4):1086–1097.
 97. Nanda AM, et al. (2014) Analysis of SOS-Induced Spontaneous Prophage Induction in *Corynebacterium glutamicum* at the Single-Cell Level. 196(1):180–188.
 98. Miller C, et al. (2004) SOS Response Induction by Beta -Lactams and Bacterial Defense Against Antibiotic Lethality. *Science (80-)* 305(September):1629–1632.
 99. Foster PL (2005) Stress responses and genetic variation in bacteria. *Mutat Res* 569:3–11.
 100. Janion C (2008) Inducible SOS response system of DNA repair and mutagenesis in *Escherichia coli*. *Int J Biol Sci* 4(6):338–44.
 101. Alves de Matos AP, Lehours P, Timóteo A, Roxo-Rosa M, Vale FF (2013) Comparison of induction of B45 *Helicobacter pylori* prophage by acid and UV radiation. *Microsc Microanal* 19(S4):27–28.
 102. Choi J, Kotay SM, Goel R (2010) Various physico-chemical stress factors cause prophage induction in *Nitrosospira multiformis* 25196- an ammonia oxidizing bacteria. *Water Res* 44(15):4550–4558.
 103. Sekiguchi M, Takagi Y (1960) Effect of mitomycin C on the synthesis of bacterial and viral deoxyribonucleic acid. *BBA - Biochim Biophys Acta* 41(3):434–443.

| General introduction |

104. Fortier LC, Moineau S (2007) Morphological and genetic diversity of temperate phages in *Clostridium difficile*. *Appl Environ Microbiol* 73(22):7358–7366.
105. Erez Z, et al. (2017) Communication between viruses guides lysis – lysogeny decisions. *Nat Publ Gr* 541(7638):488–493.
106. de Jonge PA, Nobrega FL, Brouns SJJJ, Dutilh BE (2019) Molecular and Evolutionary Determinants of Bacteriophage Host Range. *Trends Microbiol* 27(1):51–63.
107. Ross A, Ward S, Hyman P (2016) More Is Better: Selecting for Broad Host Range Bacteriophages. *Front Microbiol* 7(September):1–6.
108. Adams M (1959) Assay of phage by agar layer method. *Bacteriophages Intersci Publ Inc, New York, NY*:450–454.
109. Hyman P, et al. (2009) Bacteriophages. 502(June 2014):81–85.
110. Koskella B, Meaden S (2013) Understanding bacteriophage specificity in natural microbial communities. *Viruses* 5(3):806–823.
111. Wang K, Chen F (2008) Prevalence of highly host-specific cyanophages in the estuarine environment. *Environ Microbiol* 10(2):300–312.
112. Kudva IT, Jelacic S, Tarr PI, Youderian P, Hovde CJ (1999) Biocontrol of *Escherichia coli* O157 with O157-Specific Bacteriophages. *Appl Environ Microbiol* 65(9):3767–3773.
113. Gross RJ, Cheasty T, Rowe B (1977) Isolation of Bacteriophages Specific for the Ki Polysaccharide Antigen of *Escherichia coli*. *J Clin Microbiol* 6(6):548–550.
114. Hsieh S, Lo H, Chen S, Lee M, Tseng Y (2011) Wide Host Range and Strong Lytic Activity of *Staphylococcus aureus*. *Appl Environ Microbiol* 77(3):756–761.
115. O'Flaherty S, et al. (2005) Potential of the Polyvalent Anti- *Staphylococcus* Bacteriophage K for Control of Antibiotic-Resistant *Staphylococci* from Hospitals. *Appl Environ Microbiol* 71(4):1836–1842.
116. Malki K, et al. (2015) Bacteriophages isolated from Lake Michigan demonstrate broad host-range across several bacterial phyla. *Virology* 1–5.
117. Canchaya C, Fournous G, Chibani-Chennoufi S, Dillmann ML, Brüssow H (2003) Phage as agents of lateral gene transfer. *Curr Opin Microbiol* 6(4):417–24.
118. Lopez CA, et al. (2012) Phage-mediated acquisition of a type III secreted effector protein boosts growth of salmonella by nitrate respiration. *MBio* 3(3):1–10.
119. Waldor MK, Mekalanos JJ (1996) Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* 272(5270):1910–4.
120. Davies E V., Winstanley C, Fothergill JL, James CE (2016) The role of temperate bacteriophages in bacterial infection. *FEMS Microbiol Lett* 363(5):1–10.
121. Cheetham BF, Katz ME (1995) A role for bacteriophages in the evolution and transfer of bacterial virulence determinants. *Mol Microbiol* 18(2):201–208.
122. Obeng N, Pratama AA, Elsas JD Van (2016) The Significance of Mutualistic Phages for Bacterial Ecology and Evolution. *Trends Microbiol* 24(6):440–449.
123. Harrison E, Brockhurst MA (2017) Ecological and Evolutionary Benefits of Temperate Phage : What Does or Doesn ' t Kill You Makes You Stronger. *BioEssays* 39.
124. Haaber J, et al. (2016) Bacterial viruses enable their host to acquire antibiotic resistance genes from neighbouring cells. *Nat Commun* 7:1–8.
125. Wang X, et al. (2010) Cryptic prophages help bacteria cope with adverse environments. *Nat Commun* 1(9):147–149.
126. Susskind MM, Wright A, Botstein D (1971) Superinfection Exclusion *Salmonella* by Prophage in lysogens of typhimurium for Two Bacterial Strains and Phages. *Virology* 45:638–652.

| General introduction |

127. Hofer B, Ruge M, Dreiseikelmann B (1995) The Superinfection Exclusion Gene (sieA) of Bacteriophage P22 : Identification and Overexpression of the Gene and Localization of the Gene Product. *J Bacteriol* 177(11):3080–3086.
128. Lu M, Henning U (1994) Superinfection exclusion by T-even-type coliphages. *Trends Microbiol* 2(4):137–139.
129. Mahony J, Mcgrath S, Fitzgerald GF, Sinderen D Van (2008) Identification and Characterization of Lactococcal-Prophage-Carried Superinfection Exclusion Genes □ †. *Appl Environ Microbiol* 74(20):6206–6215.
130. Li X-Y, et al. (2017) Temperate phages as self-replicating weapons in bacterial competition. *J R Soc Interface* 14(137):20170563.
131. Gama JA, et al. (2013) Temperate Bacterial Viruses as Double-Edged Swords in Bacterial Warfare. *PLoS One* 8(3):e59043.
132. Davies E V, et al. (2016) Temperate phages enhance pathogen fitness in chronic lung infection. *ISME J* 10(10):2553–2555.
133. De Paepe M, et al. (2016) Carriage of λ Latent Virus Is Costly for Its Bacterial Host due to Frequent Reactivation in Monoxenic Mouse Intestine. *PLoS Genet* 12(2):1–20.
134. Cortes M, Krog J (2019) Optimality of the spontaneous prophage induction rate. *bioRxiv*:1–24.
135. Avrani S, Schwartz DA, Lindell D (2012) Virus-host swinging party in the oceans: Incorporating biological complexity into paradigms of antagonistic coexistence. *Mob Genet Elements* 2(2):88–95.
136. Thingstad TF (2000) Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *45(6):1320–1328.*
137. Weinbauer MG (2004) Ecology of prokaryotic viruses. *FEMS Microbiol Rev* 28(2):127–181.
138. Louca S, Doebeli M (2017) Taxonomic variability and functional stability in microbial communities infected by phages. *Environ Microbiol* 19(10):3863–3878.
139. Scanlan PD, et al. (2015) Coevolution with bacteriophages drives genome-wide host evolution and constrains the acquisition of abiotic-beneficial mutations. *Mol Biol Evol* 32(6):1425–1435.
140. Koskella B (2018) Resistance gained, resistance lost: An explanation for host-parasite coexistence. *PLoS Biol* 16(9):e3000013.
141. Buckling A, Rainey PB (2002) Antagonistic coevolution between a bacterium and a bacteriophage. *Proc R Soc L* 269:931–936.
142. Garneau JE, et al. (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* 468:67.
143. Meeske AJ, Nakandakari-Higa S, Marraffini LA (2019) Cas13-induced cellular dormancy prevents the rise of CRISPR-resistant bacteriophage. *Nature*.
144. Rostøl JT, Marraffini L (2019) (Ph)ighting Phages: How Bacteria Resist Their Parasites. *Cell Host Microbe* 25(2):184–194.
145. Samson JE, Magadán AH, Sabri M, Moineau S (2013) Revenge of the phages: defeating bacterial defences. *Nat Rev Microbiol* 11(10):675.
146. Gómez P, Buckling A (2011) Bacteria-phage antagonistic coevolution in soil. *Science (80-)* 332(6025):106–109.
147. Bull JJ (1994) VIRULENCE. *Evolution (N Y)* 48(5):1423–1437.
148. Hamilton WD (1980) Sex versus Non-Sex versus Parasite. *Oikos* 35(2):282–290.

149. Pal C, Maciá MD, Oliver A, Schachar I, Buckling A (2007) Coevolution with viruses drives the evolution of bacterial mutation rates. *Nature* 450:1079.
150. Letters E (2002) Local adaptation and the geometry of host – parasite coevolution. *Ecol Lett* 5:246–256.
151. Paterson S, et al. (2010) Antagonistic coevolution accelerates molecular evolution. *Nature* 464:275–278.
152. Marston MF, et al. (2012) Rapid diversification of coevolving marine *Synechococcus* and a virus. *PNAS* 109(12):4544–4549.
153. Woolhouse MEJ, Webster JP, Domingo E, Charlesworth B, Levin BR (2002) Biological and biomedical implications of the co-evolution of pathogens and. *Nat Genet* 32:569–577.
154. Brockhurst MA, Koskella B (2013) Experimental coevolution of species interactions. *Trends Ecol Evol* 28(6):367–375.
155. Gaba S, Ebert D (2009) Time-shift experiments as a tool to study antagonistic coevolution. *Trends Ecol Evol* 24(4):226–232.
156. Gandon S, Buckling A, Decaestecker E, Day T (2008) Host – parasite coevolution and patterns of adaptation across time and space. *J EVOL BIOL* 21:1861–1866.
157. Lennon JT, Khatana SAM, Marston MF, Martiny JBH (2007) Is there a cost of virus resistance in marine cyanobacteria? *ISME J* 1:300–312.
158. Buckling A, Wei Y, Massey RC, Brockhurst MA, Hochberg ME (2006) Antagonistic coevolution with parasites increases the cost of host deleterious mutations. *Proc R Soc B Biol Sci* 273(1582):45–49.
159. Labrie SJ, Samson JE, Moineau S (2010) Bacteriophage resistance mechanisms. *Nat Rev Microbiol* 8(5):317–327.
160. Pingoud A, Fuxreiter M, Pingoud V, Wende W (2005) Type II restriction endonucleases : structure and mechanism. *Cell Mol Life Sci* 62:685–707.
161. Sorek R, Kunin V, Hugenholtz P (2008) CRISPR — a widespread system that provides acquired resistance against phages in bacteria and archaea. *Nat Rev Microbiol* 6:181–186.
162. Molineux IJ (1991) Host-parasite interactions: recent developments in the genetics of abortive phage infections. *New Biol* 3(3):230–236.
163. Smith HS, Pizer LI, Pylkas L, Lederberg S (1969) Abortive Infection of *Shigella dysenteriae* P2 by T2 Bacteriophage. *J Virol* 4(2):162–168.
164. Durmaz E, Klaenhammer TR (2007) Abortive Phage Resistance Mechanism *AbiZ* Speeds the Lysis Clock To Cause Premature Lysis of Phage-Infected *Lactococcus lactis* □. *J Bacteriol* 189(4):1417–1425.
165. Bertozzi Silva J, Storms Z, Sauvageau D (2016) Host receptors for bacteriophage adsorption. *FEMS Microbiol Lett* 363(4):1–11.
166. Cvirkaite V, Daugelavicius R, Bamford JKH, Bamford DH (2006) The Entry Mechanism of Membrane-Containing Phage Bam35 Infecting *Bacillus thuringiensis*. *J Bacteriol* 188(16):5925–5934.
167. Groups O, Walls C (1971) O-Acetyl Groups as a Component of the Bacteriophage Receptor on *Staphylococcus aureus*. *J Bacteriol* 108(1):584–585.
168. Wendlinger G, Loessner MJ, Scherer S (1996) Bacteriophage receptors on *Listeria monocytogenes* cells are the N-acetylglucosamine and rhamnose substituents of teichoic acids or the peptidoglycan itself. *Microbiology* 142:985–992.
169. Ainsworth S, et al. (2014) Differences in Lactococcal Cell Wall Polysaccharide Structure

- Are Major Determining Factors in Bacteriophage Sensitivity. *MBio* 5(3):1–11.
170. Douglas LJ, Wolin MJ (1971) Wall of. *Cell Wall Polym Phage Lysis Lact Plant* 10(9):1551–1555.
 171. Beveridge TJ (1999) Structures of Gram-Negative Cell Walls and Their Derived Membrane Vesicles. *J Bacteriol* 181(16):4725–4733.
 172. Rakhuba D V, Kolomiets EI, Dey ES, Novik GI (2010) Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. *Pol J Microbiol* 59(3):145–155.
 173. Hantke K (1978) Major Outer Membrane Proteins of E. coli K12 Serve as Receptors for the Phages T2 (Protein I a) and 434 (Protein I b). *Molec gen Genet* 164:131–135.
 174. Verhoef C, Graaff PJ De, L EJJ (1977) Short Communication Mapping of a Gene for a Major Outer Membrane Protein of Escherichia coli K12 with the Aid of a Newly Isolated Bacteriophage. *Molec gen~ Genet* 150:103–105.
 175. Morona R, Henning ULF (1984) Host Range Mutants of Bacteriophage Ox2 Can Use Two Different Outer Membrane Proteins of Escherichia coli K-12 as Receptors. *J Bacteriol* 2(20):579–582.
 176. Hashemolhosseini S, Holmes Z, Mutschler B, Henning U (1994) Alterations of Receptor Specificities of Coliphages of T2 Family. *J Mol Biol* 240:105–110.
 177. Lindberg AA (1973) BACTERIOPHAGE RECEPTORS. *Annu Rev Microbiol* 27:205–241.
 178. Mutoh N, Furukawa H, Mizushima S (1978) Role of Lipopolysaccharide and Outer Membrane Protein of Escherichia coli K-12 in the Receptor Activity for. *J Bacteriol* 1(2):693–699.
 179. Garbe J, et al. (2010) Characterization of JG024 , a pseudomonas aeruginosa PB1-like broad host range phage under simulated infection conditions. *BMC Microbiol* 10(301):1–10.
 180. Shin H, et al. (2012) Receptor Diversity and Host Interaction of Bacteriophages Infecting Salmonella enterica Serovar Typhimurium. *PLoS One* 7(8):e43392.
 181. Datta DB, Arden B, Henning ULF (1977) Major Proteins of the Escherichia coli Outer Cell Envelope Membrane as Bacteriophage Receptors. *J Bacteriol* 131(3):821–829.
 182. Chaturongakul S, Ounjai P (2014) Phage-host interplay: Examples from tailed phages and Gram-negative bacterial pathogens. *Front Microbiol* 5(AUG):1–8.
 183. Choi Y, Shin H, Lee J-HH, Ryua S, Ryu S (2013) Identification and Characterization of a Novel Flagellum-Dependent Salmonella-Infecting Bacteriophage, iEPS5. *Appl Environ Microbiol* 79(16):4829–4837.
 184. Zhang H, Li L, Zhao Z, Peng D, Zhou X (2016) Polar flagella rotation in Vibrio parahaemolyticus confers resistance to bacteriophage infection. *Sci Rep* 6(May):1–12.
 185. Jensen EC, et al. (1998) Prevalence of Broad-Host-Range Lytic Bacteriophages of. *Microbiology* 64(2):575–580.
 186. Sabouri S, Sepehrizadeh Z, Amirpour-rostami S, Skurnik M (2017) International Journal of Food Microbiology A minireview on the in vitro and in vivo experiments with anti-Escherichia coli O157 : H7 phages as potential biocontrol and phage therapy agents. *Int J Food Microbiol* 243:52–57.
 187. Goodridge L, Gallaccio A, Griffiths MW (2003) Morphological , Host Range , and Genetic Characterization of Two Coliphages. 69(9):5364–5371.
 188. Yu S, Ko K, Chen C, Chang Y (2000) Characterization of the Distal Tail Fiber Locus and Determination of the Receptor for Phage AR1 , Which Specifically Infects Escherichia coli O157 : H7. 182(21):5962–5968.

| General introduction |

189. Meaden S, Paszkiewicz K, Koskella B (2015) The cost of phage resistance in a plant pathogenic bacterium is context-dependent. *Evolution (N Y)* 69(5):1321–1328.
190. Augustin R, Fraune S, Bosch TCG (2010) How Hydra senses and destroys microbes. *Semin Immunol* 22(1):54–58.
191. Bosch TCG, et al. (2009) Uncovering the evolutionary history of innate immunity: The simple metazoan Hydra uses epithelial cells for host defence. *Dev Comp Immunol* 33(4):559–569.
192. Zhang L, Gallo RL (2016) Antimicrobial peptides. *Curr Biol* 26(1):R14–R19.
193. Siebert S, Bosch TCG (2009) Identification of a kazal-type serine protease inhibitor with potent anti-staphylococcal activity as part of Hydra ' s innate immune system. *Dev Comp Immunol* 33:830–837.
194. Brown SP, Le Chat L, De Paepe M, Taddei F (2006) Ecology of Microbial Invasions: Amplification Allows Virus Carriers to Invade More Rapidly When Rare. *Curr Biol* 16(20):2048–2052.
195. Lachnit T, Thomas T, Steinberg P (2016) Expanding our Understanding of the Seaweed Holobiont : RNA Viruses of the Red Alga *Delisea pulchra*. *Front Microbiol* 6(January):1–12.
196. Gierer A (2012) The Hydra model - a model for what ? *Int J Dev Biol* 56:437–445.
197. COLLINS AG, CARTWRIGHT P, MCFADDEN CS, SCHIERWATER B (2005) Phylogenetic Context and Basal Metazoan Model Systems. *INTEGRCOMPBIOL* 45:585–594.
198. Campbell RD (1983) Identifying Hydra Species. *Hydra: Research Methods*, ed Lenhoff HM (Springer US, Boston, MA), pp 19–28.
199. Hemmrich G, Anokhin B, Zacharias H, Bosch TCG (2007) Molecular phylogenetics in Hydra, a classical model in evolutionary developmental biology. *Mol Phylogenet Evol* 44(1):281–290.
200. Bosch TCG (2013) Cnidarian-Microbe Interactions and the Origin of Innate Immunity in Metazoans. *Annu Rev Microbiol* 67:499–518.
201. Schwentner M, Bosch TCG (2018) Revisiting the age , evolutionary history and species level diversity of the genus Hydra (Cnidaria : Hydrozoa) Molecular Phylogenetics and Evolution Revisiting the age , evolutionary history and species level diversity of the genus Hydra (Cnidaria : Hy. *Mol Phylogenet Evol* 91(May 2015):41–55.
202. Wittlieb J, Khalturin K, Lohmann JU, Anton-Erxleben F, Bosch TCG (2006) Transgenic Hydra allow in vivo tracking of individual stem cells during morphogenesis. *Proc Natl Acad Sci* 103(16):6208–6211.
203. Böttger A, et al. (2012) Horizontal Gene Transfer Contributed to the Evolution of Extracellular Surface Structures : The Freshwater Polyp Hydra Is Covered by a Complex Fibrous Cuticle Containing Glycosaminoglycans and Proteins of the PPOD and SWT (Sweet Tooth) Families. *PLoS One* 7(12):e52278.
204. Schröder K, Bosch TCG (2016) The Origin of Mucosal Immunity : Lessons from the Holobiont Hydra. *MBio* 7(6):1–9.
205. Fraune S, Bosch TCG (2007) Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. *Pnas* 104(32):13146–13151.
206. Brooks AW, Kohl KD, Brucker RM, van Opstal EJ, Bordenstein SR (2017) Phyllosymbiosis: Relationships and Functional Effects of Microbial Communities across Host Evolutionary History (PLOS Biology, (2016), 14, 11, (e2000225), 10.1371/journal.pbio.2000225). *PLoS Biol* 15(1):1–29.
207. Murillo-Rincon AP, et al. (2017) Spontaneous body contractions are modulated by the

| General introduction |

- microbiome of Hydra. *Sci Rep* 7:15937.
208. Ozaki H, Hori M, Kinoshita K, Ohama T (2005) Intestinal dysmotility in inflammatory bowel disease: Mechanisms of the reduced activity of smooth muscle contraction. *Inflammopharmacology* 13(1):103.
 209. Buffie CG, Pamer EG (2013) Microbiota-mediated colonization resistance against intestinal pathogens. *Nat Rev Immunol* 13:790.
 210. McNally L, Brown SP (2016) Ecology of stable gut communities. *Nat Microbiol* 1:15016.
 211. Foster KR, Bell T (2012) Competition , Not Cooperation , Dominates Interactions among Culturable Microbial Species. *Curr Biol* 22(19):1845–1850.
 212. Mitri S, Foster KR (2013) The Genotypic View of Social Interactions in Microbial Communities. *Annu Rev Genet* 47:247–273.
 213. Li X-Y, et al. (2015) Which games are growing bacterial populations playing? *J R Soc Interface* 12(108):20150121.
 214. Weynberg KD, Wood-Charlson EM, Suttle CA, van Oppen MJH, Oppen MJH van (2014) Generating viral metagenomes from the coral holobiont. *Front Microbiol* 5(MAY):1–11.
 215. Rascovan N, Duraisamy R, Desnues C (2016) Metagenomics and the Human Virome in Asymptomatic Individuals. *Annu Rev Microbiol* 70:125–141.
 216. Abeles SR, et al. (2014) Human oral viruses are personal , persistent and gender-consistent. *ISME J* 8(9):1753–1767.
 217. Doss J, Culbertson K, Hahn D, Camacho J, Barekzi N (2017) A Review of Phage Therapy against Bacterial Pathogens of Aquatic and Terrestrial Organisms. *Viruses* 9(50).
 218. Hemmrich G, et al. (2012) Molecular Signatures of the Three Stem Cell Lineages in Hydra and the Emergence of Stem Cell Function at the Base of Multicellularity Research article. *Mol Biol Evol* 29(11):3267–3280.

Chapter I: The neglected part of the metaorganism: Prophages in *Hydra*-associated bacteria

Abstract

Every eukaryote is considered a metaorganism or holobiont, due to its association with a complex microbiome. Therefore, several studies focus on the characterization, importance and benefits of bacteria in metaorganism physiology and health. Even though phages, as the most abundant biological entity, are well-known drivers of diversity and shapers within microbial communities, not much is known about the prophage communities within holobionts. Here, I give insights into the prophage communities of several bacterial isolates that are associated with the metaorganism *Hydra*. I show that those bacteria contain prophages that vary significantly in their morphotype, inducibility under environmental stress and host range. Two of the identified temperate phages show a broad host range, infecting bacteria from different families and classes. Moreover, they can infect bacteria from various *Hydra* species, giving an *in vitro* indication for the importance of temperate phages within microbial communities of metaorganisms.

Introduction

All eukaryotic organisms are considered metaorganisms or holobionts, consisting of a multicellular host and its associated microbiome (1, 2). This microbiome and especially bacteria have been shown to have a major impact on their hosts fitness and physiology (3). Bacteria are able to extent health and fertility of several metaorganisms, such as worms, flies and mice (4). In humans, bacteria can modulate the health of the host, by providing their host with important metabolites, resulting in an improved metabolism and reduced inflammatory reactions (3, 5). Moreover, commensal bacteria, such as *Staphylococcus hominis*, can protect its host against invading pathogens (6), ensuring the preservation of the initial microbiome. Similar observations can be made in the freshwater polyp and metaorganism *Hydra*, where the species-specific bacterial community (7, 8) provides resistance against the lethal fungus *Fusarium* sp. (9).

In *Hydra* polyps, bacteria grow in a mucus layer that is called glycocalyx and located on the ectodermal surface (9). The mucus layer represents an important interface for host-microbe crosstalk and is considered to be a nutrient-rich environment (3). Since bacteria are located in the outer layer of the *Hydra*, influences from the environment that affect the polyp, inevitably also impact the *Hydra* microbiome. There, bacteria might even act as a filter for signals and stressors from the environment (10).

In addition to bacteria, also bacteriophages (short: phages) are occupying this niche (11). Bacteriophages are viruses that infect bacterial cells and dominate the species-specific virome of *Hydra* (11). Interestingly, the phage community seems to reflect *Hydras* respective bacteriome (11), which means that *Hydra* colonizing bacteria are likely to be targets for the identified phages (11). Moreover, Grasis *et al.* suggest that most associated phages favoured the temperate lifestyle (11).

In contrast to their lytic counterparts, temperate or lysogenic phages do not obligatory kill their bacterial host but integrate into the bacterial host genome. For this reason, they are transferred from one generation to the next (12, 13) and considered as major reservoirs for horizontal gene transfer (14). They endow their host bacterium with genes that can be beneficial and included in processes like colonization, virulence, coping with adverse environments and superinfection exclusion (15, 16). Superinfection exclusion is the protection of the host against their own phage and other, often closely related phages (15).

Adversely for their bacterial host, temperate phages can switch from the lysogenic to the lytic cycle. This can either happen spontaneously or through extrinsic, environmental triggers (17).

Hydras natural habitats are lakes and ponds, where eutrophication has become a major problem (18). Therefore, exposure to environmental changes like increased temperature, a shift to an alkaline pH and an elevated nutritional level (19) is not uncommon. Since a shift in temperature, pH and nutritional factors are well-known phage-inducers (17), a prophage can easily become a burden to its bacterial host, depending on the (spontaneous) prophage induction rate (20). However, a low level of lysis has been shown to be beneficial for the surviving lysogenized community, due to a higher competitiveness against other bacteria (21–23). Released temperate phages are able to interact with other bacteria in the same niche or with invading competitors. If a phage is able to interact with bacteria depends on their host range. Host range describes the range of bacteria the phage can infect and is a typical characteristic of bacteriophages (24, 25). Additionally, the host range of one phage can give insights into the protective potential of another prophage.

Temperate phages are widely distributed in natural environments (26–30), including metaorganisms (11, 31, 32). However, (temperate) phages are mostly described in relative abundance, while less is known about their respective genomic potential, natural inducing factors and their characteristics within holobionts.

Since *Hydra* has a simple bacteriome (7, 8) with several bacterial genomes available, it is the perfect model to identify temperate phages within metaorganism-associated bacteria. I screened the genomes of the bacterial culture collection of *Hydra* for the presence of prophages and could identify prophage signatures in six bacteria, of which five phages could be induced by Mitomycin C. To identify potential natural inducers of these phages, I analyzed the stability of lysogeny under several environmental stressors, including shifts in temperature and pH, UV radiation and elevated nutrition. Moreover, I described the host range of those phages. Two of the temperate phages feature a broad host range and could infect bacteria from different families and even orders.

Material and Methods

Bacterial strains and culture conditions

All bacterial strains, used in this study, were isolated from different *Hydra* species and can be found in Supplementary Table S1. Normal culture conditions for all bacteria include growth in R2A broth media (3 g/l, Sigma-Aldrich) under shaking conditions at 250 rpm and 18°C.

Identification and purification of prophages

First, I screened the available genomes of *Hydra*-associated bacteria (Supplementary Table S1) for the presence of prophage signatures using the online software PHASTER (Phage Search Tool Enhanced Release) (33). Second, I used Mitomycin C assays to test the induction capacity of the possible identified prophages. Therefore, exponentially growing bacterial cultures were inoculated with 0.05 µg/ml Mitomycin C to induce phage replication (34). The growth of each bacterial strain was observed in a microplate reader (Tecan Spark) to examine a possible decrease as an indicator of phage induction. After an incubation time of 16 h the phages were purified by removing the bacterial cells with two consecutive low-speed-centrifugation steps at 4,266 x g in a ThermoScientific Heraeus Multifuge 3SR at 4°C for 30 min. The supernatant was filtered 0.2 µm and phage particles were pelleted by ultracentrifugation at 25,000 rpm (72,700 x g) in a Beckman 45 Ti rotor at 4°C for 2h. The pellet was re-suspended in 3 ml SM-Buffer (50mM Tris; 100 mM NaCl; 8 mM MgSO₄; pH 7.5).

Transmission electron microscopy (TEM)

Purified phages (see above) were layered onto a pre-formed Cesium chloride gradient consisting of 5 different densities (2ml: 1.7; 1.5; 1.3; 1.2 g/cm³ and 1 ml 1.1 g/cm³) in SM-buffer and centrifuged at 28.000 rpm (135,000 x g) in a Beckman SW 41 rotor at 4°C for 2 h. The band containing phages was removed by syringe, diluted 1:3 in SM-Buffer and pelleted by centrifugation at 22 000 rpm (83,000 x g) in a Beckman SW 41 rotor at 4°C for 2 h. Sub-samples of phages (5 µl) were characterized morphologically by negative staining in 2% (w/v) aqueous uranyl acetate and visualized by transmission electron microscopy (TEM) using a Technai Bio TWIN at 80 kV and a magnification of 40 000–100 000.

Environmental stressors

To analyze if the prophage can be induced under more natural factors than Mitomycin C, the prophage harboring bacteria were exposed to several different environmental stressors. Therefore, 5 ml of exponentially grown bacterial cultures from each strain were exposed in replicates to either UVA (2500 J) - and UVB-light (100 J), alkaline pH (9.5) or elevated nutrition (4x higher R2A concentration= 12 g/l) (n=3), while the rest of the environmental conditions remain normal. Bacteria growing under standard conditions or exposed to Mitomycin C (0.05 µ/ml) served as negative and positive control, respectively. After an incubation of 16 h, I verified phage induction via Spectrophotometry method (see below).

Phage quantification

Phages were quantified by spectrophotometry, following the protocol of Antibody Design Labs (35). Therefore, 1500 µl of the bacterial cultures mentioned above were transferred to microfuge tube to remove bacteria and concentrate phages in the supernatant (2 min, 13000 g, 4°C). 1200 µl of the supernatant were transferred to a new microfuge tube and 300 µl PEG/NaCl (20 %PEG-8000 20%, 2.5 M NaCl) were added. The mixture was then inverted several times and chilled on ice for 30 min. Afterwards the virions were pelleted by a centrifugation step for 3 min at 13000 g. The supernatant was carefully removed and discarded, and the tube was centrifuged again for 1 min at 13000 g. The virus pellet was resuspended in 120 µl SM-Buffer and incubated on ice for at least 1 h. After incubation the phage suspension was vortexed vigorously and was cleared by microcentrifugation at 1 min at 13000 g. The supernatant was then transferred to a new tube for quantification. Therefore, the absorbance of the phage solution was measured at 269 nm and 320 nm at a Nanodrop. The virions per ml were determined with the following formula:

$$\text{Virions/ml} = \frac{(A_{269} - A_{320}) \times 6 \times 10^{16}}{\text{number of bases / virion}}$$

Crossinfection experiments

To analyze the host range of the phages from *Pelomonas* AEP2.2, *Undibacterium* C1.1, *Curvibacter* Mag1.1, *Pseudomonas* Mag2.2 and *Pseudomonas* Oli1.2, cross-infection experiments were conducted, using the double-layer method (36). Therefore, all six phages were induced with Mitomycin C and purified via ultracentrifugation (see above). Meanwhile, log-phase cultures of all bacteria of the bacterial culture collection

of *Hydra* were mixed into 5 ml Top-agar (R2A medium with 0.4% Agarose, 0.5mM CaCl₂, 5 mM MgCl) and poured on R2A-Agar-plates (R2A, 1.5 % Agar-Agar). The plates dried for at least 30 min. Afterwards 10 µl of phages were spotted on each bacteria lawn and the plates dried for 20 min. The plates were incubated at 18°C and checked for plaque formation after 24 – 48 h (n=4).

Data and statistical analysis

The data was statistically analyzed using the statistical program R (R version 3.5.2 (2018-12-20) -- "Eggshell Igloo" Copyright (C) 2018) (37) and the integrated packages multcomp, stat and car. The data was tested for homogeneity using the Levene's test. Since all data sets were equally distributed, they were analyzed using an ANOVA, followed by a Post-Hoc test for multiple comparisons, or a Linear Model (LM).

Results

Identification of bacteriophages from *Hydra*-associated bacteria

All available genomes of *Hydra*-associated bacteria were screened for prophage signatures. Of these, *Duganella* C1.2, *Pelomonas* AEP2.2 and *Curvibacter* Mag1.1 revealed incomplete prophages, while the genomes of *Undibacterium* C1.1, *Pseudomonas* Oli1.2 and *Pseudomonas* Mag2.2 displayed complete prophage signatures (Fig 1 & Fig 2).

The bio-computational analysis software PHASTER (33) predicted an intact prophage, featuring 39.1 kB, within the region 2197-41336 bp, in *Undibacterium* C1.1 (Fig 1 A). Here, 54 proteins could be annotated including typical phage genes, such as Transposases, assembly genes for head- and tail and endolysins (Fig 1 A). Several bacteria-derived genes could be observed in the phage region, including genes coding for hypothetical proteins and one tRNA gene.

Another complete prophage signature was located in *Pseudomonas* Mag2.2 within the region of 1-28662, containing 28.6 kb and 43 Proteins (Fig. 1 B). Here, Enzymes like Integrase, exonuclease, and DNA helicases and phage-like proteins coding for phage morphogenesis and early-lysis-protectors were displayed. I could also observe hits against hypothetical bacteria proteins were also observed in this prophage region (Fig. 1 B).

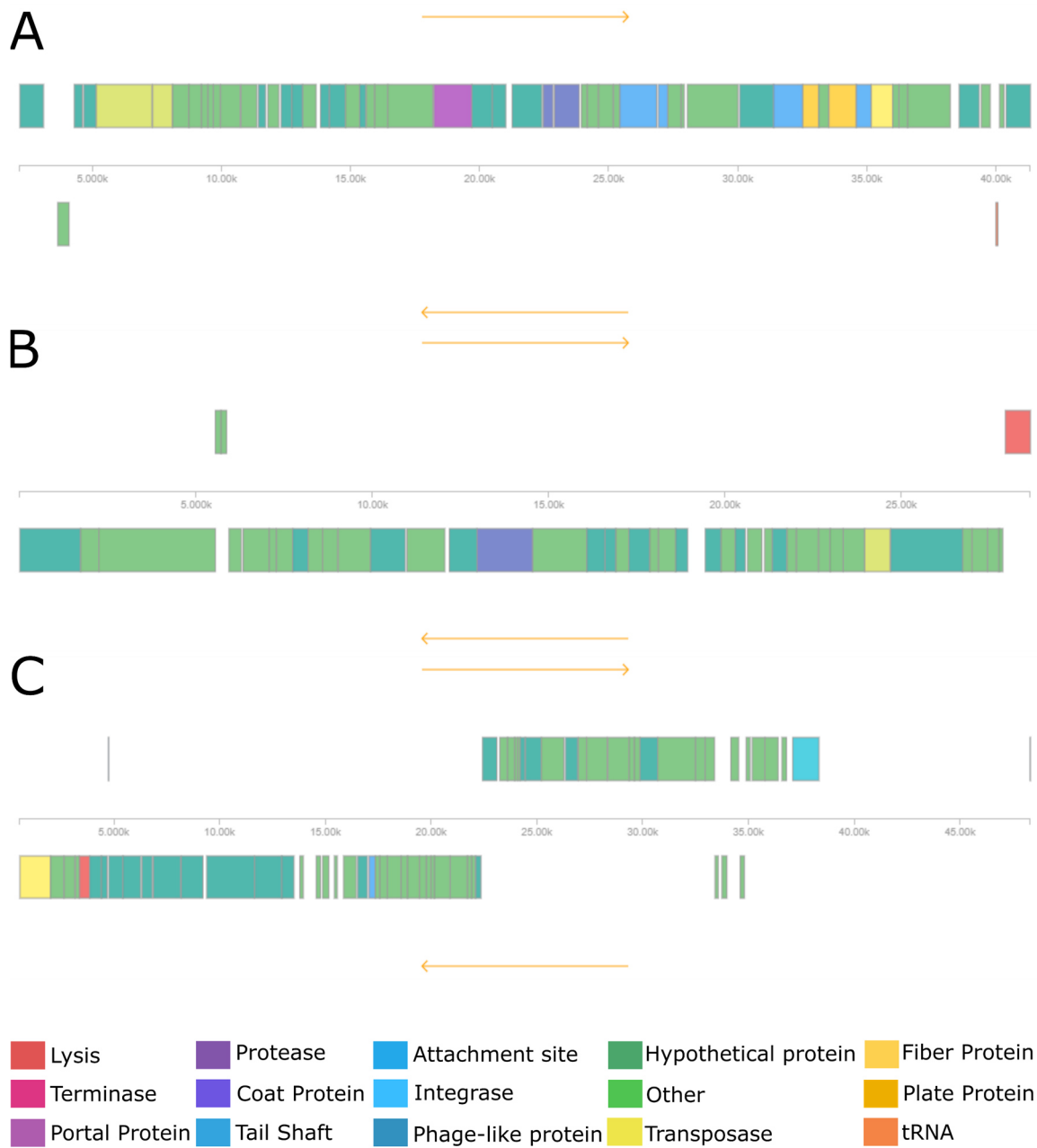


Figure .1: By PHASTER predicted intact prophage regions and genes of **(A)** *Undibacterium* C1.1, **(B)** *Pseudomonas* Mag2.2 and **(C)** *Pseudomonas* Oli1.2.

The third complete prophage could be found in *Pseudomonas* Oli1.2 (Fig. 1 C). This prophage signature is 47.7 kB and located within the region 563-48315 bp. The 62 annotated genes include genes coding for Integrases, exonucleases, tail fibers and several phage-like proteins. In addition, also this phage seems to possess several bacterial genes, including hypothetical proteins and attR and attL attachment sites (Fig. 1 C).



Figure 2: By PHASTER predicted incomplete prophage regions and genes of **(A, B, C)** *Pelomonas* AEP2.2 and **(D)** *Curvibacter* Mag1.1.

Three incomplete prophage signatures were detectable in the genome of *Pelomonas* AEP2.2. Two of them (45487-55265 kb, 372608-382590 bp) feature only 9.7 kb and 9.9 kb and 7 and 9 proteins, respectively, mostly identified as hypothetical and putative proteins (Fig. 2 A & B). The third incomplete phage was located in region 228950-260290 bp, consisted of 31.3 kb and displayed 7 proteins (Fig. 2 C). Coding proteins include a recombinase, transposases, phage-like proteins and hits for bacterial attR and attL attachment sites (Fig. 2 C).

The incomplete prophage of *Curvibacter* Mag1.1 was detectable in the region 460580-466750 bp, was 6.1 kb and contained 7 proteins, including a helicase and several hypothetical proteins (Fig. 2 D).

Three incomplete phages were located within the regions 81912-16229 bp, 36491-44665 bp, and 7706-15161 bp of *Duganella* C1.2, featuring 7.3 kb, 8.1 kb and 7.4 kb and 8, 7 and 8 proteins, respectively (Supplementary Fig. S1).

To evaluate if the predicted prophages can be induced, each bacterium was exposed to Mitomycin C. Already a low concentration of Mitomycin C (0.05 µg/ml) was enough to decrease the growth of *Undibacterium* C1.1 (Fig. 3 A) (Linear Model (LM): $F= 1174$, $p < 0.001$), *Pelomonas* AEP2.2 (Fig. 3 B) (LM: $F= 570$, $p < 0.001$), *Curvibacter* Mag1.1 (Fig 3 C) (LM: $F= 285.9$, $p < 0.001$), *Pseudomonas* Mag2.2 (LM: $F= 726.5$, $p < 0.001$) (Fig 3 D) and *Pseudomonas* Oli1.2 (LM: $F= 386.7$, $p < 0.001$) (Fig. 3 E). *Duganella* (LM: $F= 0.137$, $p= 0.711$) was not affected by the Mitomycin C treatment (Supplementary Fig. S2). A decrease in growth is a strong indication for prophage induction. Nevertheless, all potential prophage solutions were purified, by using a caesium chloride gradient, and the absence or presence of phages was verified with a transmission electron microscope (TEM). For all bacteria showing a Mitomycin C triggered decrease in growth, I observed phages via TEM, displaying a variety of morphotypes. Since no universal method for virus classification is established, phages are mostly characterized by their morphology (38).

Temperate phage of *Undibacterium* C1.1 consisted of an icosahedral head capsid with 50 nm diameter and a characteristic long tail, 120 nm in length, which classifies this phage as *Siphoviridae*. No tail fibers or shafts were visible at the end of the tail (Fig. 3 F).

The temperate phage, isolated from *Pelomonas* AEP2.2, showed a polyhedral shape, displaying only an icosahedral head (50 nm). Since no tail structures were visible on TEM pictures, this temperate phage can be categorized as *Corticoviridae* (Fig. 3 G).

The temperate phage of *Curvibacter* Mag1.1 displayed morphological similarity to *Myoviridae* featuring an isometric head of 50 nm in diameter and a tail of 40 nm. Small tail fibers and tail shaft were visible at the bottom of the tail (Fig. 3 H).

The *Pseudomonas* Mag2.2 phage showed a high similarity to *Siphoviridae*, featuring a characteristic long tail structure, 100 nm in length, and a circular head of 40 nm in diameter. There were no tail fibers or a tail shaft visible (Fig. 3 J).

The phage which was found in association with *Pseudomonas* Oli1.2 consisted of an icosahedral head (50 nm diameter) and a small tail (20 nm), classifying this phage as *Podoviridae*. A tail shaft and tail fibers are located at the bottom of the tail (Fig. 3 K).

As the growth curves already suggested, there were no phages or virus-like particles visible in the supernatant of *Duganella* C1.2.

Environmental influence on prophages of *Hydra*-associated bacteria

To test the intrinsic phage-bacteria-stability and inducibility of the identified prophages to more natural, experimental stress than Mitomycin C, I exposed their bacteria hosts to several environmental stressors, including UVA- and UVB radiation, an increased pH (9.5) and increased nutrition (4x higher concentration of R2A than regular in liquid culture).

The prophage of *Undibacterium* C1.1 was inducible by three environmental conditions. Beside Mitomycin C (ONE-WAY-ANOVA, $F= 11.35$, $p= 0.02$), both UVA (ONE-WAY-ANOVA, $F= 11.35$, $p= 0.003$) and UVB radiation (ONE-WAY-ANOVA, $F= 11.35$, $p= 0.015$) lead the induction of this phage (Fig. 3 L). The second prophage analyzed from *Hydra vulgaris* AEP- associated bacterium, was the phage of *Pelomonas* AEP2.2. This prophage was induced by to two environmental stressors, including UVA radiation (ONE-WAY-ANOVA, $F= 6.162$, $p= 0.024$) and Mitomycin C (ONE-WAY-ANOVA, $F= 6.162$, $p= 0.020$). UVB radiation did not lead to a prophage induction (ONE-WAY-ANOVA, $F= 6.162$, $p= 0.65$) (Fig. 3 M). The prophage of Mag1.1 showed the highest sensitivity to environmental changes. This prophage was induced by UVA- (ONE-WAY-ANOVA, $F= 20.73$, $p= 0.02$) and UVB radiation (ONE-WAY-ANOVA, $F= 20.73$, $p= 0.003$), Mitomycin C (ONE-WAY-ANOVA, $F=20.73$, $p < 0.001$) and an increased

pH (ONE-WAY-ANOVA, $F=20.73$, $p < 0.001$) (Fig. 3 N). The second prophage associated with a bacterium from *Hydra magnipapillata* was the prophage of *Pseudomonas* Mag2.2. It was induced by three environmental factors, including UVB light (ONE-WAY-ANOVA, $F= 15.1$, $p < 0.001$), Mitomycin C (ONE-WAY-ANOVA, $F= 15.1$, $p= 0.003$) and an increased pH of 9.5 (ONE-WAY-ANOVA, $F= 15.1$, $p= 0.003$). Interestingly, UVA light did not show a significant phage increase in comparison to the control (ONE-WAY-ANOVA, $F= 15.1$, $p= 0.12$) (Fig. 3 O). The most resistant phage to environmental stress was the prophage of *Pseudomonas* Oli1.2. This prophage was only inducible by the artificial treatment with Mitomycin C (ONE-WAY-ANOVA, $F= 22.95$, $p < 0.001$) and did not get triggered by any other environmental stressor (Fig. 3 P).

An increased nutrition did not result in prophage induction in any of the tested bacteriophages.

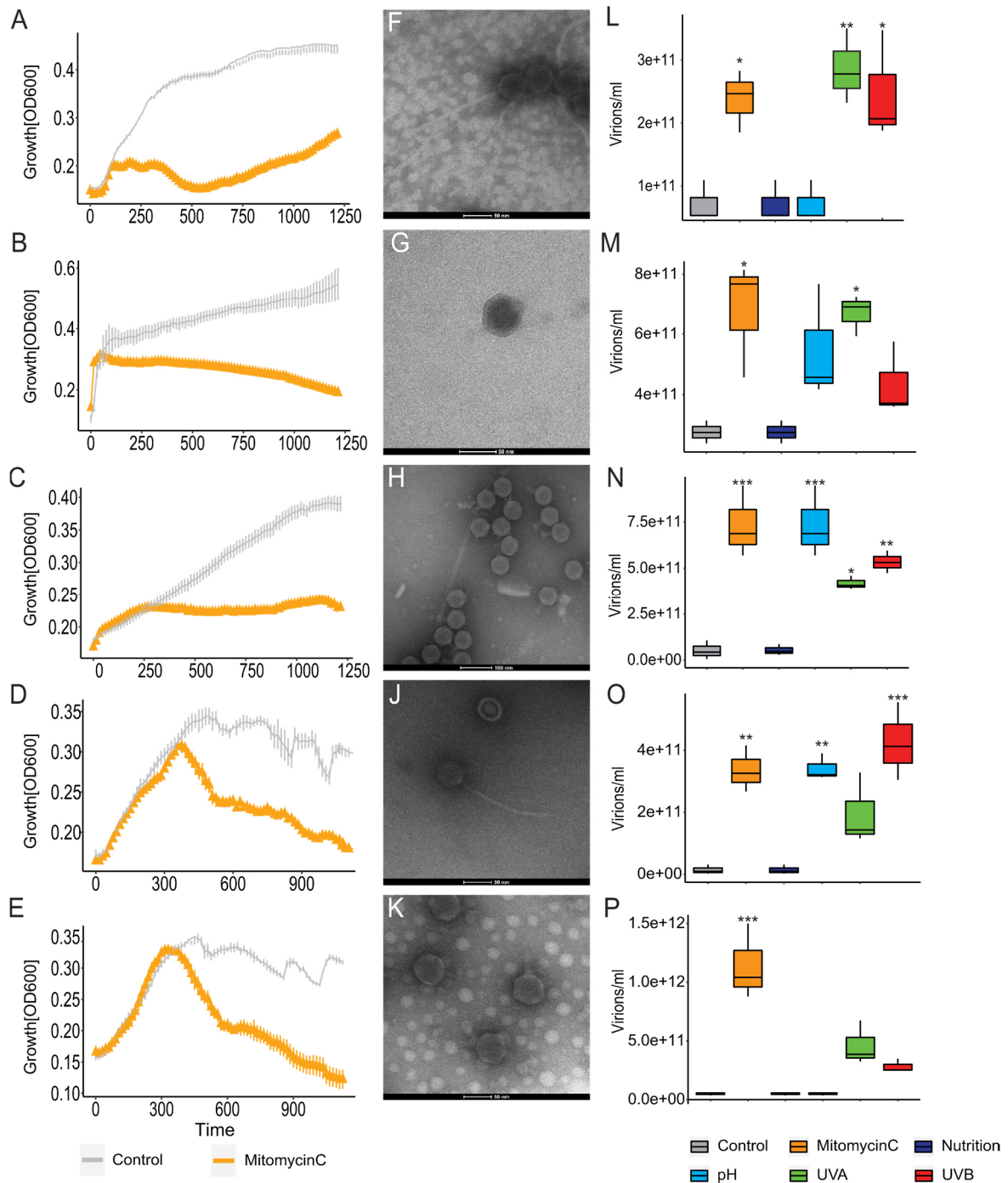


Figure 3: Mean growth (OD600) (\pm SE) over time (min) of (A) *Undibacterium* C1.1, (B) *Pelomonas* AEP2.2, (C) *Curvibacter* Mag1.1, (D) *Pseudomonas* Mag2.2, and (E) *Pseudomonas* Oli1.2 without (Control, grey) and with Mitomycin treatment (orange) (n= 4). Transmission Electron micrographs of (F) *Undibacterium* C1.1 phage, (G) *Pelomonas* AEP2.2 phage, (H) *Curvibacter* Mag1.1 phage, (J) *Pseudomonas* Mag2.2 phage and (K) and *Pseudomonas* Oli phage negative stained with 2% (w/v) aqueous uranyl acetate. Scale bars represent 50 nm (F,G,J,K) or 100 nm (H). Boxplot of Virions/ml after exposure to the environmental stressors (Mitomycin C, Nutrition, elevated pH, UVA- and UVB radiation) of (L) *Undibacterium* C1.1 phage, (M) *Pelomonas* AEP2.2 phage, (N) *Curvibacter* Mag1.1 phage, (O), *Pseudomonas* Mag2.2 phage and (P) *Pseudomonas* Oli1.2 phage (n=3). Stars (*=p<0.05, **= p<0.01) indicate significantly differences between treatment and control (One-way-ANOVA).

Cross infectivity of temperate bacteriophages of *Hydra*-associated bacteria

The phage host range was determined with a Spot-Assay and tested against all naturally occurring bacteria from *Hydra* that were culturable (Fig. 4, Supplementary Table S1).

The prophage of *Pelomonas* AEP2.2 showed lytic activity against 3 of the 13 tested bacterial strains. This temperate phage could lyse bacteria that are closely related to its original host (*Commamodaceae*), including two *Curvibacter* strains (Mag1.1, Hvu1). This phage could also infect *Duganella* Oli1.1, that affiliates to a different family than its bacterial host (*Oxalobacteraceae*) (Fig. 4).

The phage of *Curvibacter* Mag1.1 could infect 2 of the 13 tested bacteria, all classed within the same genus (*Curvibacter*) as its host bacterium (Fig. 4).

The prophages of *Pseudomonas* Mag2.2 and *Undibacterium* C1.1 did not show lytic activity against any of the tested bacteria (Fig. 4).

The prophage from *Pseudomonas* Oli1.2 displayed a broad host range. Here, no lytic activity was observed against bacteria from the same family or genus (*Pseudomonaceae*). Instead this prophage was able to infect all three tested *Curvibacter* strains, belonging to a different bacterial family and class than its original host (Fig. 4, Supplementary Table S1).

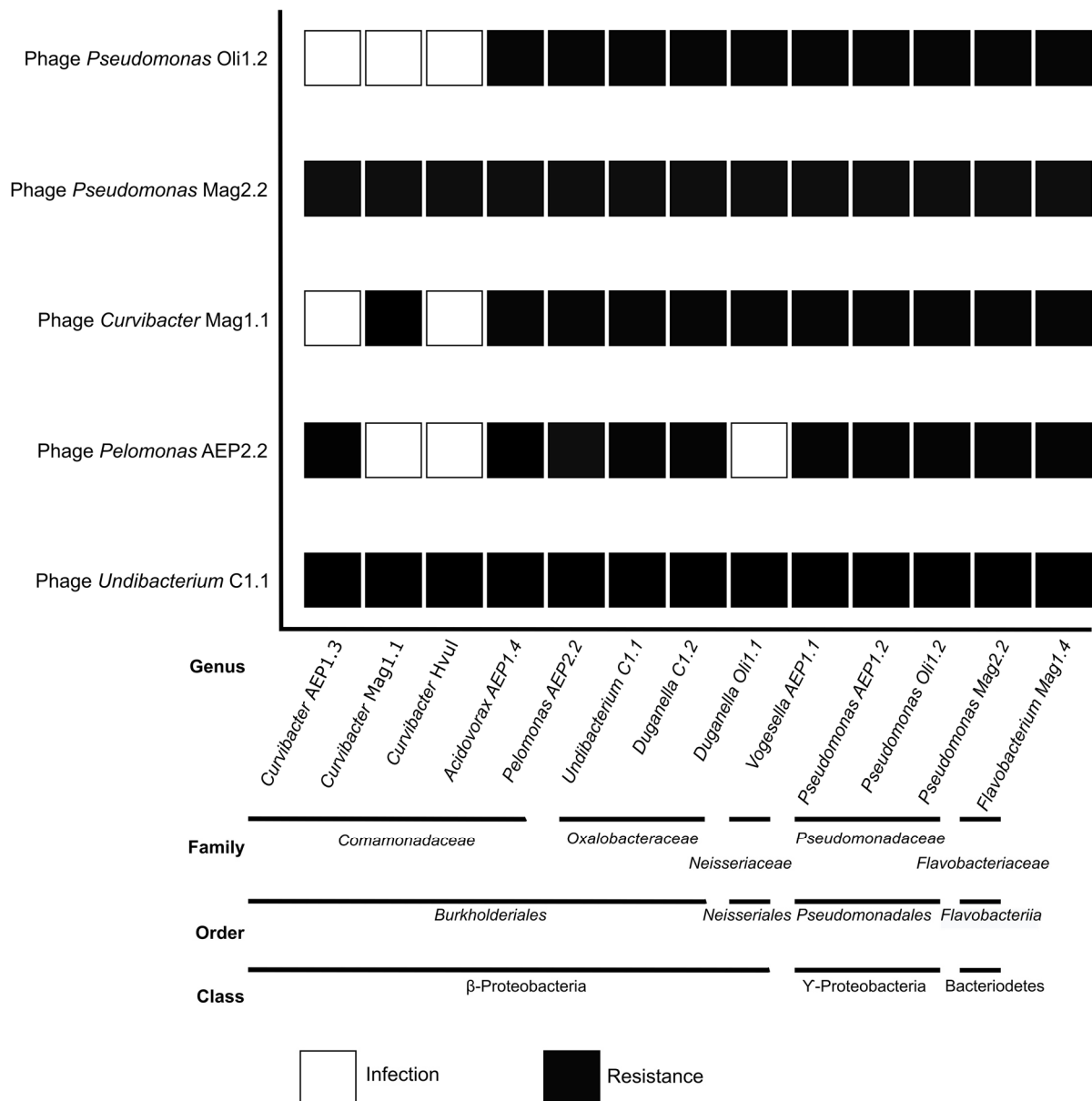


Figure 4: Host range of the temperate phages of *Undibacterium* C1.1, *Pelomonas* AEP2.2, *Curvibacter* Mag1.1, *Pseudomonas* Mag2.2 and *Pseudomonas* Oli1.2 on *Hydra*-associated bacteria. White boxes indicate a plaque formation and an infection of the bacterium, while black boxes indicate no plaque formation and resistance to phage infection.

Discussion

Phages are highly abundant in nature (39) and prophages or temperate phages are widespread throughout several environments (23). Overall, lysogeny is suggested to be rather common in bacteria (40). It is reported that 47% of 12000 strains from a medical and non-marine environment, and 43 % of marine bacteria are lysogenic (40, 41). Moreover, it has been suggested that 60-70 % of all sequenced bacterial genomes contain prophages and two-third of all sequenced γ -proteobacteria harbour prophages (40, 42). Several prophage signatures are also present in core gut-associated bacteria of metaorganism *Ciona intestinalis* (43) and it has been shown that temperate phages most likely represent the majority of the human gut phageome (26–29). Since prophages are present in a diverse phylogenetic range of host bacteria (44), it is not surprising that I could identify several prophage signatures within *Hydra*-associated bacteria from different families, whereof five phages were inducible.

Temperate phages are well known agents of horizontal gene transfer (45, 46) and therewith alter and influence bacteria genetic diversity (45, 46). Beside transferring different kinds of genes to their host bacterium (45–54), phages may also unintentionally acquire host genetic material, called transduction (55–57). Specialized transduction occurs during prophage excision from its specific integration site. This results in packaging both, phage and bacterial DNA, in one phage particle (55). I could observe several hits for bacterial genes in the phage genomes, which suggests that specialized transduction could have taken place. Beside hypothetical proteins, a bacterial tRNA gene was detected in the genome of the prophage of *Undibacterium* C1.1. This is interesting, since phages mostly rely on their host machinery for replication (58). Nevertheless, acquiring those genes can complement with the host machinery and might be a fitness advantage for the respective phage (59). Those phage-encoded tRNA genes are widely spread in prophages (60), but seem to be different from the bacterial genomic tRNA gene (61). Often used as integration sites for phages (60), it has been suggested that those phage-encoded tRNA compensates the compositional differences between phage and host genomes (59). Therefore, the bacteria acquired tRNA gene from the phage of *Undibacterium* C1.1 might be a fitness advantage during the lytic cycle or helps the phage to integrate into the host bacterium more easily.

Similar bacterial genes that are related to attaching or integration could be observed in the genomes of the *Pseudomonas* Oli1.2 and *Pelomonas* AEP2.2 prophages. Here, the bacterial genes for *attL* and *attR* could be observed. Those *att*-regions are well-known integration sites for prophages. In λ -phages, λ can recognize the bacterial host attachment site *attB*. This site recombines with a phage attachment site, whereby two hybrid sets, *attL* and *attR*, emerge (62). Therefore, the bacteria acquired genes in prophages of *Undibacterium* C1.1, *Pseudomonas* Oli1.2 and *Pelomonas* AEP2.2 were probably acquired during excision from a different host and are probably of great importance for integration into their own, but also new bacterial hosts.

Prophages are known to react to environmental stressors (17). Changing environmental conditions can lead to DNA damage of the bacterial host, which in turn triggers the SOS response and results in prophage induction (63, 64). UV light is known to cause severe DNA damage (65). Therefore, it is not surprising that four of the five tested phages in this study were induced by UV radiation. Besides, a shift towards an acidic pH is well known to result in prophage induction of *Helicobacter pylori* and *Nitrosospira multiformis* phages (66, 67). In this study, a shift towards an alkaline pH leads to a prophage excision of both bacteriophages that are associated with bacteria of *Hydra magnipapillata*, probably also triggered by an activated SOS response in the respective bacterium.

No identified prophage in this study was inducible by an elevated nutrition. Similar observations could be made in marine *Synechococcus* phages (68). Moreover, it was suggested that nutrient increase even has an inhibitory effect on the inducibility and viral production (68). Given the fact that mucus layers, including the *Hydra* glycocalyx, are normally nutrient-rich environments (69), this comes with no surprise. Probably adapted to the nutrient-rich environment *in vivo*, an increased concentration of R2A did not result in prophage induction.

Intriguingly, neither altered nutrition and pH, nor UV radiation lead to prophage induction of *Pseudomonas* Oli1.2 phage. Only the artificial treatment with Mitomycin C triggers phage induction. This suggests that lysogeny is maintained in this bacterium under various environmental conditions.

The benefit of prophages highly depends on their induction rate: The higher the induction rate, the lower the advantage for the bacterial host (70). Spontaneous prophage induction is rather rare (13), but if phages are induced by a high number of

environmental stress, prophages can quickly become detrimental, due to a constant lysis of their bacterial host (13). Therefore, phages are considered molecular time bombs in the marine environment (40). In gut-associated *Escherichia coli*, carriage of prophage λ is costly, due to a frequent reactivation in monoxenic mouse guts, probably triggered by DNA damage (70).

In the wild, *Hydra* and the associated bacteria are exposed to several different environmental changes in freshwater environments, especially in consideration of the increasing eutrophication, that includes shifts in pH levels (18, 71). This might imply that in association with *Hydra*, a stable establishment of lysogeny under several changing environmental conditions would be from great importance for the bacterial host. Corresponding, the phages of *Pelomonas* AEP2.2 and *Pseudomonas* Oli1.2 showed low or no reaction to natural stressors. In contrast, the phages of *Curvibacter* Mag1.1, *Pseudomonas* Mag2.2 and *Undibacterium* C1.1 were inducible by the majority of the tested environmental stressors. Here, the constant lysis might become a disadvantage for the bacterial hosts, comparable to λ -carrying *E. coli* strains (70). Constant lysis might reduce their abundance and their colonization ability on the *Hydra* host. While this is not the case for *Curvibacter* Mag1.1, which is the main colonizer of *Hydra magnipapillata*, the induction rate might be an explanation why *Undibacterium* C1.1 only makes 2.1 % of the bacterial population on *Hydra vulgaris* AEP and why *Pseudomonadaceae* are only sparsely represented on *Hydra magnipapillata* (7, 9). Nevertheless, the fact that the phages were still active, implement that the benefits of those prophages prevail. If phages are too costly due to reoccurring induction, bacteria should lose or inactivate these costly prophages (70). This is supported by the large numbers of cryptic, inactive prophages, which implement a regular selection for or against active prophages in the favor of the bacterium (16, 70, 72, 73). It has been shown that induced prophages can give their host an advantage. When susceptible bacteria are present, the prophage indirectly benefits its carrier and a strong selection to higher induction rates takes place (70, 74). The lysogen itself is resistant against its own phage, due to transferred superinfection exclusion genes (15), while other competing bacteria might be killed by the released temperate phage (70, 74). Therefore, a propensity for prophage-induction is not generally a disadvantage. The balance between loss through induction and fighting competitors defines the utility.

To convey a benefit, phages are in need of a certain host range. Most phages are considered specific and seem to target a single bacterial species and even only a few strains within that species (75, 76). Accordingly, temperate phage of *Curvibacter* Mag1.1 only lyses bacteria within the same genus as its host bacterium (*Curvibacter*). On the other hand, the phage of *Pelomonas* features a broader host range, infecting bacteria from the same family as its host bacterium (*Comamonadaceae*) but also bacteria from the *Oxalobacteriaceae* family. Similar broad host ranges are well known for other phages (77, 78), including freshwater bacteriophages recovered from lake Michigan, that demonstrate a host range spanning several phyla of bacteria (79). Interestingly, the temperate phage of *Pseudomonas* Oli1.2 only infected bacteria that belong to a different bacteria family than its original host and none of the tested *Pseudomonas* strains. This polyvalency can be observed in several bacteriophages that infect *Burkholderiales* (80, 81). Moreover, it has been suggested that phage host range is not a stable property. It rather changes over time, probably due to bacteria-phage coevolution and the resulting resistances and counter-adaptations (77), which is a possible explanation for the infection pattern of the *Pseudomonas* Oli1.2 phage. The host range of phages isolated from leaves of horse chest nut tree shows that phages are more efficient in infecting bacteria from the same tree, compared to bacteria from other trees (82). This suggests that phages are rather locally adapted and environment specific than bacteria specific (82). In this study, I could observe a contradicting observation. Here, temperate phages seem to show lytic activity against bacteria from other *Hydra* species. However, I only tested a few isolates from the different *Hydra* strains. Therefore, it might be that the proper bacterial target from the same *Hydra* species was missing in the spectrum of tested bacteria. Nevertheless, the cross-infectivity and broad host ranges suggest a shaping role of temperate phages in the metaorganism *Hydra*. Considering that wild *Hydra* lives in exposure to the surrounding water and also to other *Hydra* species (71), it is likely that *Hydra* is confronted with bacteria from other *Hydra* species and other invading bacteria from the water. Therefore, the role of temperate phages might include protection against invading bacteria from the environment. Consistently, several models describe a protective role of prophages, including the “Piggyback-the-winner” model (83) and “Bacteriophage adhering to mucus” (BAM) model (84). The latter suggests that phages provide a non-host derived immunity against pathogens (84). Here, phages are

enriched in mucus layer of their host, via binding interactions between the host mucin and phage capsid, and are now able to encounter invading bacteria from the environment (84). On the other hand, those temperate phages might be able to influence the host-associated community. Given the high amount of prophages in *Hydra*-associated bacteria, which can also be observed in other metaorganism like humans (29, 85), and their inducibility and host range, they might be able to shape the metaorganism associated bacterial community.

The phages of *Undibacterium* C1.1 and *Pseudomonas* Mag2.2 do not show any lytic activity against the tested bacteria. It might be that the proper bacterial target for both phages was missing in the spectrum of tested bacteria or the method for testing the phage host range was incompatible with the long tail of the *Siphoviridae* phages.

Our results emphasize the wide variety of prophages that are associated with bacteria from the metaorganism *Hydra*. Those temperate phages vary greatly in their inducibility under environmental stress and host range. The fact that already a basal metazoan like *Hydra* is associated with this assortment of temperate phages, hint to their importance in more complex animals, such as mice and humans. Two of the five phages display a broad host range, infecting bacteria throughout different bacterial families and classes. These results give a first *in vitro* hint for the shaping utility of temperate phages in metaorganism-associated bacterial communities but need to further be validated with *in vivo* experiments.

Supplementary

Table S1: List of all culturable, *Hydra*-associated bacteria, used in this study, including the *Hydra* species they were isolated from, their genus, their strain name and if their genomes were available.

<i>Hydra</i> host	Genus	Strain	Genome	Inducible prophage
<i>Hydra vulgaris</i> (AEP)	<i>Curvibacter</i> sp.	AEP1.3	Yes	Yes (other study)
<i>Hydra magnipapillata</i>	<i>Curvibacter</i> sp.	Mag1.1	Yes	Yes
<i>Hydra vulgaris</i>	<i>Curvibacter</i> sp.	Hvul	Yes	No
<i>Hydra vulgaris</i> (AEP)	<i>Undibacterium</i> sp.	C1.1	Yes	Yes
<i>Hydra vulgaris</i> (AEP)	<i>Acidovorax</i> sp.	AEP1.4	Yes	No
<i>Hydra vulgaris</i> (AEP)	<i>Pelomonas</i> sp.	AEP2.2	Yes	Yes
<i>Hydra vulgaris</i> (AEP)	<i>Duganella</i> sp.	C1.2	Yes	No
<i>Hydra oligactis</i>	<i>Duganella</i> sp.	Oli1.1	No	No
<i>Hydra vulgaris</i> (AEP)	<i>Pseudomonas</i> sp.	AEP2.2	Yes	Yes
<i>Hydra magnipapillata</i>	<i>Pseudomonas</i> sp.	Mag2.2	Yes	Yes
<i>Hydra oligactis</i>	<i>Pseudomonas</i> sp.	Oli1.2	Yes	Yes
<i>Hydra magnipapillata</i>	<i>Flavobacterium</i> sp.	Mag1.4	Yes	No
<i>Hydra vulgaris</i> (AEP)	<i>Vogesella</i> sp	AEP1.1	Yes	No

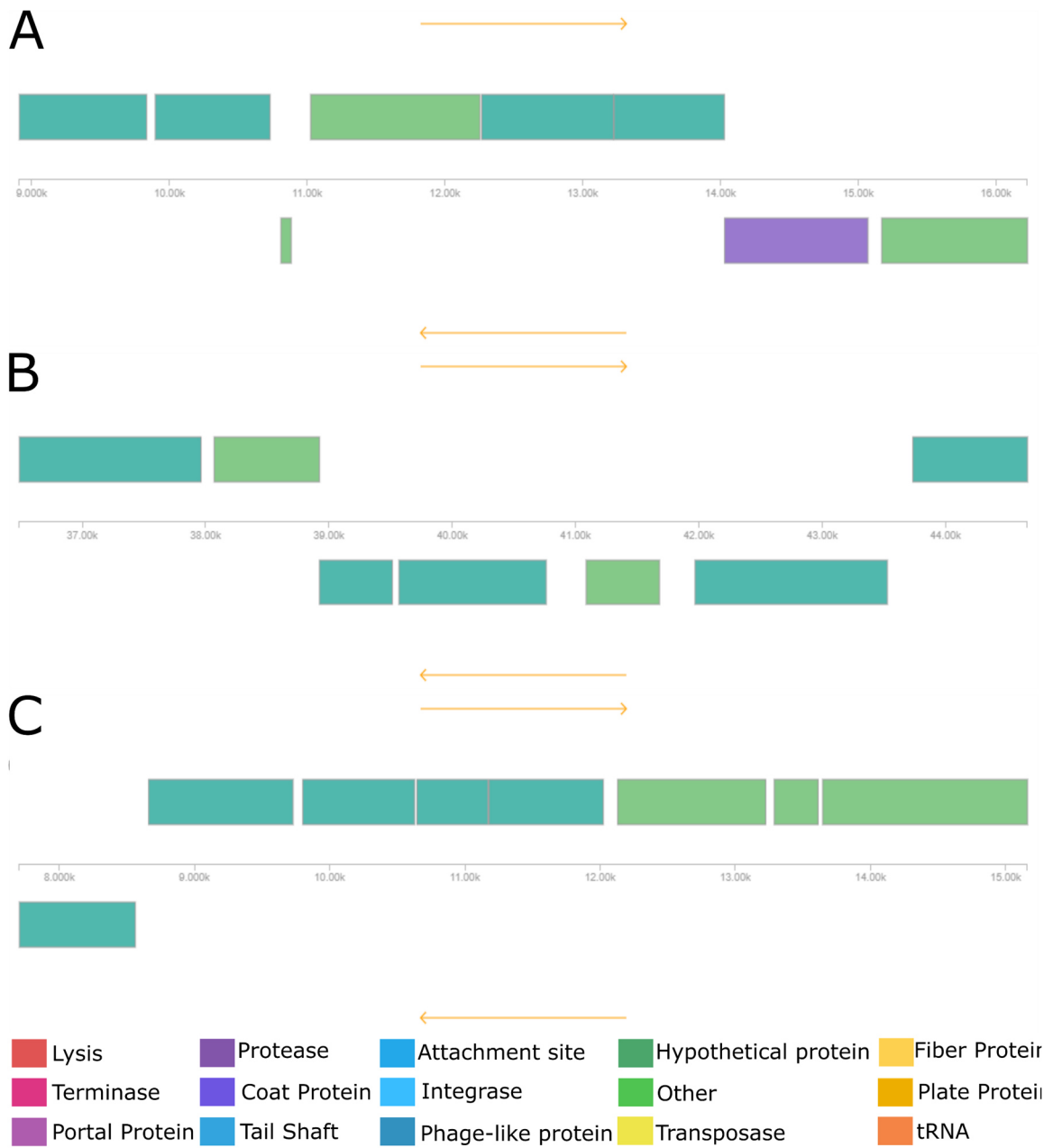


Figure S1: By PHASTER predicted incomplete prophage regions and genes of *Duganella* C1.2 (**A**, **B**, **C**).

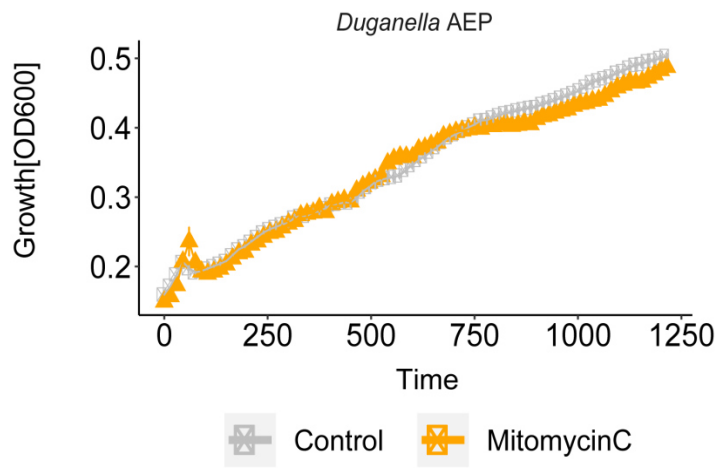


Figure S2: Growth (OD₆₀₀) of *Duganella* C1.2 over time with Mitomycin C treatment (orange) or in the control group (grey) (n=4).

References

1. Bosch TCG, McFall-Ngai MJ (2011) Metaorganisms as the new frontier. *Zoology* 114(4):185–190.
2. Zilber-rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants : the hologenome theory of evolution. *FEMS Microbiol Rev* 32:723–735.
3. Esser D, et al. (2018) Functions of the Microbiota for the Physiology of Animal Metaorganisms. *J Innate Immun.* 11:393-404
4. Sonowal R, et al. (2017) Indoles from commensal bacteria extend healthspan. *Proc Natl Acad Sci U S A* 114(36):E7506–E7515.
5. Rios-Covian D, Salazar N, Gueimonde M, de Los Reyes-Gavilan CG (2017) Shaping the Metabolism of Intestinal Bacteroides Population through Diet to Improve Human Health. *Front Microbiol* 8:376.
6. Nakatsuji T, et al. (2017) Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Sci Transl Med* 9(378):eaah4680.
7. Franzenburg S, et al. (2013) Distinct antimicrobial peptide expression determines host species-specific bacterial associations. *Proc Natl Acad Sci U S A* 110:E3730-8.
8. Fraune S, Bosch TCG (2007) Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. *Pnas* 104(32):13146–13151.
9. Fraune S, et al. (2015) Bacteria-bacteria interactions within the microbiota of the ancestral metazoan Hydra contribute to fungal resistance. *ISME J* 9:1543–1556.
10. Brown JM, Hazen SL (2015) The Gut Microbial Endocrine Organ: Bacterially Derived Signals Driving Cardiometabolic Diseases. *Annu Rev Med* 66(1):343–359.
11. Grasis JA, et al. (2014) Species-specific viromes in the ancestral holobiont hydra. *PLoS One* 9(10):e109952.
12. Koskella B, Brockhurst MA (2014) Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS Microbiol Rev* 38(5):916–931.
13. De Paepe M, Leclerc M, Tinsley CR, Petit M-A (2014) Bacteriophages: an underestimated role in human and animal health? *Front Cell Infect Microbiol* 4(March):1–11.
14. Keen EC (2015) A century of phage research: bacteriophages and the shaping of modern biology. *Bioessays* 37(1):6–9.
15. Bondy-Denomy J, Davidson AR (2014) When a Virus is not a Parasite : The Beneficial Effects of Prophages on Bacterial Fitness. *J Microbiol* 52(3):235–242.
16. Wang X, et al. (2010) Cryptic prophages help bacteria cope with adverse environments. *Nat Commun* 1(9):147–149.
17. Nanda AM, Thormann K, Frunzke J (2015) Impact of spontaneous prophage induction on the fitness of bacterial populations and host-microbe interactions. *J Bacteriol* 197(3):410–419.
18. Withers P, Neal C, Jarvie H, Doody D (2014) Agriculture and Eutrophication: Where Do We Go from Here? *Sustainability* 6(9):5853–5875.
19. Jensen HS, Andersen FO (1992) Importance of temperature, nitrate, and pH for phosphate release from aerobic sediments of four shallow, eutrophic lakes. *Limnol Oceanogr* 37(3):577–589.

20. Cortes MG, Krog J, Balázsi G (2019) Optimality of the spontaneous prophage induction rate. *J Theor Biol* 483:110005.
21. Brown SP, Le Chat L, De Paepe M, Taddei F (2006) Ecology of Microbial Invasions: Amplification Allows Virus Carriers to Invade More Rapidly When Rare. *Curr Biol* 16(20):2048–2052.
22. Burns N, James CE, Harrison E (2014) Polylysogeny magnifies competitiveness of a bacterial pathogen in vivo. *Evol Appl* 8(4):346–351.
23. Harrison E, Brockhurst MA (2017) Ecological and Evolutionary Benefits of Temperate Phage: What Does or Doesn't Kill You Makes You Stronger. *BioEssays* 39.
24. de Jonge PA, Nobrega FL, Brouns SJJJ, Dutilh BE (2019) Molecular and Evolutionary Determinants of Bacteriophage Host Range. *Trends Microbiol* 27(1):51–63.
25. Hyman P, Abedon ST (2010) Bacteriophage Host Range and Bacterial Resistance, pp 217–248.
26. Stern A, Mick E, Tirosh I, Sagy O, Sorek R (2012) CRISPR targeting reveals a reservoir of common phages associated with the human gut microbiome. *Genome Res* 22(10):1985–1994.
27. Minot S, et al. (2011) The human gut virome: Inter-individual variation and dynamic response to diet. *Genome Res* 21:1616–1625.
28. Manrique P, Dills M, Young MJ (2017) The human gut phage community and its implications for health and disease. *Viruses* 9(6):9–11.
29. Furuse K, et al. (1983) Bacteriophage distribution in human faeces: Continuous survey of healthy subjects and patients with internal and leukaemic diseases. *J Gen Virol* 64(9):2039–2043.
30. Armon R (2011) Soil Bacteria and Bacteriophages. *Biocommunication in Soil Microorganisms*, pp 403–412.
31. Jahn MT, et al. (2019) A Phage Protein Aids Bacterial Symbionts in Eukaryote Immune Evasion. *Cell Host Microbe* 26(4):542–550.
32. Brüwer JD, Voolstra CR (2018) First insight into the viral community of the cnidarian model metaorganism *Aiptasia* using RNA-Seq data. *PeerJ* 6:e4449.
33. Arndt D, et al. (2016) PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res* 44(W1):W16–W21.
34. Sekiguchi M, Takagi Y (1960) Effect of mitomycin C on the synthesis of bacterial and viral deoxyribonucleic acid. *BBA - Biochim Biophys Acta* 41(3):434–443.
35. Antibody Design labs (2013) Quantification of Bacteriophage by Spectrophotometry. <http://www.abdesignlabs.com/technical-resources/bacteriophage-spectrophotometry/>.
36. Adams M (1959) Assay of phage by agar layer method. *Bacteriophages Intersci Publ Inc, New York, NY*,:450–454.
37. R Core Team (2018) R: A Language and Environment for Statistical Computing.
38. Ackermann H-W (2009) Phage Classification and Characterization. *Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions*, eds Clokie MRJ, Kropinski AM (Humana Press, Totowa, NJ), pp 127–140.
39. Suttle C a (2007) Marine viruses--major players in the global ecosystem. *Nat Rev Microbiol* 5(10):801–812.
40. Paul JH (2008) Prophages in marine bacteria: Dangerous molecular time bombs or the key to survival in the seas? *ISME J* 2(6):579–589.
41. Ackermann H-W, DuBow MS (1987) *Viruses of prokaryotes* (CRC press, Boca Raton). 1st Ed.

42. Brüssow H, Canchaya C, Hardt W, Bru H (2004) Phages and the Evolution of Bacterial Pathogens : from Genomic Rearrangements to Lysogenic Conversion Phages and the Evolution of Bacterial Pathogens : from Genomic Rearrangements to Lysogenic Conversion. *Microbiol Mol Biol Rev* 68(3):560–602.
43. Leigh BA, Djurhuus A, Breitbart M, Dishaw LJ (2018) The gut virome of the protochordate model organism, *Ciona intestinalis* subtype A. *Virus Res* 244:137–146.
44. Hendrix RW, Smith MCM, Burns RN, Ford ME, Hatfull GF (1999) Evolutionary relationships among diverse bacteriophages and prophages: All the world's a phage. *Proc Natl Acad Sci U S A* 96(5):2192–2197.
45. Touchon M, Moura de Sousa JA, Rocha EP (2017) Embracing the enemy: The diversification of microbial gene repertoires by phage-mediated horizontal gene transfer. *Curr Opin Microbiol* 38:66–73.
46. Canchaya C, Fournous G, Chibani-Chennoufi S, Dillmann ML, Brüssow H (2003) Phage as agents of lateral gene transfer. *Curr Opin Microbiol* 6(4):417–24.
47. Muniesa M, Colomer-Lluch M, Jofre J (2013) Potential impact of environmental bacteriophages in spreading antibiotic resistance genes. *Future Microbiol* 8(6):739–751.
48. von Wintersdorff CJH, et al. (2016) Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. *Front Microbiol* 7(February):173.
49. Haaber J, et al. (2016) Bacterial viruses enable their host to acquire antibiotic resistance genes from neighbouring cells. *Nat Commun* 7:1–8.
50. Davis BM, Waldor MK (2003) Filamentous phages linked to virulence of *Vibrio cholerae*. *Curr Opin Microbiol* 6(1):35–42.
51. Bille E, et al. (2017) A virulence-associated filamentous bacteriophage of *Neisseria meningitidis* increases host-cell colonisation. *PLoS Pathog* 13(7):1–23.
52. McCarthy AJ, Witney AA, Lindsay JA (2012) *Staphylococcus aureus* Temperate Bacteriophage: Carriage and Horizontal Gene Transfer is Lineage Associated. *Front Cell Infect Microbiol* 2(February):1–10.
53. Wagner PL, Waldor MK (2002) Bacteriophage Control of Bacterial Virulence. *Infect Immunity* 70(8):3985–3993.
54. Cheetham BF, Katz ME (1995) A role for bacteriophages in the evolution and transfer of bacterial virulence determinants. *Mol Microbiol* 18(2):201–208.
55. Thierauf A, Perez G, Maloy, Stanley (2009) Generalized Transduction. *Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions*, eds Clokie MRJ, Kropinski AM (Humana Press, Totowa, NJ), pp 267–286.
56. Sternberg NL, Maurer R (1991) [2] Bacteriophage-mediated generalized transduction in *Escherichia coli* and *Salmonella typhimurium*. *Bacterial Genetic Systems, Methods in Enzymology*. (Academic Press), pp 18–43.
57. ZINDER ND, LEDERBERG J (1952) Genetic exchange in *Salmonella*. *J Bacteriol* 64(5):679–99.
58. Ofir G, Sorek R (2018) Contemporary Phage Biology: From Classic Models to New Insights. *Cell* 172(6):1260–1270.
59. Bailly-Bechet M, Vergassola M, Rocha E (2007) Causes for the intriguing presence of tRNAs in phages. *Genome Res* 17(10):1486–1495.
60. Canchaya C, Fournous G, Brüssow H (2004) The impact of prophages on bacterial chromosomes. *Mol Microbiol* 53(1):9–18.

| Chapter I |

61. Ventura M, Canchaya C, Pridmore RD, Brüssow H (2004) The prophages of *Lactobacillus johnsonii* NCC 533: comparative genomics and transcription analysis. *Virology* 320(2):229–242.
62. Fogg PCM, Colloms S, Rosser S, Stark M, Smith MCM (2014) New applications for phage integrases. *J Mol Biol* 426(15):2703–2716.
63. Mardanov A V., Ravin N V. (2007) The antirepressor needed for induction of linear plasmid-prophage N15 belongs to the SOS regulon. *J Bacteriol* 189(17):6333–6338.
64. Howard-Varona C, Hargreaves KR, Abedon ST, Sullivan MB (2017) Lysogeny in nature: Mechanisms, impact and ecology of temperate phages. *ISME J* 11(7):1511–1520.
65. Janion C (2008) Inducible SOS response system of DNA repair and mutagenesis in *Escherichia coli*. *Int J Biol Sci* 4(6):338–44.
66. Alves de Matos AP, Lehours P, Timóteo A, Roxo-Rosa M, Vale FF (2013) Comparison of induction of B45 *Helicobacter pylori* prophage by acid and UV radiation. *Microsc Microanal* 19(S4):27–28.
67. Choi J, Kotay SM, Goel R (2010) Various physico-chemical stress factors cause prophage induction in *Nitrosospira multiformis* 25196- an ammonia oxidizing bacteria. *Water Res* 44(15):4550–4558.
68. L. M, J.H. P (2005) Effect of nutrient addition and environmental factors on prophage induction in natural populations of marine *Synechococcus* species. *Appl Environ Microbiol* 71(2):842–850.
69. Sicard J-F, Le Bihan G, Vogeleeer P, Jacques M, Harel J (2017) Interactions of Intestinal Bacteria with Components of the Intestinal Mucus. *Front Cell Infect Microbiol* 7.
70. De Paepe M, et al. (2016) Carriage of λ Latent Virus Is Costly for Its Bacterial Host due to Frequent Reactivation in Monoxenic Mouse Intestine. *PLoS Genet* 12(2):1–20.
71. Quinn B, Gagné F, Blaise C (2012) Hydra, a model system for environmental studies. *Int J Dev Biol* 56(6–8):613–625.
72. Wang X, Wood TK (2016) Cryptic prophages as targets for drug development. *Drug Resist Updat* 27:30–38.
73. Ragunathan PT, Vanderpool CK (2019) Cryptic-Prophage-Encoded Small Protein DicB Protects *Escherichia coli* from Phage Infection by Inhibiting Inner Membrane Receptor Proteins. *J Bacteriol* 201(23):e00475-19.
74. Berngruber TW, Froissart R, Choisy M, Gandon S (2013) Evolution of Virulence in Emerging Epidemics. *PLoS Pathog* 9(3):e1003209.
75. Koskella B, Meaden S (2013) Understanding bacteriophage specificity in natural microbial communities. *Viruses* 5(3):806–823.
76. Hyman P, et al. (2009) Bacteriophages. 502(June 2014):81–85.
77. Ross A, Ward S, Hyman P (2016) More Is Better: Selecting for Broad Host Range Bacteriophages. *Front Microbiol* 7(September):1–6.
78. Uchiyama J, et al. (2008) Isolation and characterization of a novel *Enterococcus faecalis* bacteriophage Φ EF24C as a therapeutic candidate. *FEMS Microbiol Lett* 278(2):200–206.
79. Malki K, et al. (2015) Bacteriophages isolated from Lake Michigan demonstrate broad host-range across several bacterial phyla. *Virology* 1–5.
80. Langley R, Kenna DT, Vandamme P, Ure R, Govan JRW (2003) Lysogeny and bacteriophage host range within the *Burkholderia cepacia* complex. *J Med Microbiol* 52(6):483–490.

| Chapter I |

81. Kawasaki T, et al. (2009) Genomic characterization of *Ralstonia solanacearum* phage ϕ RSB1, a T7-like wide-host-range phage. *J Bacteriol* 91(1):422–427.
82. Koskella B, Thompson JN, Preston GM, Buckling A (2011) Local biotic environment shapes the spatial scale of bacteriophage adaptation to bacteria. *Am Nat* 177(4):440–51.
83. Silveira CB, Rohwer FL (2016) Piggyback-the-Winner in host-associated microbial communities. *npj Biofilms Microbiomes*:1–5.
84. Barr JJ, et al. (2013) Bacteriophage adhering to mucus provide a non-host-derived immunity. *Proc Natl Acad Sci U S A* 110(26):10771–6.
85. Waller AS, et al. (2014) Classification and quantification of bacteriophage taxa in human gut metagenomes. *ISME J* 8(7):1391–1402.

Chapter II – Prophage TJ1 acts as an internal regulator of the *Hydra* microbiome

Abstract

Many multicellular organisms are closely associated with a specific bacterial community and therefore considered “metaorganisms”. Controlling the bacterial community composition is essential for the stability and function of metaorganisms, but the factors contributing to the maintenance of host specific bacterial colonization are poorly understood. Here, I demonstrate that in *Hydra* the most dominant bacterial colonizer *Curvibacter sp.* is associated with an intact prophage which can be induced by different environmental stressors both *in vitro* and *in vivo*. Differences in the induction capacity of *Curvibacter* phage TJ1 in culture (*in vitro*) and on *Hydra* (*in vivo*) imply that the habitat of the prokaryotic host and/or bacterial frequency dependent factors influence phage inducibility. Moreover, I show that phage TJ1 features a broad host range against other bacterial colonizers and is directly capable of affecting bacterial colonization on *Hydra*. From these results I conclude that prophages are a hidden part of the microbiome interfering with bacteria-bacteria interactions and have the potential to influence the composition of host associated bacterial communities.

Introduction

Eukaryotic organisms are living in a close relationship with a complex microbial community, composed of bacteria, fungi, viruses and protists. This close association can be beneficial for both partners and forms a complex unit, termed metaorganism or holobiont (1, 2). Disturbance or loss of the natural associated bacterial community can facilitate the invasion of pathogens and lead to reduced host fitness (3–5).

Host genetics and innate immunity play an important role in establishing and maintaining the microbial composition of metaorganisms. For some animal species including *Hydra* it has been shown that the eukaryotic host actively selects and shapes its specific bacterial community (6–9). Therefore, it is not surprising that *Hydra* contains a stable, species-specific bacterial community that consists of five main colonizers: *Curvibacter* sp. (76%), *Duganella* sp (11%), *Undibacterium* sp. (2.1%), *Acidovorax* sp. (0.3%) and *Pelomonas* sp. (0.2%) (5, 10). Interestingly, bacteria-bacteria interactions are an important component contributing to the fitness of the metaorganism *Hydra* (5). The main colonizer *Curvibacter* can protect the animal host from fungal infection, if *Duganella* sp., *Undibacterium* sp. or *Pelomonas* sp. is also present (5). Co-occurrence of these bacteria is thus essential to provide this beneficial antifungal host defense. Li *et al.* (2015) investigated the interaction between the two most abundant bacterial colonizers of *Hydra*, *Curvibacter* sp. and *Duganella* sp., *in vitro* and observed that in mono-culture *Duganella* sp. features higher growth rate than *Curvibacter* sp.. In contrast to the expectation that *Duganella* sp. would outcompete *Curvibacter* sp. in co-culture experiments, a frequency dependent suppression of *Duganella* sp. was detected (11). The observation of a frequency dependent growth rate indicates that the interactions among bacteria in co-culture are beyond a simple case of direct competition. It has been predicted by modelling that this interaction is mediated by a temperate phage integrated in the genome of *Curvibacter* sp. (prophage) (12). The fresh water polyp *Hydra* is not only associated with a host specific bacterial community (10) but also features a host specific viral community of which more than 50% are bacteriophages (13). Due to the fact that phages are often obligate killers to their host cells but also to other bacteria, they have a strong selective effect on bacterial populations and are hypothesized to shape whole bacterial communities (14–17). While lytic phages infect bacteria, get multiplied and kill their bacterial host (18), temperate phages can become lysogenic, where the phage genome is replicated

along with the genome of its host and is transferred vertically (19). By this they can increase the fitness of its bacterial host by altering the bacterial geno- and phenotype (20). During stress for the bacterial host cell, temperate phages can switch from lysogenic to lytic cycle (prophage induction) (21, 22). Free phages are then released to the environment (23, 24). In this manner excised prophages may function as weapons in competition with other (susceptible) bacteria (25, 26). In a previous study I could demonstrate that the prophage of *Curvibacter* sp. is inducible and can lytically infect the second most abundant colonizer of *Hydra Duganella* sp. *in vitro* (12). In the present study I further investigate the function of *Curvibacter* phage (TJ1) in the host context and hypothesized that phage TJ1 interferes with bacteria-bacteria interactions and has the potential to influence the composition of host associated bacterial community. To test this hypothesis, I investigated the presence and induction capacity of the prophage associated with *Curvibacter* sp. and its ability to cross-infect and downregulate other *Hydra*-associated bacteria in culture (*in vitro*) and on *Hydra* (*in vivo*).

My results demonstrate that *Curvibacter* phage (TJ1) can be induced in culture (*in vitro*) and on *Hydra* (*in vivo*). In association with *Hydra*, *Curvibacter* sp. was more susceptible for phage induction at reduced water temperature, suggesting an impact of the host environment on phage bacteria interactions. Finally, I could demonstrate that phage TJ1 has a broad host range against other bacterial colonizers and has the potential to regulate bacterial colonization on *Hydra*.

Results

Curvibacter* sp. (strain AEP1.3) harbours a prophage that can be induced in culture and on *Hydra

Bio-computational analysis of the genome of *Curvibacter* sp. (9) (strain AEP1.3) predicted an intact prophage within the region 444644-484825 bp and an incomplete prophage within the region (547656-566074). To test the induction capacity and to visualize the *Curvibacter* phage TJ1, I first induced the phage with Mitomycin C. Low concentrations of Mitomycin C between 0.05 and 0.1 µg/ml were sufficient to reduce the growth of the bacterial host *Curvibacter* (Fig. 1 A). The decline in growth is an indicator for phage induction and replication. I verified the presence of phage TJ1 by

gradient ultracentrifugation, negative staining with uranyl acetate and visualization via transmission electron microscopy (TEM) (Fig. 1B). Transmission electron micrographs displayed morphological similarity of phage TJ1 to *Myoviridae* featuring an isometric head of 50 nm in diameter and a contractile tail of 80 by 20 nm with small tail fibers (Fig. 1 B).

Sequencing the linear double-stranded DNA of phage TJ1 (Supplementary Fig. S1) revealed that the 37079 bp long phage genome featured 54 proteins including capsid protein, phage tail, tail sheath and tail fiber proteins as well as transposase and lytic murein transglycosylase (Supplementary Table S1). The second prophage seems to be inactive as I did not observe different morphotypes by TEM or detected it in my sequencing data. I compared phage TJ1 to other known phages, using Seed viewer. This analysis, centered on a phage capsid protein, indicated highest similarity to *Burkholderia* phage KS10 with 25 proteins in common (Fig. 1 C).

Similarity searches of phage TJ1 by BLASTP revealed the presence of TJ1 predicted proteins in several other bacterial strains including Betaproteobacteria but also Gammaproteobacteria. Screening the genomes of these bacteria by PHASTER I could reveal that these proteins were located within putative intact prophage regions. Phylogenetic analysis of these prophage sequences and phage TJ1 by VICTOR using the GENOME-BLAST Distance Phylogenetic method (GBDP) revealed *Rhodoferrax fermentans* prophage as closest relative. The presence of phylogenetic similar phages in distantly related bacteria implies a broad host range of phage TJ1, infecting Betaproteobacteria as well as Gammaproteobacteria (Supplementary Fig. S2).

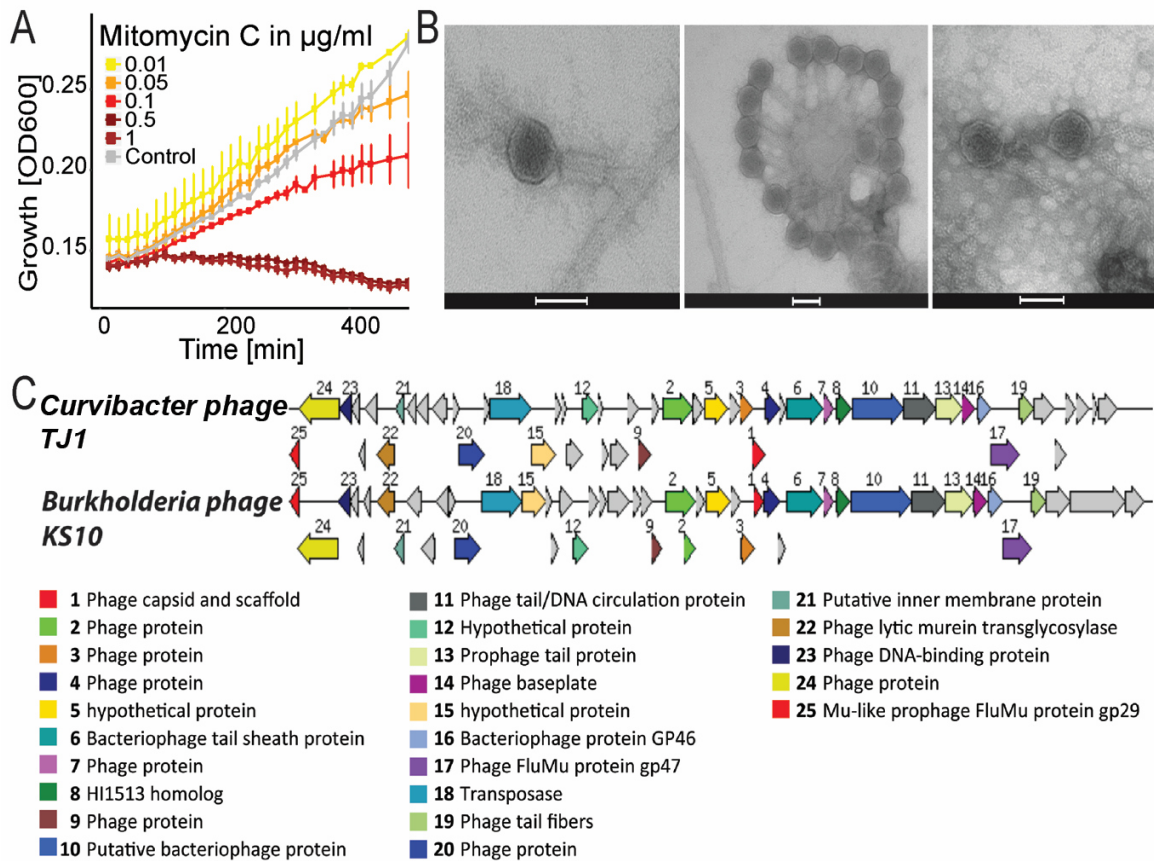


Figure 1. A: Mean growth (OD600) (\pm SE) of *Curvibacter* sp. without treatment (Control, grey) and when exposed to 5 different concentrations of mitomycin C ($n=3$). **B:** Transmission Electron micrographs of *Curvibacter* phage TJ1 negative stained with 2% (w/v) aqueous uranyl acetate. Scale bars represent 50 nm. **C:** Annotation and comparison of phage TJ1 to *Burkholderia* phage KS10 (NCBI: Reference Sequence: NC_011216.1). The graphic is centered on the phage capsid coding region colored in red and numbered with 1. Genes present in both phages are highlighted with the same number and color.

To test if the prophage of *Curvibacter* sp. can be excised from the host genome under more natural conditions I tested the impact of different environmental factors including temperature, pH and nutrition on prophage induction both in culture (*in vitro*) (Fig. 2 A) and in association with *Hydra* (*in vivo*) (Fig. 2 B). I quantified phages and *Curvibacter* via qPCR and calculated phage/*Curvibacter* ratio to exclude the effect of different colonization successes which is highly influenced by the polyp size. When *Curvibacter* sp. was grown in bacterial growth media in the absence of the eukaryotic host, environmental stressors, like altered temperature, higher pH or higher nutrition had no significant effect on prophage induction in comparison to the control. Phage TJ1 could only be induced by Mitomycin C (Wilcoxon-Rank-Sum-test: $W=0$, $p=0.007$) (Fig. 2 C).

Living in association with *Hydra*, phage TJ1 could also be induced by Mitomycin C (Wilcoxon-Rank-Sum-test: $W=0$, $p=0.01193$ (Fig. 2 D). The amount of phage TJ1 produced under Mitomycin C induction (One-Way-ANOVA, $F=10.13$ $p=0.002$) and in total (One-Way-ANOVA, $F=10.13$ $p=0.002$) was significantly higher when its bacterial host *Curvibacter* was living in association with the *Hydra* epithelium compared to the *in vitro* experiment. Interestingly, a temperature reduction from 18°C to 12°C affected the number of induced phages only if the bacterial host *Curvibacter sp.* lived in associated with *Hydra* (Wilcoxon-Rank-Sum-test: $W=25$, $p=0.01193$). Changes in pH-values, elevated temperature or nutrition did not lead to a significant prophage induction, neither *in vitro*, nor *in vivo* (Fig. 2 C, 2 D).

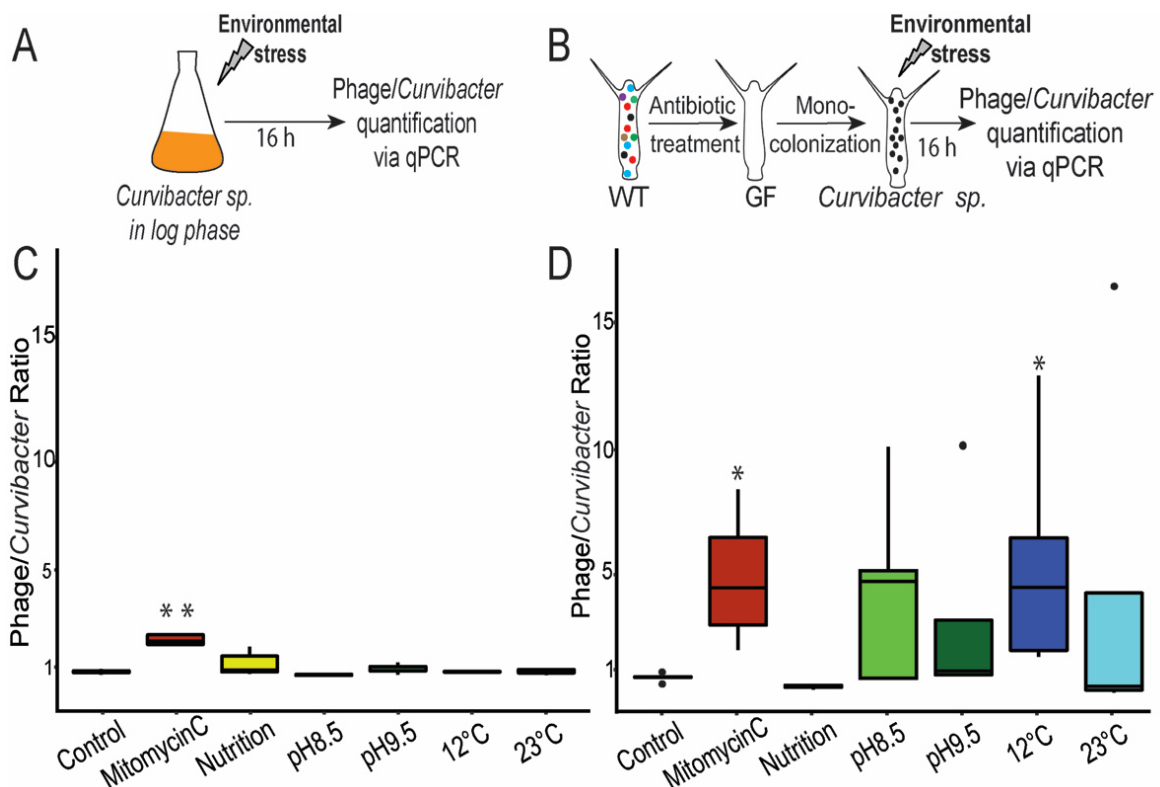


Figure 2: A: *In vitro* experimental set-up. *Curvibacter sp.* were grown at log phase and exposed to several environmental stressors. Prophage induction was quantified via qPCR (n=5). **B:** *In vivo* experimental set-up. WT polyps were treated with antibiotics to generate germfree *Hydra*. These germfree polyps were recolonized in mono-association with *Curvibacter* and exposed to different environmental stressors. Prophage induction was quantified via qPCR (n=5); **C:** Boxplot of phage/*Curvibacter* ratio after exposure to the environmental stressors. Stars (*= $p < 0.05$, **= $p < 0.01$) indicate significant differences between treatment and control (Wilcoxon rank sum test). **D:** Boxplot of phage/*Curvibacter* ratios after the polyps were exposed to the environmental stressors. Stars (*= $p < 0.05$, **= $p < 0.01$) indicate significant differences between treatment and control (Wilcoxon rank sum test).

Curvibacter phage TJ1 can infect a broad range of Hydra-associated bacteria

To test the ability of phage TJ1 to cross-infect other bacteria I spotted purified phages onto a lawn of *Hydra*-associated bacteria and used plaque formation as sign of infection success. For a variety of bacteria belonging to Betaproteobacteria I could observe plaque formation, while no plaque formation was visible for Gammaproteobacteria and *Bacteroidetes* (Supplementary Table S2). Phage TJ1 could not only infect closely related bacterial strains affiliating to the same family as their original bacterial host (*Comamonadaceae*) but seems to be lytic in a broad spectrum of bacteria. This includes *Undibacterium sp.* and *Duganella sp.*,

(*Oxalobacteraceae*), as well as *Vogesella* sp., a less abundant bacterium of *Hydra*, belonging to the order *Neisseriales* (Supplementary Table S2). To examine whether phage TJ1 can cross-infect bacteria not only *in vitro* but also when they are living on *Hydra*, I monocolonized germfree *Hydra* with the most abundant bacterial colonizers (*Curvibacter* sp., *Duganella* sp., *Undibacterium* sp., *Acidovorax* sp. and *Pelomonas* sp. (5)) and subsequently inoculated them with phage TJ1. After 0 h, 24 h and 72 h post phage infection I quantified living bacterial cells by counting colony forming units (CFU/polyp) and estimated phage/bacteria ratio by qPCR (Fig. 3 A).

While the phage did not interfere with the growth of *Curvibacter* sp. and *Acidovorax* sp., I observed a significant reduction of *Duganella* sp. and *Undibacterium* sp. after 72 h post phage exposure (One-way ANOVA, *Duganella*: $F= 4.111$; $p<0.006$, *Undibacterium*: $F=3.973$; $p=0.013$) (Fig. 3 B). Interestingly, I observed a significant reduction of CFUs in polyps that were monocolonized with *Pelomonas* sp. 72 h post phage infection (One-way ANOVA, $F = 22.92$, $p< 0.001$) (Fig. 3 B), although *Pelomonas* sp. could not be infected by phage TJ1 in *in vitro* experiments (Supplementary Table S2). The impact of phage TJ1 on bacterial numbers (Fig. 3 B) was accompanied by an elevated amount of phages (Fig. 3C). The number of phages increased significantly over time in polyps which were recolonized with *Undibacterium* (GLM= 2.7373, $p= 0.0103$), *Duganella* (GLM, $F= 2.7373$, $p=0.0246$) and *Pelomonas* (GLM, $F = 2.7373$, 0-24h: $p= 0.0011$; 0-72h: $p< 0.0024$) (Fig. 3 C). This implies a successful infection and replication of phage TJ1.

There was no significant increase of phages detectable in polyps recolonized with *Curvibacter* sp. or *Acidovorax* sp. (Fig. 3 C).

| Chapter II |

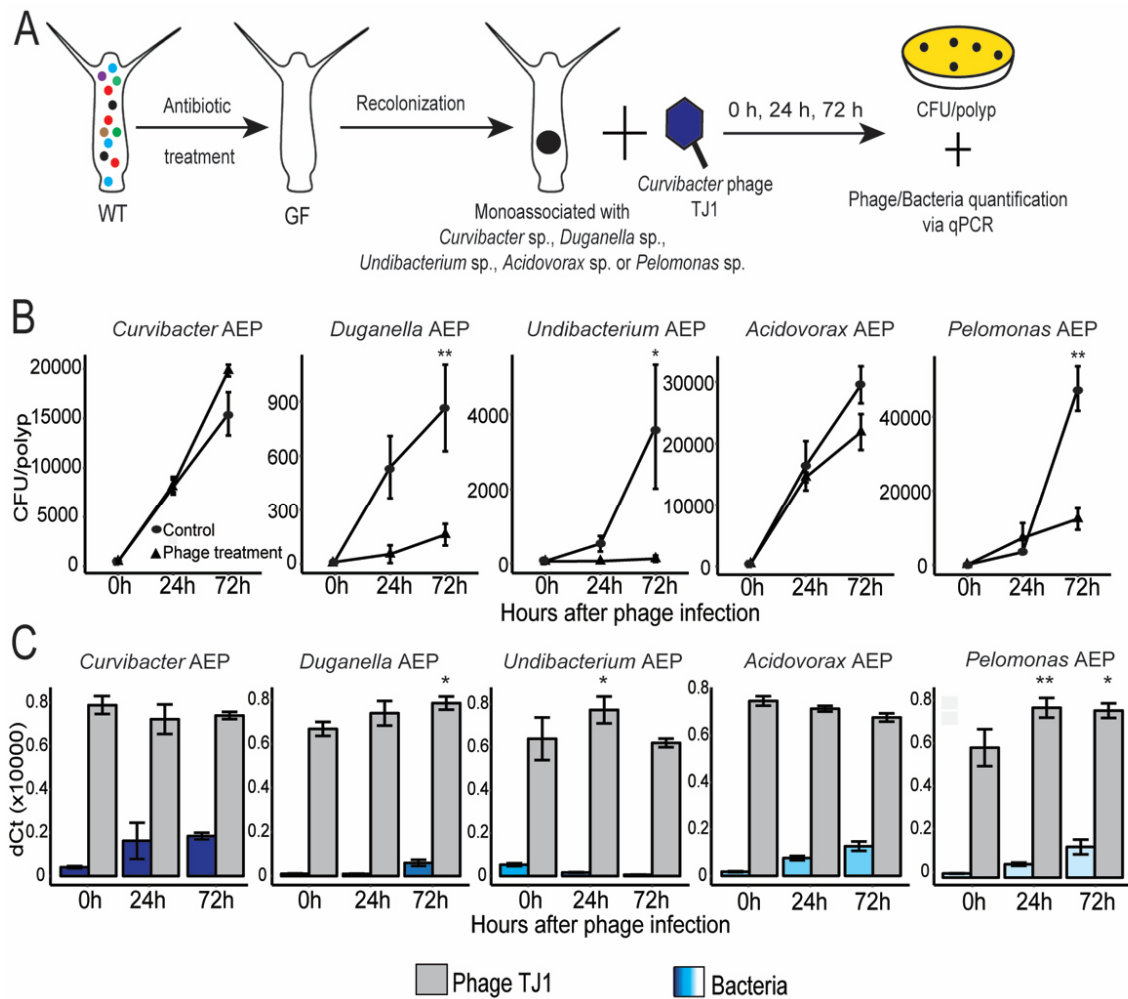


Figure 3: A: Experimental set-up. WT polyps were treated with antibiotics to generate germfree *Hydra*. These germfree polyps were recolonized in mono-association with one of the 5 main colonizer and either treated with phage TJ1 or with SM-Buffer as a control. The bacteria were quantified via plating and counting the CFU/polyp and the phages were quantified via qPCR. **B:** Mean CFU/polyp (± SE) over time of *Curvibacter* sp., *Duganella* sp., *Undibacterium* sp., *Acidovorax* sp. or *Pelomonas* sp. on *Hydra* with phages (▲) or without (●) (n=5). Stars (*=p<0.05, **= p<0.01) indicate significantly difference between phage treatment and control (One-way-ANOVA). **C:** Bar plot of delta CT values of *Curvibacter* (blue) and phage (grey) over time in the phage TJ1 treated polyps monocolonized with *Curvibacter* sp., *Duganella* sp., *Undibacterium* sp., *Acidovorax* sp. or *Pelomonas* sp. (n = 5). Stars (*=p<0.05, **= p<0.01) indicate significantly difference between the time of incubation in comparison to 0 h (GLM).

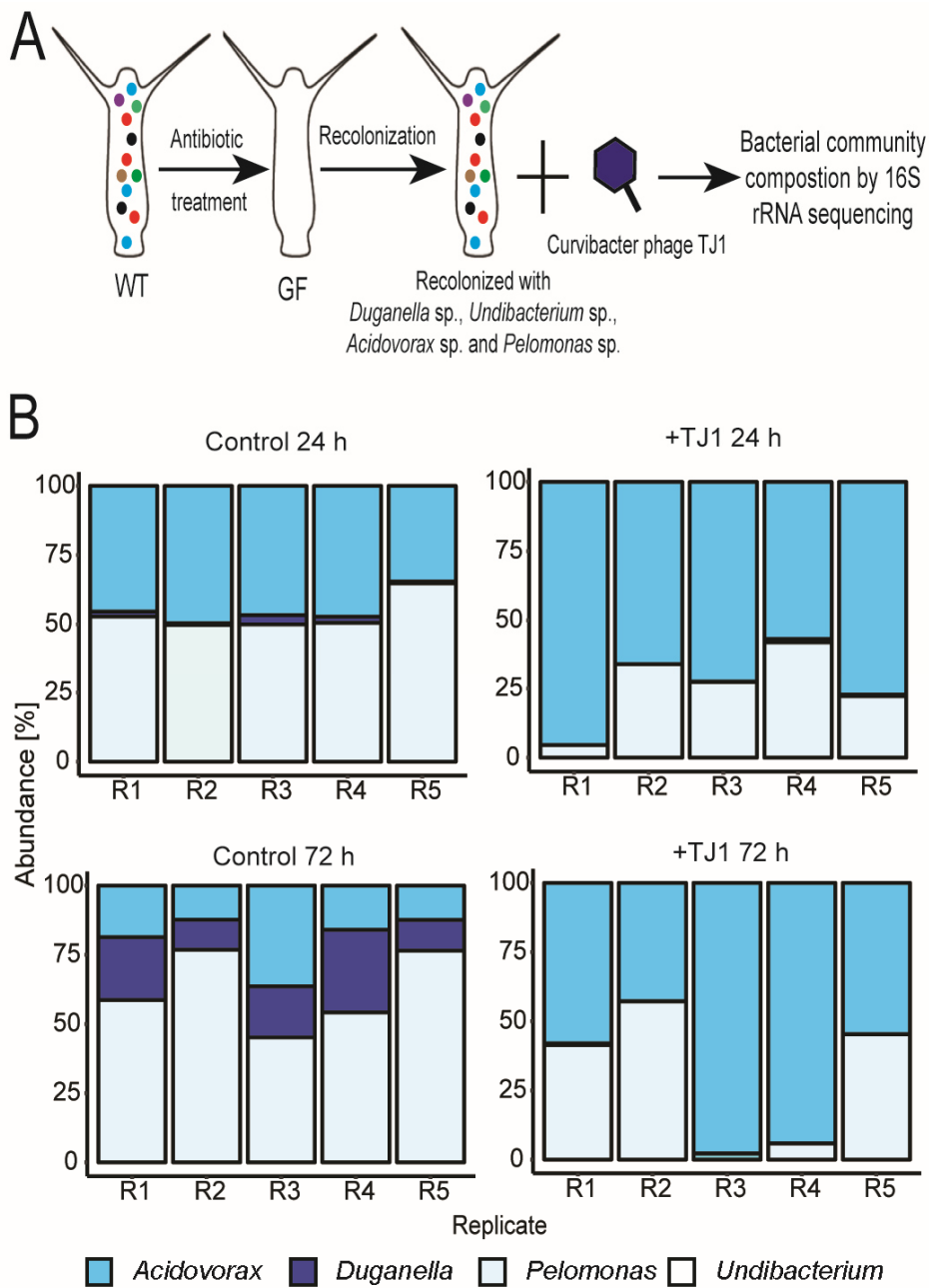


Figure 4: **A:** Experimental set-up; WT polyps were treated with antibiotics to generate germfree *Hydra*. Germfree polyps were recolonized with *Duganella*, *Undibacterium*, *Acidovorax* and *Pelomonas* and in absence of *Curvibacter*. Recolonized polyps were exposed to phage TJ1. After 24 h and 72 h hours bacterial community composition was analyzed by 16S rRNA amplicon sequencing (n=5). Recolonized polyps that were only exposed to *Hydra* medium served as a control group; **B:** Barplots illustrate the relative abundance of *Duganella* sp., *Undibacterium* sp., *Acidovorax* sp. and *Pelomonas* sp. of controls and phage treated polyps after 24 h and 72 h.

Impact of *Curvibacter* phage TJ1 on Microbial community composition on *Hydra*

To validate the impact of phage TJ1 on a bacterial community level, I exposed polyps that were recolonized with *Duganella*, *Undibacterium*, *Acidovorax* and *Pelomonas* to phage TJ1. After 24 h and 72 h I analyzed the bacterial community composition by 16S rRNA amplicon sequencing (Fig 4 A) and observed a reduction in the relative abundance of *Duganella* (GLM, $F= 14.077$, 72 h: $p=0.0233$) and *Pelomonas* (GLM, $F= 14.077$, 24 h: $p= 0.0064$, 72 h: $p<0.001$) in the phage treatments compared to the control (Fig. 4 B). This reduction was accompanied by an increase of the phage-resistant bacterium *Acidovorax* (GLM, $F= 14.077$, 24 h: $p< 0.001$, 72 h: $p<0.001$) (Fig. 4 B). The recolonization of *Undibacterium* was not successful and therefore absent in treatment and control.

***Curvibacter* can use phage TJ1 to shape the *Hydra*-associated microbiota**

Given the above results, phage TJ1 may contribute to the regulation of the microbial composition. However, to exert its regulatory function phage TJ1 must switch from a lysogenic to a lytic life cycle even under normal environmental conditions. To test if the presence of different bacterial strains is sufficient to exert the regulatory function of phage TJ1, I monocolonized *Hydra vulgaris* (strain AEP) with *Curvibacter* sp. and exposed them separately to five bacterial strains commonly found on *Hydra vulgaris* (strain AEP): *Curvibacter* sp. (double recolonization as control), *Duganella* sp., *Undibacterium* sp., *Acidovorax* sp. and *Pelomonas* sp.. After 16 h I quantified phages, *Curvibacter* and all bacteria by qPCR (Fig. 5 A). Intriguingly, I observed a significant higher phage/*Curvibacter* ratio compared to the control treatment, when *Curvibacter* mono-associated polyps were exposed to phage TJ1 sensitive bacteria including *Duganella* (Generalized linear model (GLM), $F= 6.9518$, $p=0.03723$), *Pelomonas* (GLM, $F = 6.9518$, $p = 0.02432$) and *Undibacterium* (GLM, $F = 6.9518$, $p = 0.00299$) (Fig. 5 B).

The addition of bacterial strains that were resistant to phage infection, such as *Curvibacter* sp. and *Acidovorax* sp., did not lead to significant changes in the ratio between phage and *Curvibacter* sp. (Fig. 5 B). An elevated prophage induction in *Curvibacter* sp. or cross-infectivity of these bacterial strains therefore can be excluded.

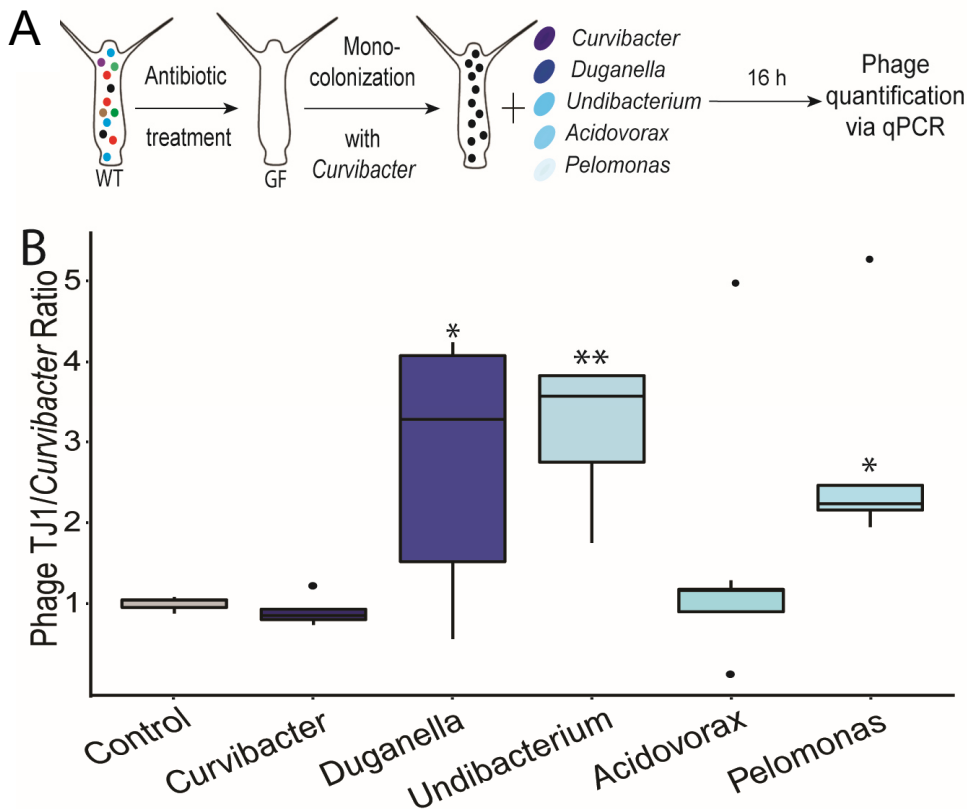


Figure 5: A: Experimental set-up: WT polyps were treated with antibiotics to generate germfree *Hydra*. These germfree polyps were recolonized in mono-association with *Curvibacter* and exposed to one of *Hydras* main colonizer (*Curvibacter*, *Duganella*, *Undibacterium*, *Acidovorax* or *Pelomonas*) or sterile *Hydra* culture medium as control. Phages were quantified via qPCR (n=5); **B:** Boxplot of phage/*Curvibacter* ratio after the polyps were exposed to the bacteria. Stars (*= $p < 0.05$, **= $p < 0.01$) indicate significant differences between treatment and control (Generalized linear model).

Discussion

This study elucidates the potential regulatory role of phage TJ1, a prophage of the most abundant bacterial colonizer of *Hydra*, *Curvibacter* sp. This prophage could be induced *in vitro* and *in vivo* and therewith has the potential to directly affect and shape the *Hydra*-associated microbiota. Observed differences in both, prophage excision from the host genome and infectivity of phage TJ1 between experiments in culture and on *Hydra* imply that the habitat of the prokaryotic host and/or bacterial frequency dependent factors influence phage-bacteria interactions.

The observation that neither changes in pH-values, elevated temperature nor nutrition lead to a significant prophage induction *in vitro* and *in vivo* suggests that lysogenesis

is maintained, and phages are not induced under a natural range of variable environmental conditions. As phage TJ1 was not detectable in the virome data set of *Hydra* (13), ambient or elevated water temperature also seems to have no effect on prophage induction on polyps, that are associated with a complex, natural bacterial community. However, the observation that the prophage can be induced *in vivo* by lowering the water temperature is interesting since temperature serves as an important environmental cue in *Hydra* and can affect many developmental processes including body size and budding (27–29), as well as onset of sexual reproduction (30). It is commonly known that prophage excision is expected to be strongest when bacteria are exponentially growing (31), due to the high expression of genes involved in the SOS response of the bacterium (32). Comparing growth rates *in vivo* and *in vitro* showed that *Curvibacter* grow equally well on *Hydra* compared to liquid media (33). This suggests that the symbiotic bacterium *Curvibacter* is well adapted to the epithelial environment of its host. Nevertheless, those differences in the induction capacity add support to the view that the host environment has a strong influence on phage-bacteria interactions (34). Important factors in the epithelial-derived mucus layer, including nutrients as well as components of the innate immune system (9, 35) may contribute to this finding. However, there are also other factors that might play a role in the differential induction capacity. A recent mathematical model predicts that at low frequencies of *Curvibacter* phage TJ1 is produced via the lytic pathway, while at high densities the lysogenic cycle is favored (12). Therefore, dilution or density dependent issues may lead to a lower induction rate *in vitro* compared to *in vivo* conditions.

In contradiction to non-host associated environments, such as marine sediments (36) or plankton communities (16), which suggest a phage-bacteria ratio of 10:1, I observed a relatively low phage-bacteria ratio of 2:1. Interestingly, similar low phage-bacteria ratios have been reported for the human gut (37), suggesting beside genomics (38) and phylogenetics (38, 39), phage induction and abundance may also be affected by bacteria lifestyle.

Intriguingly, phage TJ1 features a broad host range, by infecting bacteria, belonging to different bacterial families than its original host. Similar broad host ranges (polyvalency) have been described for other phages that belong to the *Myoviridae* (40) and other freshwater phages (41). Polyvalency has been suggested to be linked to poor-nutrient conditions as an adaptation to low host cell numbers in aquatic

environments (42) and is also wide spread in bacteriophages that infect *Burkholderiales* (43, 44). In this context the *Burkholderia cepacia complex* (BCC) group of bacteria is intensively studied. This group of bacteria includes beneficial and pathogenic bacteria of plants but also human opportunistic pathogens, such as *Burkholderia cenocepacia* causing pulmonary infection in the background of cystic fibrosis and chronic granulomatous disease (45). Several phages have been characterized for their ability to control *B. cenocepacia* among those *Burkholderia* phage KS10 (46), which shared 25 of 54 proteins with phage TJ1. The presence of phylogenetic similar prophages in distantly related Betaproteobacteria as well as Gammaproteobacteria (Supplementary Fig. S2) supports my findings of a broad host range.

The observation that phage TJ1 can infect and kill other bacterial colonizer of *Hydra vulgaris* AEP as well as *Curvibacter* strains from different *Hydra* species, supports the hypothesis that prophage TJ1 may function as a weapon against other bacteria securing its bacterial host *Curvibacter* the dominant position on *Hydra*. Moreover, my data provide direct evidence that the presence of already one temperate phage within a bacterial population can elicit tremendous shifts in the community composition.

Thus, already the addition of phage sensitive bacteria to the surrounding of *Hydra* leads to phage replication clearly shows that phage TJ1 is active on *Hydra* without any additional artificial stimulation of prophage induction. It can be speculated that phages are already present at low abundances and therewith able to cross-infect new colonizing bacteria or that small signal molecules released by bacteria stimulate prophage induction (47). Another explanation could be that the exposure of *Hydra* to new bacterial colonizers elicit an immune response of the eukaryotic host (8), which in turn stimulate prophage induction in *Curvibacter*.

Considering that all host associated bacterial communities also feature a diverse phage community e.g. the human gut (48), complex phage regulated processes of the bacterial community can be expected. My results demonstrate that environmental factors and bacterial frequency can affect lysogenic or lytic decision of phages which may impair experimental outcomes. Moreover, cross-infection can interfere with bacterial colonization and can affect the bacterial community composition. Therewith, phages should be taken into account when studying bacteria-bacteria as well as bacteria-host interactions.

Material and Methods

Identification and induction of *Curvibacter* prophage

First, I screened the bacterial genome of *Curvibacter* sp. (strain AEP1.3; GenBank:CP015698.1) for the presence of prophage signatures using the online software PHASTER (Phage Search Tool Enhanced Release) (49). Second, I used Mitomycin C assay to test the induction capacity of the phage. Therefore, *Curvibacter* sp. was grown in liquid culture containing 3% (w/v) R2A broth media (Sigma-Aldrich) under shaking conditions at 250 rpm at 18°C. Exponentially growing bacterial cultures (OD₆₀₀= 0.2) were inoculated with 0.05 µg/ml Mitomycin C to induce phage replication (50) and incubated at 18°C for 16 h. During incubation bacterial growth was quantified by measuring optical density at 600 nm in a plate reader (Tecan Spark). Afterwards bacterial cells were removed by two consecutive low-speed-centrifugation steps at 4,266 x g in a ThermoScientific Heraeus Multifuge 3SR at 4°C for 30 min. The supernatant was filtered 0.2 µm and phage particles were pelleted by ultracentrifugation at 25,000 rpm (72,700 x g) in a Beckman 45 Ti rotor at 4°C for 2h. The pellet was re-suspended in 3 ml SM-Buffer (50mM Tris; 100 mM NaCl; 8 mM MgSO₄; pH 7.5). Re-suspended phages were layered onto a pre-formed Cesium chloride gradient consisting of different densities (2ml: 1.7; 1.5; 1.3; 1.2 g/cm³ and 1 ml 1.1 g/cm³) and centrifuged at 28.000 rpm (135,000 x g) in a Beckman SW 41 rotor at 4°C for 2 h. The band containing phages was removed by syringe, diluted 1:3 in SM-Buffer and pelleted by centrifugation at 22 000 rpm (83,000 x g) in a Beckman SW 41 rotor at 4°C for 2 h. Phages were re-suspended in 200 µl SM-Buffer and stored at -80°C until DNA extraction. Sub-samples of phages (5 µl) were further characterized morphologically by negative staining in 2% (w/v) aqueous uranyl acetate and visualized by transmission electron microscopy (TEM) using a Technai Bio TWIN at 80 kV and a magnification of 40 000–100 000.

To exclude possible Mitomycin C effects on plaque formation, I also used UV radiation to induce phage TJ1 according to the protocol described by Aucouturier *et al.* (2018) (51) with minor changes. Briefly, I exposed 50 ml of *Curvibacter* culture in exponential growth phase (OD=0.2) to 60 J/m² for 8 sec. After 16 h, 10% (v/v) Chloroform was added and mixed by vortexing. The phages were purified as described above and used for Plaque-assays (see below).

Phage DNA extraction and Sequencing

Phage DNA was extracted from two-hundred microliters of purified phages according to the protocol developed by Thurber and colleagues (52) with minor modifications. In brief, 22 µl 2M Tris-HCL (pH 8.5)/0.2 M EDTA, 10 µl 0.5 M EDTA and 268 µl formamid were added to the samples and incubated for 30 min at room temperature. DNA was precipitated by adding two volumes of ethanol and incubation at -20°C overnight. DNA was pelleted by centrifugation at 13,000 x g at 4°C for 20 min. The pellet was washed with 70% (v/v) ethanol. 100 µl SDS extraction buffer (1% SDS; 100 mM Tris; 20 mM EDTA; pH 7.5), 1% 2-mercaptoethanol and proteinase K (0.5 mg/ml) were added to the pellet and incubated for 30 min at 36°C and 15 min at 56°C. Twice the amount of DNA-extraction buffer (100 mM Tris, pH 8.0; 1.4 M NaCl; 20 mM EDTA; 1% 2-mercaptoethanol; 2% (w:v) CTAB) were added followed by an incubation step at 65°C for 10 min. An equal volume of chloroform isoamyl alcohol (24:1) was added to the warm solution, mixed thoroughly and centrifuged at 13,000 × g at room temperature for 5 min. Supernatant was transferred into a new tube and DNA was precipitated by the addition of 0.7 volume of isopropanol. After an incubation at -20°C for 2 h DNA was pelleted by centrifugation at 13,000 x g at 4°C for 20 min. DNA was washed with 70% (v/v) ethanol and dissolved in 50 µl water. Nextera XT kit (Illumina) was used for library preparation and 2 x 150 bp paired-end sequencing was conducted on a MiSeq platform (Illumina) at Centre for molecular biology in Kiel. Trimmomatic V.0.36 (53) was used for sequence adaptor removal and read trimming. Trimmed and quality controlled reads were finally assembled using SPAdes V.3.1.11 (54). The assembled *Curvibacter* phage TJ1 genome is publically available under the GenBank accession number MH766655 in the NCBI database.

Phage annotation and comparison

Phage TJ1 was annotated using the genome annotation service RAST (Rapid Annotation using Subsystem Technology). The phage capsid protein was compared with different phages by Psi-Blast. The most closely related was *Burkholderia* phage KS10 (NCBI: Reference Sequence: NC_011216.1) by SEED Viewer (55). Gene organization and sequence similarity between *Curvibacter* phage TJ1 and *Burkholderia* phage KS10 was graphically illustrated by SEED Viewer centered on the phage capsid protein (55).

In a next step I controlled RAST predicted ORFs by GeneMark (56) and conducted similarity searches by BLASTP (57). Based on BLASTP results I downloaded genomes from those bacteria which showed the highest similarity and coverage to my phage TJ1 from the National Centre for Biotechnology Information (NCBI). Afterwards, I screened the selected bacterial genomes for the presence of prophages by PHASTER (Phage Search Tool Enhanced Release) (49). Prophage sequences were extracted and submitted together with the sequence of *Burkholderia* phage KS10 to VICTOR (58) for phylogenetic analysis using the GENOME-BLAST Distance Phylogeny method (GBDP) (58).

Phage quantification

TJ1 phages were quantified during *in vitro* and *in vivo* induction and cross infection experiments by qPCR (24, 59). In order to take into account that phage sequences derived from either induced phages or from bacterial cells carrying integrated prophages in their genome I quantified both phage TJ1 and bacteria via qPCR. I calculated the ratio between phage and bacteria to exclude the effect of different polyp size and the resulting difference in bacterial recolonization and growth success. For the quantification of phage TJ1 a specific set of primers targeting the phage tail gene (F: 5'-GCTTTGACCTGTCGTTTCATCC-3' and R: 5'-CGGGTTTGTGGATAGGTCGT-3') was designed. For bacteria quantification I used either primer specific for the *recA* gene of *Curvibacter* sp. (strain AEP1.3) (F: 5'-TTCGGCAAGGGCACCATC-3' and R: 5'-ACGACTCCGGGCCATAGA-3') or Eubacterium Primer (F: 5'-CCTACGGGAGGCAGCAG-3' and R: 5'-ATTACCGCGGCTGCTGGC-3') for quantification of the other bacterial colonizer (cross-infection experiment *in vivo*). QPCR reactions were performed in a 25 µl volume with 12.5 µl GoTaq qPCR MasterMix (Promega), 10 pmol/µl forward and reverse primers and 10 ng/µl template DNA. Cycler (Applied Biosystems 7300 Real Time PCR System) conditions were as follows: 95 °C for 2 min, 40 cycles of 95 °C for 15 s, 59 °C for 30 s, 60 °C for 35 s, followed by a dissociation step. At the end of each cycle at 60 °C the fluorescence was measured.

Purified TJ1 phages that were used in infection assays (*in vitro* and *in vivo* cross infection experiments) (see below) were quantified by the double agar layer method (60). Exponentially growing *Curvibacter* strain Hvul was mixed into 5 ml preheated top-

agar (R2A medium, 5 mM CaCl₂, 5 mM MgCl₂, 0.4% Agarose) and poured onto R2A agar plates (R2A medium, 1.5% Agar). Plates were dried for at least 30 min and 10 µl of a dilution series of purified phages were spotted onto the bacterial lawn. Plaque formation was checked after incubation at 18°C for 24-48 h and single spots were counted (Supplementary Fig. S3).

Environmental stress induction of phage TJ1 *in vitro*

Curvibacter sp. log phase cultures were grown in R2A media until they reached an optical density (OD₆₀₀) of 0.26. Subsequently 6 ml bacterial cultures were exposed separately in replicates (n = 5) to one of the following conditions, while the rest of the environmental conditions remain normal: altered temperature (23°C, 12°C), elevated pH (9.5, 8.5), higher nutrition (4 x R2A medium (12 g/l)) or normal conditions (18°C, pH 7) serving as control. After an incubation time of 16 h, DNA was extracted using the DNA blood and tissue kit (Qiagen) followed by the quantification of phage and *Curvibacter* sp. by qPCR (see above).

Recolonization of *Hydra*

Experiments were carried out using *Hydra vulgaris* (AEP) (61). Prior to recolonization animals were cultured under constant laboratory conditions including *Hydra* culture medium (0.28 mM CaCl₂, 0.33 mM MgSO₄, 0.5 mM NaHCO₃ and 0.08 mM KCO₃), food (first instar larvae of *Artemia salina*, fed four times per week) and constant water temperature at 18°C, according to standard procedures (62). Germfree *Hydra* were generated by exposing animals to an antibiotic cocktail containing 50 µg/ml of Ampicillin, Rifampicin, Streptomycin, Spectinomycin and Neomycin as previously described (8). Antibiotic solutions were exchanged every second day. After 2 weeks of antibiotic treatment animals were transferred to antibiotic free sterile *Hydra* culture medium for 3 days. Sterility was confirmed by plating homogenized, germfree polyps on R2A plates and by negative PCR, using eubacterial primers (F: 5'-CCTACGGGAGGCAGCAG-3' and R: 5'-ATTACCGCGGCTGCTGGC-3') (10). 5000 CFU/ml of the corresponding bacterial strain was added to the surrounding water (50 ml) of germfree polyps and incubated for one day. Afterwards polyps were transferred into new container with fresh, sterile *Hydra* medium, in order to remove unattached bacteria in the surrounding medium and used for subsequent experiments.

Environmental stress induction of phage TJ1 *in vivo*

Curvibacter sp. monocolonized *Hydra* polyps (see above) were transferred to 1.5 ml tubes with 200 μ l *Hydra* culture medium. After 3 days of settlement recolonized polyps were exposed separately and in replicates (n=5) to one of the following environmental conditions: altered temperature (23°C, 12°C), higher pH (9.5, 8.5) and elevated nutrition (10% R2A diluted in sterile *Hydra* culture medium) or normal conditions (18°C, pH 7) serving as control. After 16 h incubation time DNA was extracted from the polyp including surrounding water. Phages and *Curvibacter* were quantified by qPCR.

Cross-infection and host range assay *in vitro*

Cross-infectivity of phage TJ1 was tested by spot assays in double agar layer (60) against the bacterial culture collection of *Hydra* consisting of diverse bacterial strains isolated from different *Hydra* species (see Supplementary Table S2). Exponentially growing bacterial strains were mixed into 5 ml preheated top-agar (R2A medium, 5 mM CaCl₂, 5 mM MgCl₂, 0.4% agarose) and poured onto R2A agar plates (R2A medium, 1.5% agar). Plates were dried for at least 30 min and 10 μ l of purified phages and 10 μ l sterile *Hydra* culture medium as control were spotted onto each bacterial lawn (n = 3). Plaque formation was checked after incubation at 18°C for 12-24 h.

Cross-infection assay *in vivo*

This experiment was conducted with a subset of bacterial strains specific for the colonization of *Hydra vulgaris* (AEP). Germfree *Hydra vulgaris* (AEP) polyps were monocolonized with either *Curvibacter* sp., *Duganella* sp., *Undibacterium* sp., *Acidovorax* sp. or *Pelomonas* sp. (see above) in log-phase. After 3 days of settlement, the recolonized polyps were transferred separately to 1.5 ml tubes, containing 200 μ l sterile *Hydra* culture medium. Phage TJ1 was purified from 100 ml culture and resuspended in sterile *Hydra* culture medium and quantified using the double agar layer method (60). In total, 1×10^4 PFU/ml were then added to 5 monocolonized polyps. Five monocolonized polyps that received an equal volume of pure sterile *Hydra* culture medium served as treatment control. After 0 h, 24 h and 72 h post infection the amount of colonizing bacteria and the amount of phages were quantified. For bacterial quantification polyps were homogenized separately, diluted and plated out onto R2A agar plates (n = 5) and incubated at 18°C. CFU per polyp were counted after 2-4 days of incubation. For the quantification of phages that were released to the surrounding

water, DNA was extracted from the polyps and their surrounding *Hydra* culture medium, using the DNA Blood and Tissue Kit (Qiagen). Phages and bacteria were quantified by qPCR (see above).

Impact of phage TJ1 on microbial community

Germfree *Hydra vulgaris* (AEP) polyps were recolonized with *Duganella* sp., *Undibacterium* sp., *Acidovorax* sp. and *Pelomonas* sp. (see above). After three days of settlement I added phage TJ1 at a concentration of 1×10^4 PFU/ml to the recolonized polyps and sterile S-Medium to polyps that served as control (n=5). After 24 h and 72 h I extracted DNA as described above. Bacterial community composition was analyzed by amplicon sequencing of the variable region V1-V2 of the 16S rRNA gene using the forward primer 27F (5'-AATGATACGGCGACCACCGAGATCTACAC XXXXXXXX TATGGTAATTGT AGAGTTTGATCCTGGCTCAG-3') and reverse primer 338R (5'-CAAGCAGAAGACGGCATAACGAGAT XXXXXXXX AGTCAGTCAGCC TGCTGCCTCCCGTAGGAGT-3') containing the Illumina adaptor p5 (forward) and p7 (reverse) and unique MIDs (designated as XXXXXXXX). PCR reactions were performed in duplicates using Phusion Hot Start DNA Polymerase (Finnzymes, Espoo, Finland). PCR cycling conditions were: 98°C for 30 s, 30 × [98°C – 9 s, 55°C – 30 s, 72°C – 90 s], 72°C – 10 min. PCR products were combined and purified by MinELute Gel Extraction Kit (Qiagen) after agarose gel electrophoresis. Sequencing was performed on the Illumina MiSeq platform at the sequencing facility of the Kiel Institute for Clinical Molecular Biology (IKMB). Sequence data were analyzed using the MOTHUR packages(63) according to the MiSeq SOP (64). In Brief, MiSeq paired-end reads were assembled and quality controlled finally resulting in 9837 sequences per sample. Sequences were grouped into operational taxonomic units (OTU) using a 97 % similarity threshold. Sequences were aligned to SILVA 128 Database and taxonomically classified by RDP classifier. Sequence data are deposited at Sequence Read Archive (SRA-ID: PRJNA530779).

Phage induction by different bacterial colonizers *in vivo*

In order to test if phage TJ1 is already active or can be induced only in the presence of different bacterial colonizers, *Hydra* polyps were monocolonized with exponentially growing *Curvibacter* and after three days of settlement they were transferred to 1.5 ml tubes. The recolonized polyps were subsequently exposed separately and in

replicates (n = 5) to one of the five main bacterial colonizers: *Curvibacter* sp., *Duganella* sp., *Undibacterium* sp., *Pelomonas* sp. and *Acidovorax* sp. Concentration of the inoculum was 5000 CFU/ml. After 16 h incubation time DNA of the polyp and its surrounding water was extracted. Bacteria and phages were finally quantified via qPCR (see above).

Statistical data analysis

All statistical analyses were conducted using the statistic program R® (version 3.2.3 (2015-12-10) "Wooden Christmas-Tree" (Copyright (C) 2015 The R Foundation for Statistical). The Levene's test was used to check for homogeneity of variance. Some data did not fulfill the criteria of normality. Therefore, the effect of environmental stressors on phage induction was analyzed by using a Wilcoxon-Rank-Test. The amount of phages over time and the effect of other colonizers on prophage induction were analyzed by using a generalized linear model (GLM), with a following Tukey HSD post-hoc test for multiple comparisons or pairwise comparisons. The statistical analysis of the cross-infection *in vivo* was conducted by ANOVA, with a following Tukey HSD post-hoc test for multiple comparisons.

Supplementary

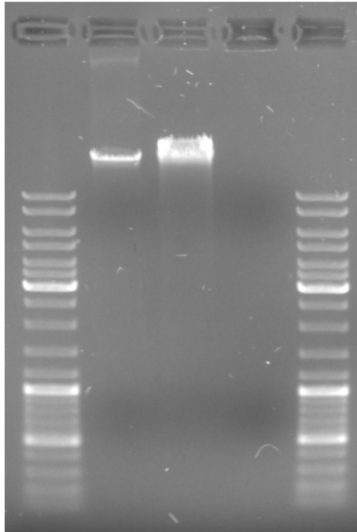


Figure S1: 0.8% Agarose gel of phage TJ1 DNase digestion (From left to right: 1kb DNA ladder; phage TJ1 DNA; S1 nuclease digestion of phage TJ1 DNA; Turbo DNase digestion of phage TJ1 DNA; 1kb DNAladder)

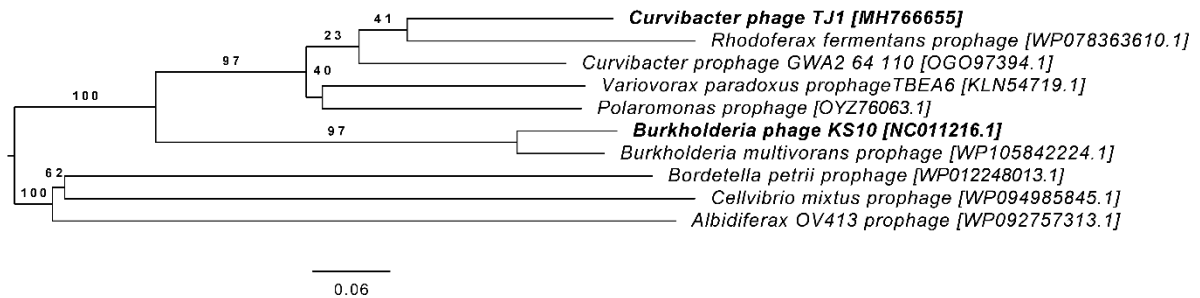


Figure S2: Phylogenetic analysis of *Curvibacter phage TJ1*, *Burkholderia phage KS10* and other prophages. Prophage sequences were identified by PHASTER (Phage Search Tool Enhanced Release) in bacterial genomes featuring proteins highly similar to *Curvibacter phage TJ1*. Phylogenetic tree was calculated by VICTOR using the GENOME-BLAST Distance Phylogeny method (GBDP). Branch length are scaled in terms of the GBDP distance formula d_0 . Average support values are presented in percent.

| Chapter II |

Table S1: ORFs located within phage TJ1 of *Curvibacter* sp.

ORF	Strand	Coding region (bp)	Size (aa)	Possible function	hmm name	blastp results	GenBank accession no.
1	-	365-1594	409	Putative capsid assembly protein F	Phage_Mu_F	<i>Curvibacter</i> sp. GWA2 64 110	OGO97394.1
2	-	1587-1910	107	Uncharacterized protein	DUF4406	<i>Curvibacter</i> sp. GWA2 64 110	OGO97393.1
3	-	1907-2059	50	Uncharacterized protein		<i>Vibrio phage vB VpS PG07</i>	AXQ66778.1
4	-	2066-2347	93	Uncharacterized protein		<i>Herbaspirillum</i> sp. K1R23-30	WP_119769014.1
5	-	2360-3919	519	Portal protein	DUF935	<i>Curvibacter</i> sp. GWA2 64 110	OGO97391.1
6	-	3916-5508	530	Uncharacterized protein		<i>Curvibacter</i> sp. GWA2 64 110	OGO97390.1
7	-	5508-6017	169	Uncharacterized protein	DUF1804; HTH_23; UPF0147	<i>Curvibacter</i> sp. GWA2 64 110	OGO97389.1
8	-	6021-6326	101	Uncharacterized protein		<i>Rhodoferax fermentans</i>	WP_078363610.1
9	-	6323-6535	70	RNA polymerase-binding transcription factor DksA	zf-dskA_traR; HrpB7; ZF-HD_dimer	<i>Curvibacter</i> sp. GWA2 64 110	OGO97387.1
10	-	6538-7056	172	Uncharacterized protein		<i>Pandoraea apista</i>	WP_052765636.1
11	-	7053-7775	240	Membrane-bound lytic murein transglycosylase F	SLT	<i>Curvibacter</i> sp. GWA2 64 110	OGO97385.1
12	-	7777-8121	114	Uncharacterized protein	Holin_2-3; Psbj	<i>Polaromonas</i> sp.	OYZ76063.1
13	-	8220-8642	140	Uncharacterized endonuclease HI_1296	SNase	<i>Hydrogenophaga</i> sp. H7	OPF63875.1
14	-	8643-9014	123	Uncharacterized protein		<i>Idiomarina zobellii</i>	WP_053953164.1
15	-	9259-9888	209	Putative HTH-type transcriptional regulator	Peptidase_S24; HTH_31	<i>Curvibacter</i> sp. GWA2 64 110	OGO97381.1
16	+	10128-10331	67	Uncharacterized protein		<i>Curvibacter</i> sp. GWA2 64 110	OGO97407.1
17	+	10355-11383	342	Uncharacterized protein	Tektin	<i>Curvibacter</i> sp. GWA2 64 110	OGO97380.1
18	+	11380-11580	66	Uncharacterized protein	rve	<i>Lasius niger</i>	KMQ84808.1
19	+	11617-13272	551	Uncharacterized protein	rve; Mu-transpos_C	<i>Curvibacter</i> sp. GWA2 64 110	OGO97379.1

| Chapter II |













20	+	13308-14264	318	Mu-like prophage FluMu DNA transposition protein B	AAA_22; TniB; AAA_30	<i>Curvibacter sp. GWA2 64 110</i>	OGO97378.1
21	+	14264-14485	73	Uncharacterized protein		<i>Rhodofera fermentans</i>	WP_078363622.1
22	+	14524-14697	57	Uncharacterized protein		<i>Curvibacter sp. GWA2 64 110</i>	OGO97376.1
23	+	14694-15362	222	Uncharacterized protein		<i>Curvibacter sp. GWA2 64 110</i>	OGO97375.1
24	+	15520-15975	151	Uncharacterized protein	DUF3164	<i>Curvibacter sp. GWA2 64 110</i>	OGO97374.1
25	+	16002-16178	58	Uncharacterized protein		<i>Cellvibrio mixtus</i>	WP_094985845.1
26	+	16175-16354	59	Uncharacterized protein		<i>Sinomonas humi</i>	WP_081998439.1
27	+	16475-17176	233	Uncharacterized protein	DUF2786; Histone_HN S	<i>Curvibacter sp. GWA2 64 110</i>	OGO97372.1
28	+	17173-17625	150	Uncharacterized protein	DUF1018	<i>Variovorax paradoxus</i>	KLN54719.1
29	+	17622-18104	160	Uncharacterized protein	Mor; HTH_31	<i>Rhodofera fermentans</i>	WP_078363629.1
30	+	18157-18462	101	Uncharacterized protein	CENP-B_N; HTH_23; Sigma70_r4; LacI; HTH_3	<i>unknown</i>	
31	+	18620-19795	391	Uncharacterized protein	Mu-like_Pro; H-kinase_dim	<i>Curvibacter sp. GWA2 64 110</i>	OGO97369.1
32	+	19832-20215	127	Uncharacterized protein	DUF2190	<i>Rhodofera fermentans</i>	WP_078363633.1
33	+	20276-21232	318	Major capsid protein	Phage_cap_E	<i>Rhodofera fermentans</i>	WP_078363634.1
34	+	21293-21742	149	Uncharacterized protein		<i>unknown</i>	
35	+	21749-22264	171	Uncharacterized protein	DUF1320	<i>Curvibacter sp. GWA2 64 110</i>	OGO97365.1
36	+	22264-22710	148	Uncharacterized protein	Phage_tail_S	<i>Variovorax paradoxus</i>	WP_047785772.1
37	+	22707-23309	200	Uncharacterized protein	DUF1834	<i>Curvibacter sp. GWA2 64 110</i>	OGO97363.1
38	+	23320-23544	74	Uncharacterized protein		<i>Curvibacter sp. GWA2 64 110</i>	OGO97362.1
39	+	23603-25078	491	Mu-like prophage FluMu tail sheath protein		<i>Curvibacter sp. GWA2 64 110</i>	OGO97361.1
40	+	25113-25484	123	Uncharacterized protein		<i>Curvibacter sp. GWA2 64 110</i>	OGO97360.1
41	+	25586-26209	207	Uncharacterized protein	Phage_TAC_7	<i>Curvibacter sp. GWA2 64 110</i>	OGO97406.1

| Chapter II |

42	+	26250-28307	685	Probable tape measure protein	PhageMin_T ail	<i>Rhodoferax fermentans</i>	WP_078363.642.1
43	+	28307-29623	438	Mu-like prophage FluMu DNA circularization protein	DNA_circ_N	<i>Curvibacter sp. GWA2 64 110</i>	OGO97358.1
44	+	29623-30738	371	Baseplate hub protein gp44		<i>Rhodoferax fermentans</i>	WP_078363.644.1
45	+	30738-31226	162	Mu-like prophage FluMu protein gp45	Phage_Mu_Gp45	<i>Rhodoferax fermentans</i>	WP_078363.645.1
46	+	31284-31874	196	Baseplate protein gp46	GP46	<i>Rhodoferax fermentans</i>	WP_078363.646.1
47	+	31871-33007	378	Baseplate protein gp47	Baseplate_J	<i>Rhodoferax fermentans</i>	WP_078363.647.1
48	+	33013-33606	197	Baseplate protein gp48	DUF2313	<i>Curvibacter sp. GWA2 64 110</i>	OGO97353.1
49	+	33615-34427	270	Putative Uncharacterized protein YfdL		<i>Burkholderia multivorans</i>	WP_105842.224.1
50	+	34437-34907	156	Probable tail fiber assembly protein		<i>Bordetella petrii</i>	WP_012248.013.1
51	+	34994-35284	96	Uncharacterized protein		<i>Rhodoferax fermentans</i>	WP_078363.651
52	+	35310-35831	173	Uncharacterized protein	Phage_gp49_66	<i>Comamonas testosteroni</i>	WP_034372.990.1
53	+	35992-36147	51	Com family DNA-binding transcriptional regulator	Mu-like_Com	<i>Albiferax sp.</i>	WP_092757.313.1
54	+	36188-36934	248	Modification methylase DpnIIA	Methyltransf D12	<i>Curvibacter sp. GWA2 64 110</i>	OYY32060.1

| Chapter II |

Table S2: Host range of *Curvibacter* phage TJ1 on *Hydra*-associated bacteria. ✓ indicates a plaque formation and an infection of the bacterium with phage TJ1, while ✗ indicates no plaque formation and resistance to phage TJ1 infection. Provided plaque photos show the plaque formation when TJ1 phage induction with Mitomycin C (Mit C) and UV radiation.

Class	Order	Family	Genus	Phage TJ1	Mit C	UV
β-Proteobacteria	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Curvibacter</i> AEP 1.3	✗		
β-Proteobacteria	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Curvibacter</i> Mag 1.1	✓		
β-Proteobacteria	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Curvibacter</i> HvuI	✓		
β-Proteobacteria	<i>Burkholderiales</i>	<i>Oxalobacteraceae</i>	<i>Undibacterium</i> C1.1	✓		
β-Proteobacteria	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Acidovorax</i> sp. AEP 1.4	✗		
β-Proteobacteria	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Pelomonas</i> sp. AEP2.2	✗		
β-Proteobacteria	<i>Burkholderiales</i>	<i>Oxalobacteraceae</i>	<i>Duganella</i> C1.2	✓		
β-Proteobacteria	<i>Burkholderiales</i>	<i>Oxalobacteraceae</i>	<i>Duganella</i> Oli 1.1	✓		
γ-Proteobacteria	<i>Pseudomonaceae</i>	<i>Pseudomonas</i>	<i>Pseudomonas</i> AEP 1.2	✗		
γ-Proteobacteria	<i>Pseudomonaceae</i>	<i>Pseudomonas</i>	<i>Pseudomonas</i> Mag 2.2	✗		
γ-Proteobacteria	<i>Pseudomonaceae</i>	<i>Pseudomonas</i>	<i>Pseudomonas</i> Oli 1.2	✗		
Bacteroidetes	<i>Flavobacteriaceae</i>	<i>Flavobacterium</i>	<i>Flavobacterium</i> Mag 1.4	✗		
β-Proteobacteria	<i>Neisseriales</i>	<i>Neisseriaceae</i>	<i>Vogesella</i> sp AEP 1.1	✓		

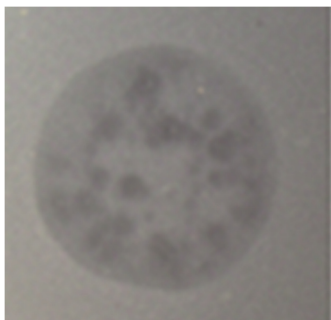


Figure S3: Picture of a TJ1-induced plaque on the *Hydra*-associated bacterium *Curvibacter* HvuI.

References

1. Bosch TCG, McFall-Ngai MJ (2011) Metaorganisms as the new frontier. *Zoology* 114(4):185–190.
2. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* 5:355–362.
3. Van Rensburg JJ, et al. (2015) The human skin microbiome associates with the outcome of and is influenced by bacterial infection. *MBio* 6(5):e01315-15.
4. Bates KA, et al. (2018) Amphibian chytridiomycosis outbreak dynamics are linked with host skin bacterial community structure. *Nat Commun* 9(1):9:693.
5. Fraune S, et al. (2015) Bacteria-bacteria interactions within the microbiota of the ancestral metazoan Hydra contribute to fungal resistance. *ISME J* 9:1543–1556.
6. Franzenburg S, et al. (2013) Bacterial colonization of Hydra hatchlings follows a robust temporal pattern. *ISME J* 7(4):781–90.
7. Goodrich JK, et al. (2014) Article Human Genetics Shape the Gut Microbiome. *Cell* 159(4):789–799.
8. Franzenburg S, et al. (2012) MyD88-deficient Hydra reveal an ancient function of TLR signaling in sensing bacterial colonizers. *Proc Natl Acad Sci* 109(47):19374–19379.
9. Pietschke C, et al. (2017) Host modification of a bacterial quorum-sensing signal induces a phenotypic switch in bacterial symbionts. *Proc Natl Acad Sci* 114(40):E8488-E8497.
10. Franzenburg S, et al. (2013) Distinct antimicrobial peptide expression determines host species-specific bacterial associations. *Proc Natl Acad Sci U S A* 110:E3730-8.
11. Li X-Y, et al. (2015) Which games are growing bacterial populations playing? *J R Soc Interface* 12(108):20150121.
12. Li X-Y, et al. (2017) Temperate phages as self-replicating weapons in bacterial competition. *J R Soc Interface* 14(137):20170563.
13. Grasis JA, et al. (2014) Species-specific viromes in the ancestral holobiont hydra. *PLoS One* 9(10):e109952.
14. Bohannan BJM, Lenski RE (2000) Linking genetic change to community evolution: Insights from studies of bacteria and bacteriophage. *Ecol Lett* 3(4):362–377.
15. Gómez P, Buckling A (2011) Bacteria-phage antagonistic coevolution in soil. *Science* (80-) 332(6025):106–109.
16. Suttle C a (2007) Marine viruses--major players in the global ecosystem. *Nat Rev Microbiol* 5(10):801–812.
17. Koskella B, Meaden S (2013) Understanding bacteriophage specificity in natural microbial communities. *Viruses* 5(3):806–823.
18. Lenski RE (1988) Dynamics of Interactions between Bacteria and Virulent Bacteriophage. *Advances in Microbial Ecology*, ed Marshall KC (Springer US, Boston, MA), pp 1–44.
19. De Paepe M, Leclerc M, Tinsley CR, Petit M-A (2014) Bacteriophages: an underestimated role in human and animal health? *Front Cell Infect Microbiol* 4(March):1–11.
20. Oakey HJ, Owens L (2000) A new bacteriophage, VHML, isolated from a toxin-producing strain of *Vibrio harveyi* in tropical Australia. *J Appl Microbiol* 89(4):702–709.
21. Ranquet C, Toussaint A, De Jong H, Maenhaut-Michel G, Geiselmann J (2005) Control of bacteriophage Mu lysogenic repression. *J Mol Biol* 353(1):186–195.

| Chapter II |

22. Nanda AM, Thormann K, Frunzke J (2015) Impact of spontaneous prophage induction on the fitness of bacterial populations and host-microbe interactions. *J Bacteriol* 197(3):410–419.
23. Livny J, Friedman DI (2004) Characterizing spontaneous induction of Stx encoding phages using a selectable reporter system. *Mol Microbiol* 51(6):1691–1704.
24. Refardt D (2012) Real-time quantitative PCR to discriminate and quantify lambdoid bacteriophages of Escherichia coli K-12. *Bacteriophage* 2(2):98–104.
25. Bossi L, Fuentes J a, Mora G, Figueroa-bossi N (2003) Prophage Contribution to Bacterial Population Dynamics Prophage Contribution to Bacterial Population Dynamics. *J Bacteriol* 185(21):6467–6471.
26. Burns N, James CE, Harrison E (2014) Polylysogeny magnifies competitiveness of a bacterial pathogen in vivo. *Evol Appl* 8(4):346–351.
27. Bode H, et al. (1973) Quantitative analysis of cell types during growth and morphogenesis in Hydra. *Wilhelm Roux Arch für Entwicklungsmechanik der Org* 171(4):269–285.
28. Bisbee BJW (1973) Size determination in Hydra: The roles of growth and budding. *J Embryol Exp Morphol* 30(1965):1–19.
29. Stiven AE (1965) The relationship between size, budding rate, and growth efficiency in three species of hydra. *Res Popul Ecol (Kyoto)* 7(1):1–15.
30. Littlefield CL, Finkemeier C, Bode HR (1991) Spermatogenesis in Hydra oligactis. *Dev Biol* 146(2):292–300.
31. Madera C, García P, Rodríguez A, Suárez JE, Martínez B (2009) Prophage induction in Lactococcus lactis by the bacteriocin Lactococcin 972. *Int J Food Microbiol* 129(1):99–102.
32. Nanda AM, et al. (2014) Analysis of SOS-Induced Spontaneous Prophage Induction in Corynebacterium glutamicum at the Single-Cell Level. 196(1):180–188.
33. Deines P, Hammerschmidt K, Bosch TC (2019) The effect of symbiosis on symbiont fitness – interactions within a simple metaorganism. *bioRxiv*:609271.
34. De Paepe M, et al. (2016) Carriage of λ Latent Virus Is Costly for Its Bacterial Host due to Frequent Reactivation in Monoxenic Mouse Intestine. *PLoS Genet* 12(2):1–20.
35. Deines P, Lachnit T, Bosch TCG (2017) Competing forces maintain the Hydra metaorganism. *Immunol Rev* 279(1):123–136.
36. Engelhardt T, Kallmeyer J, Cypionka H, Engelen B (2014) High virus-to-cell ratios indicate ongoing production of viruses in deep subsurface sediments. *ISME J*:1–7.
37. Mirzaei MK, Maurice CF (2017) Ménage à trois in the human gut : interactions between host , bacteria and phages. *Nat Rev Microbiol* 15(7):397–408.
38. Fitzpatrick BM, Allison AL (2014) Similarity and differentiation between bacteria associated with skin of salamanders (Plethodon jordani) and free-living assemblages. *FEMS Microbiol Ecol* 88(3):482–494.
39. Mohit V, Archambault P, Toupoint N, Lovejoy C (2014) Phylogenetic differences in attached and free-living bacterial communities in a temperate coastal lagoon during summer, revealed via high-throughput 16S rRNA gene sequencing. *Appl Environ Microbiol* 80(7):2071–2083.
40. Goodridge L, Gallaccio A, Griffiths MW (2003) Morphological , Host Range , and Genetic Characterization of Two Coliphages. 69(9):5364–5371.
41. Malki K, et al. (2015) Bacteriophages isolated from Lake Michigan demonstrate broad host-range across several bacterial phyla. *Virology* 1–5.

| Chapter II |

42. Chibani-Chennoufi S, Bruttin A, Dillmann ML, Brüßow H (2004) Phage-host interaction: An ecological perspective. *J Bacteriol* 186(12):3677–3686.
43. Langley R, Kenna DT, Vandamme P, Ure R, Govan JRW (2003) Lysogeny and bacteriophage host range within the Burkholderia cepacia complex. *J Med Microbiol* 52(6):483–490.
44. Kawasaki T, et al. (2009) Genomic characterization of Ralstonia solanacearum phage ϕ RSB1, a T7-like wide-host-range phage. *J Bacteriol* 91(1):422–427.
45. Mahenthiralingam E, Urban TA, Goldberg JB (2005) The multifarious, multireplicon Burkholderia cepacia complex. *Nat Rev Microbiol* 3(2):144–156.
46. Goudie AD, et al. (2008) Genomic sequence and activity of KS10, a transposable phage of the Burkholderia cepacia complex. *BMC Genomics* 9:615.
47. Ghosh D, et al. (2009) Acyl-homoserine lactones can induce virus production in lysogenic bacteria: An alternative paradigm for prophage induction. *Appl Environ Microbiol* 75(22):7142–7152.
48. Manrique P, et al. (2016) Healthy human gut phageome. *Proc Natl Acad Sci* 113(37):10400–10405.
49. Arndt D, et al. (2016) PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res* 44(W1):W16–W21.
50. Sekiguchi M, Takagi Y (1960) Effect of mitomycin C on the synthesis of bacterial and viral deoxyribonucleic acid. *BBA - Biochim Biophys Acta* 41(3):434–443.
51. Aucouturier A, Chain F, Langella P, Bidnenko E (2018) Characterization of a prophage-free derivative strain of Lactococcus lactis ssp. lactis IL1403 reveals the importance of prophages for phenotypic plasticity of the host. *Front Microbiol* 9(AUG):1–16.
52. Thurber R V, Haynes M, Breitbart M, Wegley L, Rohwer F (2009) Laboratory procedures to generate viral metagenomes. *Nat Protoc* 4:470–483.
53. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30(15):2114–20.
54. Bankevich A, et al. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19(5):455–77.
55. Wattam AR, et al. (2017) Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. *Nucleic Acids Res* 45(D1):D535–D542.
56. Borodovsky M, McIninch J (1993) GENMARK: Parallel gene recognition for both DNA strands. *Comput Chem.*
57. Altschul SF, et al. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.*
58. Meier-Kolthoff JP, Göker M (2017) VICTOR: genome-based phylogeny and classification of prokaryotic viruses. *Bioinformatics.*
59. Imamovic L, Ballesté E, Jofre J, Muniesa M (2010) Quantification of Shiga toxin-converting bacteriophages in wastewater and in fecal samples by real-time quantitative PCR. *Appl Environ Microbiol* 76(17):5693–5701.
60. Adams M (1959) Assay of phage by agar layer method. *Bacteriophages Intersci Publ Inc, New York, NY*:450–454.
61. Hemmrich G, Anokhin B, Zacharias H, Bosch TCG (2007) Molecular phylogenetics in Hydra, a classical model in evolutionary developmental biology. *Mol Phylogenet Evol* 44(1):281–290.
62. Bosch TC, Krylow SM, Bode HR, Steele RE (1988) Thermotolerance and synthesis of heat shock proteins: these responses are present in Hydra attenuata but absent in

| Chapter II |

- Hydra oligactis. *Proc Natl Acad Sci U S A* 85(21):7927–31.
63. Schloss PD, et al. (2009) Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75(23):7537–7541.
 64. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Appl Environ Microbiol* 79(17):5112–5120.

Chapter III – Evolution of temperate phage resistance interferes with bacterial colonization on *Hydra*

Abstract

Every eukaryotic organism associates with a complex microbial community to form what is termed a holobiont or a metaorganism. Regulating this multi-organism association plays an important role in health and disease. Phages impart significant influence on bacterial community composition and function, often through continuous phage-bacteria coevolution. Studying phage-bacteria interactions is of enormous importance to understand how bacterial communities are shaped and maintained in metaorganisms. In a recent study I could show that a temperate phage of *Hydra*'s main colonizer *Curvibacter* sp. is able to infect and reduce the abundance of several other *Hydra*-associated bacteria, including *Duganella* sp., *Undibacterium* sp. and *Pelomonas* sp.. In this study, I analyzed the evolution dynamics between *Curvibacter* phage TJ1 and those susceptible *Hydra*-associated bacteria (*Duganella* sp., *Undibacterium* sp. and *Pelomonas* sp.). I demonstrated that *Duganella* sp. and *Pelomonas* sp. developed resistance against phage TJ1 through mutations in Fibronectin type III domain genes or flagellum related genes, respectively. These mutations reduced the ability of resistant *Duganella* isolates to form biofilms and diminished motility in resistant *Pelomonas* strains. Interestingly, I observed that phage-resistant bacteria were less efficient at colonizing their eukaryotic host than the ancestral phage-sensitive bacteria. My results demonstrate that phage resistance acquired in a given environment can interfere with the ability of a host-associated bacterium to colonize its eukaryotic host. My data indicate that phage resistance is favoured when phage infection pressure is strong, but when phages are absent or other selection criteria predominate, resistance imposes a high fitness cost by reducing host colonization.

Introduction

Antagonistic coevolution is a process of reciprocal host defence and parasitic counter-defence that is an important driving force of adaptation and has been implicated in a number of ecological and evolutionary processes, including host-parasite population dynamics, the evolution of diversity and speciation, and the evolution of sex, mutation rates and pathogen virulence (1–4). The coevolution between bacteria and their “parasitic” bacteriophages (or “phages”, viruses that kill bacteria) is central to the ecology and evolution of microbial communities, since phages are known to alter competition between bacteria, maintain bacterial diversity and play an important role in horizontal gene transfer (5–7). Bacteria-phage interactions are often considered antagonistic but this is highly dependent on the phage’s lifestyle (8) Temperate phages can become latent by integrating into the host cell genome and form long-lasting associations with their bacterial host (5, 9). Conversely, lytic phages are obligate killers of their bacterial host (10), which can select for resistance in the bacterial host via *de novo* mutations or other diverse mechanisms (11–13). This in turn can select for counter-adaptation in the phage by increasing its infectivity (14).

Understanding antagonistic interactions between bacteria and their phages becomes especially relevant in the light of metaorganism research. Each eukaryotic organism is a metaorganism, or holobiont, living in a close relationship with a complex microbial community (15, 16). The bacterial community is known to be important for host-organism health (17, 18) and plays a role in illnesses like inflammatory bowel disease (17), diabetes and obesity (19) and even depression (20). Due to bacteria-phage interactions, phages are able to shape entire bacterial communities (5, 21) and thus are capable of influencing health and disease in eukaryotic hosts (18).

The freshwater polyp *Hydra* is an excellent organism for studying bacteria-phage-host interactions because it features a host-specific bacterial (22) and viral community (23). Even after 20 years of culturing *Hydra* under laboratory conditions, the *Hydra* microbiome still resembles polyps living in the wild (24) and remains stable even in co-culture with other *Hydra* strains that are associated with different bacteria (22, 24). Previous work has shown that *Hydra* actively selects and shapes its bacterial community via antimicrobial peptides (25, 26) and kazal 2-type protease inhibitors (26, 27). I also have identified phages as additional important factor in the maintenance of

Hydra's bacterial community composition. I demonstrated that the main bacterial colonizer of *Hydra*, *Curvibacter*, possesses an inducible prophage (TJ1) with a broad host range (21). Normally phages are considered species specific and display a narrow host range (28, 29). However, TJ1 infects and regulates the growth of three of the five most dominant bacteria associated with *Hydra*: *Duganella* sp., *Undibacterium* sp. and *Pelomonas* sp. (21, 30). This observation was surprising as I would expect that, due to coevolutionary dynamics, *Duganella* sp., *Undibacterium* sp. and *Pelomonas* sp. would have evolved to resist phage TJ1. Nonetheless, all isolated strains of those three bacteria are susceptible to *Curvibacter* phage TJ1 (21). It is well known that phage resistance can be costly and results in reduced fitness compared to ancestral phage-sensitive bacterial strains (31). This led us to ask: Are the bacteria that colonize *Hydra* able to evolve phage resistance, and if so, does phage resistance impose a fitness cost in phage absence *in vitro* and in association with the *Hydra* host? To answer these questions, I conducted evolution experiments using *Curvibacter* phage TJ1 and the susceptible *Hydra* colonizers *Duganella*, *Undibacterium* and *Pelomonas* as target bacteria. Genome sequencing and recolonization assays of phage-resistant bacteria revealed that mutations in membrane or flagella genes interfered with the initial process of eukaryotic host colonization. My results demonstrate that phage resistance acquired in an aquatic environment can become detrimental under certain environmental conditions, which may result in selection favouring bacteria that are susceptible to phage infection.

Material and Methods

Bacteria culture conditions, phage induction and concentration

The experiments were conducted using the *Hydra vulgaris* AEP-associated bacteria *Duganella* sp, *Undibacterium* sp and *Pelomonas* sp. as target bacteria for the temperate phage TJ1 of *Curvibacter* sp. All bacterial strains were cultured in 0.3 % R2A broth (Sigma-Aldrich) at 18°C and 250 rpm shaking.

Curvibacter phage TJ1 was induced using a Mitomycin C assay (32). Therefore, *Curvibacter* culture was grown to log phase (OD₆₀₀ of 0.25). Afterwards, 0.075 µg/ml Mitomycin were added and the culture was incubated with 250 rpm shaking at 18 °C for 16 h.

The next day bacterial cells were removed by two low speed centrifugation steps at 4,266 x g at 4°C for 30 min. The pellet was discarded and the supernatant was filtered using a 0.2 µm filter (Whatman). Afterwards, 10 % Polyethylene glycol (PEG 8000) and 0.4 mM NaCl were added to the supernatant to precipitate phage and incubated for 2.5 h on ice. Phages were pelleted at 4°C and 4,266 x g for 1 h and then resuspended in SM-buffer (50mM Tris; 100 mM NaCl; 8 mM MgSO₄; pH 7.5). To verify that the phage suspension was free of bacteria contaminants, 10 µl were spotted on a R2A plate (R2A medium, 1.5 % Agar-Agar). Remaining phages were used for further experiments.

Phage quantification

Phages were quantified using the double-layer method (33). Therefore, 1 ml of target bacterium in log phase (OD₆₀₀= 0.1) was added to 5 ml Overlay-Agar (R2A medium, 0.4 % Agarose, 5 mM CaCl₂, 5 mM MgCl₂), then mixed and poured on a R2A Agar plate (R2A medium, 1.5 % Agar-Agar). Plates were allowed to dry for 30 min. Subsequently 10 µl of phage suspension were spotted on the plates and incubated at 18°C. Plaque formation was monitored every 24 to 48 h.

Coevolution experiment

The coevolution experiment was carried out using *Duganella sp.*, *Undibacterium sp.* and *Pelomonas sp.* as target bacteria for *Curvibacter* phage TJ1 for 21 days (n=4). One day before the start of the experiment, a single colony of each bacterium was picked, transferred to R2A medium and grown over night at 18°C and 2000 rpm, to ensure a homogeneous bacterial culture. The next day 500 µl of each culture were transferred to 5 ml of fresh R2A medium and grown for 3-4 h. In parallel, *Curvibacter* phages were purified and concentrated using PEG-precipitation (see above) and quantified using a plaque assay by estimating the PFU/ml (33) (see above). Phages were added to the treated bacterial cultures at a concentration of 10⁴ PFU/ml. Bacteria that just received resuspension buffer without phages served as controls. Every 24 h, 500 µl of bacterial culture were transferred to 5 ml of fresh R2A medium. These transfers were repeated for 21 days in total (approx. 100 generations) (Fig. 1 A). The growth of each bacterial species over time was estimated by measuring the CFU/ml of each sample every 24 h for the first 72 h and every 48 h afterwards. Therefore, serial dilutions were prepared and two dilutions per sample (10⁻⁵/10⁻⁶) were plated.

During the experiment phages were quantified by a plaque assay (33) (see above) every 24 h for the first 72 h and afterwards every second day. For quantification I used a very susceptible bacterium *Curvibacter Hvul* in order to get optimal plaque formation. For further sequencing analysis and to identify mutations in the bacterial populations, subsamples of the coevolution experiment (500 µl) were taken daily and preserved as Cryo-stocks (sterile 25% Glycerol in H₂O) at -80 °C. Moreover, single isolates were picked every day for the first 72 h and every 48 h afterwards. Those single isolates were stored in Cryo-Stocks at -80 °C.

DNA Extraction, Library prep and Sequencing

Community samples from preserved Cryo-stocks were grown in R2A and DNA was extracted after 2 days of bacterial growth, using the DNA Blood and Tissue Kit (Qiagen) and the standard protocol for bacterial cultures. The Illumina Nextera kit and the protocol published by Baym *et al.* 2015 (34) were used to prepare the library for shotgun sequencing. The resulting sequencing libraries were loaded onto an Illumina Next-Seq at 1.8 picomolar, using Illumina's mid-output kit for 75 bp paired end sequencing.

Genome Assembly and Annotation

The reference genomes for *Undibacterium*, *Duganella* and *Pelomonas* were assembled using the reads from timepoint 0 in SPAdes, with previously sequenced genomes of each bacterium as a backbone (35). The resulting contigs were annotated using RAST (36), which uses RASTtk for gene calls (37), and visualized using Seed viewer (38).

Genomic mutation analysis

Genome mutation analysis was conducted using Breseq (39). Paired end reads for each timepoint were run through Breseq, using the respective assembled bacterial genome as reference. The polymorphism mode was used to consider different mutation frequencies throughout the community. In brief, Breseq uses Bowtie 2 to align the paired end reads to a reference genome and creates SAM files as output. Afterwards, gdttools packaged with Breseq was used to subtract all mutations that occurred in Control samples at timepoint 21 from phage treated samples; this was done to exclude from the analysis any mutations that conferred adaption to the culturing conditions. Results were annotated using the gdttool ANNOTATE and the

annotated genomes as reference. Custom R scripts (40) for the dplyr package (41) were used to filter the data and to detect non-synonymous mutations at greater than 10 % frequency (42).

Resistance assay

For analysing potential resistances of evolved bacteria to the ancestral phage TJ1, the *Curvibacter* prophage was induced and purified (see above). Bacteria from different timepoints were transferred to log phase and either treated with roughly 10^4 PFU/ml phages or SM-Buffer, which served as controls (n=8). The bacterial growth was quantified in a plate reader (Tecan Spark) and the growth rate was calculated using the following formula: $\text{LN}(\text{OD}_{\text{end}}/\text{OD}_{\text{beginning}})/\text{time}$ (in h).

Germfree Hydra

Experiments were conducted using *Hydra vulgaris* AEP. *Hydra* polyps were cultured according to standard procedure, at 18°C, constant light conditions (12 h/12 h light/dark rhythm) and in *Hydra* culture medium (0.28 mM CaCl₂, 0.33 mM MgSO₄, 0.5 mM NaHCO₃ and 0.08 mM KCO₃) (43). Before antibiotic treatment, polyps were fed twice a week with *A. salina* instar larvae. For generating germfree *Hydra*, polyps were exposed to 50 µg/ml of each of the following antibiotics: Ampicillin, Rifampicin, Streptomycin, Spectinomycin and Neomycin. The antibiotics and the surrounding medium were exchanged 3 times per week. After two weeks, all polyps were transferred to new sterile *Hydra* medium without antibiotics and incubated for three more days to remove all traces of antibiotics. Sterility was checked by two different methods. First, I plated homogenized polyps onto R2A agar plates and checked for colonies for 4 days. Second, I conducted PCR on extracted gDNA (Quiagen Blood & Tissue Kit) of the homogenized polyps, using a bacterial 16S primer set (F: 5'-CCTACGGGAGGCAGCAG-3' and R: 5'-ATTACCGCGGCTGCTGGC-3').

Recolonization capacity of (co-)evolved bacteria

Overnight cultures of evolved and ancestral bacteria were diluted and grown to log phase at 18°C and 250 rpm shaking. Bacteria were pelleted by low speed centrifugation at 4,022 x g and 18°C for 30 min. The supernatant was discarded, and bacterial cells were resuspended in sterile *Hydra* medium. In total, 5000 CFU/ml of each bacterium were added to 40 ml surrounding water of germfree *Hydra* polyps (see above) (n=4). After 48 h, 72 h, and 96 h, recolonized polyps were washed twice in

sterile *Hydra* medium, homogenized, diluted and plated onto R2A-Agar plates for bacteria quantification. CFU/polyp was counted after 2-3 days incubation at 18°C.

Biofilm quantification

To analyse the biofilm formation of phage-resistant and susceptible *Duganella* strains, I conducted a Crystal violet assay using the protocol of Álvarez *et al.* (2006) (44) and Castillo *et al.* (2015) (45) with some modifications. Cultures in static phase of all tested *Duganella* strains were diluted 1:100 in fresh R2A medium and 100 µl of the dilution were transferred to replicates in a 96-well plate (n=8). The plates were incubated in a shaking environment (225 rpm) at 18°C. After 48 h the supernatant was removed and the wells were washed twice with sterile water (200 µl) to remove unattached cells and media components. Biofilm was stained with 125 µl of a 0.1 % crystal violet solution, which was added to the wells and incubated for 15 min. The plates were rinsed with sterile water 3 times and dried for 6 h. The biofilm was resuspended in 200 µl 95 % ethanol and incubated for 15 min at room temperature. Subsequently, 150 µl of the cell solutions were transferred to a new 96-well-plate and the absorbance was measured at 595 nm in a microplate reader (Tecan Spark), using ethanol as a blank.

Swimming assay

The motilities of ancestral and resistant *Pelomonas* strains were quantified using the swimming assay from Calvio *et al.* (2005) (46) with minor changes. In brief, resistant (T13, T21), ancestral and control (T21) *Pelomonas* strains were grown to static phase in R2A medium at 18 °C and 225 rpm shaking. A 2.5 µl aliquot of each strain was spotted in the middle of a semi-solid R2A agarose plate (R2A medium, 0.3 % Agarose) and incubated at 18°C. The diameter of each bacterial colony was measured after three days.

Statistics and data analysis

Statistical analysis was conducted using the statistic programme R (version 3.5.2 "Eggshell Igloo" (2018-12-20), Copyright R Foundation for Statistical Computing, Vienna, Austria) (40) and the packages "car" (47), "multcomp" (48), "stats" (40) and DescTools (49). The Levene's test for homogeneity was conducted to analyse the distribution of samples. All normally distributed data sets were analyzed using a ONE-WAY-ANOVA, followed by a Tukey HSD Posthoc test for multiple comparisons. Data sets that did not fulfil the criteria of normality (swimming assay & *in vivo* growth rates)

were analyzed using a Kruskal-Wallis test, followed by a Dunn test for multiple comparisons, and adjustment of p-values as described by Benjamin and Hochberg (BH) (1995).

Results

Dynamics of *Hydra*-associated bacteria and Phage TJ1

I studied the growth dynamics of three *Hydra vulgaris* (strain AEP)-associated bacteria, *Pelomonas*, *Undibacterium* and *Duganella*, in presence of *Curvibacter* phage TJ1 for 21 days in the absence of the *Hydra* host. Transfers were conducted daily in 1:10 dilutions, resulting in approximately 100 generations (Fig. 1 A). After the first day, phage infection drastically reduced the initial density of cultures from all three bacteria. However, after three days, the growth of *Duganella* and *Undibacterium* recovered. A second phase of growth reduction was observed after 9 transfers in both *Duganella* and *Undibacterium* cultures. After another recovery phase, the bacterial density remained stable until the end of the experiment. The growth of *Pelomonas* recovered after 7 transfers and another phage-induced decrease in growth was not observed (Fig. 1 B). The densities of the phage over time showed patterns that were opposite of bacterial densities (Fig 1 B). In *Duganella* cultures, phage density increased after 1 day and then declined to nearly 0 after 5 transfers. At 7 and 9 transfers the density of the phage increased again but the whole phage population dropped below a detectable level afterwards. Phages in *Pelomonas* cultures showed high density after 1 and 5 transfers. Subsequently, the phage titer declined steadily and the phage was undetectable by the end of the experiment. In the case of *Undibacterium*, the phage density peaked at 1, 3 and 9 days and disappeared afterwards. At the final timepoint, bacterial populations of all three bacterial strains remained at a high density and the phage was not detectable (Fig 1 B).

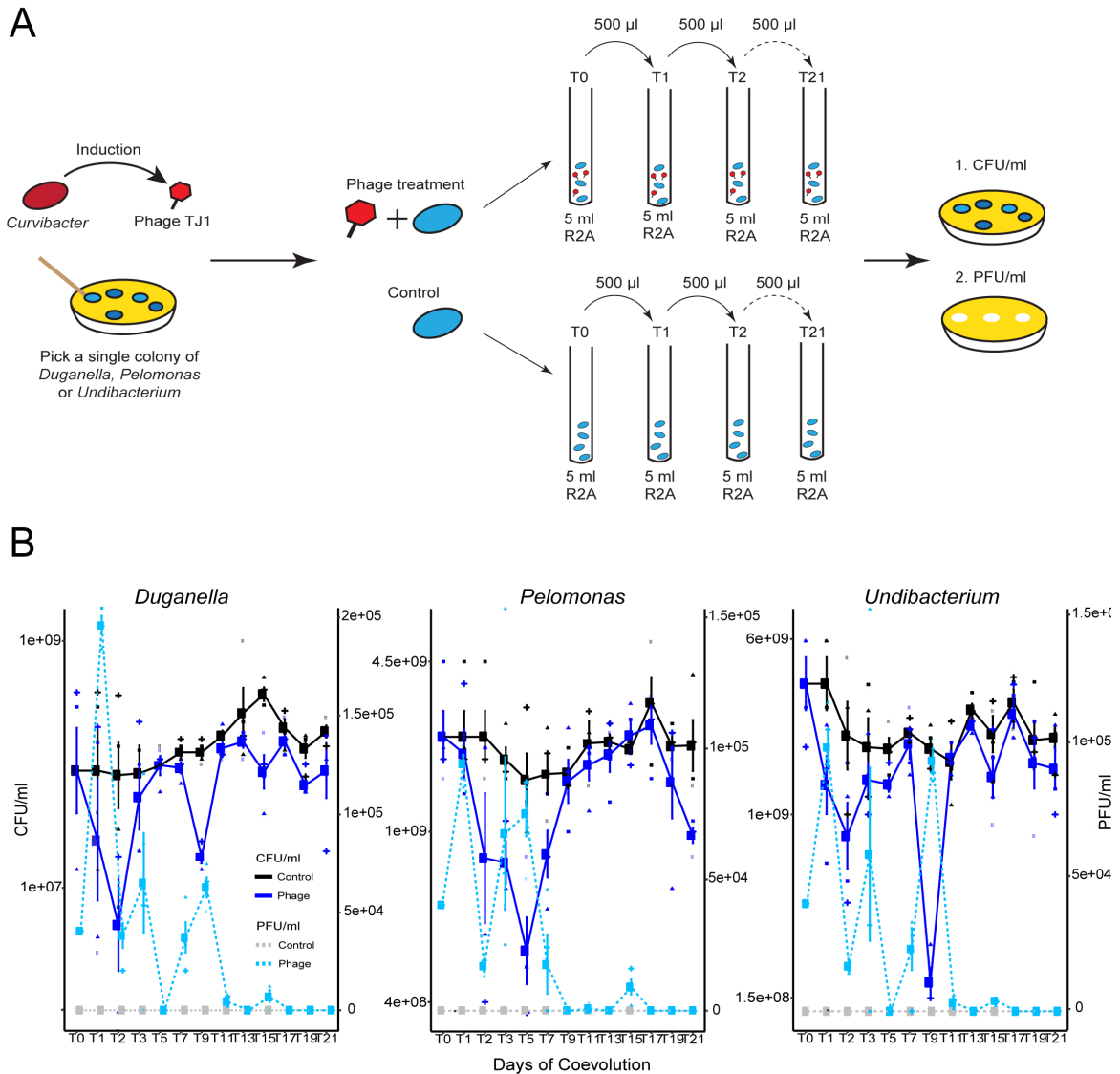


Figure 1: A: Experimental set up: Phage TJ1 was induced using Mitomycin C. One single colony of each target bacteria (*Duganella*, *Pelomonas*, *Undibacterium*) was picked from a R2A plate and inoculated overnight in liquid media ($n = 4$). Purified phage TJ1 was transferred into 5 ml R2A medium with one of the target bacteria and transferred every day to new culture medium. As a control, bacteria were grown and treated in the same way in the absence of the phage. For bacteria and phage quantification the CFU/ml and PFU/ml were counted on a regular basis. **B:** Bacteria-phage dynamics. Mean growth (CFU/ml) (\pm SE) of *Duganella*, *Pelomonas* and *Undibacterium* over time, with the phage (blue) or in the control treatment (black) and mean PFU/ml (\pm SE) of phages with target bacteria (cyan) and in the control treatment (grey) over time of the experiment ($n=4$). Note that both y-axes are log scale.

Phage resistance and growth *in vitro*

The observed bacterial growth dynamics (see above) indicated that phage and bacteria coevolved. To determine if the bacteria evolved resistance, I selected timepoints for each bacterium based on the CFU/ml counts taken during the coevolution experiment (Fig. 1 B). Due to the growth recovery of *Duganella* and *Pelomonas* after 7 and 21 days and of *Undibacterium* after 7 and 13 days, I selected those timepoints for further experiments, enhancing the chances for an evolved resistance phenotype. To check if phage resistances occurred, I estimated the growth rate of evolved bacteria isolates from these timepoints and compared it to isolates of the corresponding ancestral bacterial strain, both in the presence and absence of the ancestral phage (control) (Fig. 2 A).

Evolution of resistance against phage TJ1 was detected in *Duganella* after 13 days. This evolved resistance was still present at the end of the experiment (T21), since evolved *Duganella* from both timepoints showed the same growth rate both, the phage treatment and the control treatment (- phage) (ONE-WAY-ANOVA, $F= 8.468$, $p_{T13}=0.93$, $p_{T21}= 0.087$). The ancestral *Duganella* was susceptible to phage infection and displayed a significant decrease in growth in the phage treatment compared to the control (ONE-WAY-ANOVA, $F= 8.468$ $p< 0.001$) (Fig. 2 A).

Similar evolution of resistance was observed in *Pelomonas*. After 13 days, phage resistance occurred and was still detectable after 21 transfers (Fig. 2 A). Consistently, the evolved *Pelomonas* from timepoint 13 and 21 did not show reduction in growth when exposed to phage TJ1 compared to the control treatment without the phage (ONE-WAY-ANOVA, $F= 12.89$, $p_{T13}=0.97$, $p_{T21}= 0.99$). Again, the ancestral *Pelomonas* strain was susceptible, supported by the significant decline in growth rate when exposed to phage TJ1 (ONE-WAY-ANOVA, $F= 12.89$, $p< 0.001$) (Fig 2 A).

Surprisingly, even though coevolutionary growth dynamics were observed within the replicates of *Undibacterium* (Fig. 1 B), the evolved strains that were tested did not show resistance against *Curvibacter* phage TJ1 in the same conditions (liquid media). Growth rates of all strains were significantly reduced when exposed to phage TJ1 in comparison to the control treatment (ONE-WAY-ANOVA, $F=0.415$ $p_{T7}< 0.001$, $p_{T13}< 0.001$ $p_{WT}< 0.001$) (Fig. 2 A).

Resistant *Duganella* strains displayed the same growth rate *in vitro* as the corresponding ancestral strain (ONE-WAY-ANOVA, $F= 8.468$, $p_{T13}=0.99$, $p_{T21}= 0.29$),

suggesting that neither phage resistance, nor adaptation to the liquid culture conditions, influenced bacterial fitness *in vitro* (Fig. S1).

Similarly, resistant *Pelomonas* strains did not show higher fitness *in vitro* than the corresponding ancestral strain. Therefore, no significant differences in growth rate were observed between resistant strains and the ancestral *Pelomonas* strain (ONE-WAY-ANOVA, $F=12.89$, $p_{T13}=0.40$, $p_{T21}=0.99$) (Supplementary Fig S1).

The same could be observed in (non-resistant) evolved *Undibacterium*. No significant growth differences between (non-resistant) evolved *Undibacterium* strains and ancestral strains were detectable (ONE-WAY-ANOVA, $F=0.415$, $p_{T7}<0.063$, $p_{T13}<0.93$) (Fig S1).

Phage resistance led to an altered *Hydra* colonization pattern

Because phage resistant *Duganella*, *Undibacterium* and *Pelomonas* strains could not be observed and recovered *in vivo*, I tested whether phage resistance interferes with bacterial colonization of the *Hydra* host. Therefore, I tested whether germfree *Hydra vulgaris* AEP polyps could be colonized by resistant or ancestral *Duganella* or *Pelomonas* strains. I also tested the colonization abilities of *Duganella* and *Pelomonas* control strains that were cultured in *in vitro* conditions in the absence of the phage for 21 days, as a control for any adaptation to *in vitro* culture conditions that might negatively influence recolonization capacity.

I could show that phage resistance did interfere with host recolonization, while potential adaptations to *in vitro* culturing conditions alone did not impact the bacterial recolonization success (Fig 2 B).

Resistant *Duganella* isolates from timepoints 13 and 21 showed a significantly reduced recolonization capacity in comparison to the ancestral strain (ONE-WAY-ANOVA, $F=20.79$, $p_{T13}<0.001$, $p_{T21}<0.001$) and to the evolved control isolates (ONE-WAY-ANOVA, $F=20.79$, $p_{T13}<0.001$, $p_{T21}<0.001$) after 96 h of recolonization. Interestingly, the evolved control strain recolonized *Hydra* as efficiently as the ancestral strain of *Duganella* (ONE-WAY-ANOVA, $F=20.79$, $p=1$), suggesting that either adaptation to liquid culture conditions did not affect colonization, or the time was not sufficient to adapt to liquid culture conditions (Fig 2 B).

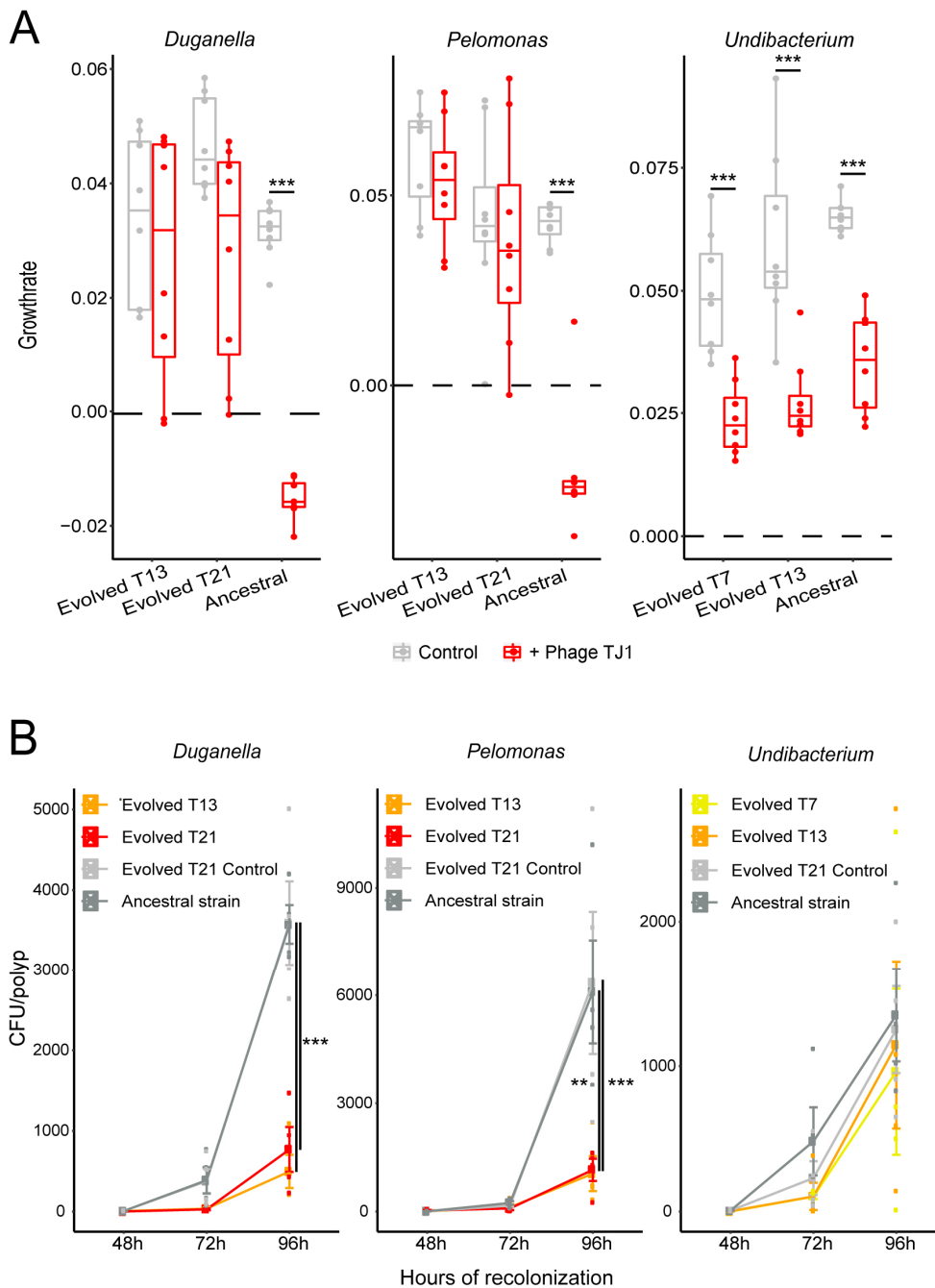


Figure 2: A: Bacterial growth rates *in vitro*: Boxplot of growth rates of evolved and ancestral *Duganella*, *Pelomonas* and *Undibacterium* strains without treatment (grey) and exposed to phage TJ1 (red) (n=8). *Duganella* and *Pelomonas* recovered after 7 and 21 days. *Undibacterium* did show phage recovery after 7 and 13 days. Therefore, the chance of a resistant phenotypes were the highest at those selected timepoints, which were chosen for further analysis and experiments. Stars (** = p<0.01, *** = p<0.001) indicate significant differences between phage treatment and control, based on ONE-WAY-ANOVA. **B:** Recolonization efficiency on *Hydra*. Mean CFU/polyp (\pm SE) over time of resistant, evolved, host control and ancestral strains of *Duganella* sp., *Undibacterium* sp. or *Pelomonas* sp. on *Hydra*. Stars (**=p<0.01, ***=p<0.001) indicate a significant difference between bacterial strains (ONE-WAY-ANOVA).

Likewise, the resistant *Pelomonas* isolates from transfer 13 (ONE-WAY-ANOVA, $F=5.424$ $p_{\text{toWT}}= 0.001$, $p_{\text{toControlT21}} = 0.0005$) and 21 (ONE-WAY-ANOVA, $F=5.424$ $p_{\text{toWT}}= 0.0014$, $p_{\text{toControlT21}} = 0.0007$) had a reduced ability to recolonize *Hydra* compared to the ancestral *Pelomonas* strain and the evolved control isolates (96 h). As with *Duganella*, adaptation to liquid culture conditions did not affect recolonization by *Pelomonas*, since there was no significant difference in the recolonization efficiency between the ancestral *Pelomonas* and the evolved control strain (ONE-WAY-ANOVA, $F= 5.424$ $p= 1$) after 96 h (Fig 2 B).

In contrast to *Duganella* and *Pelomonas*, the recolonization success of *Undibacterium* was not affected. All evolved *Undibacterium* strains, grown with and without the phage, showed the same recolonization success as the ancestor (ONE-WAY-ANOVA, $F=20.062$, $p= 0.989$) (Fig 2 B).

Interestingly, the finding of a reduced colonization of resistant bacteria compared to the ancestral strains (see above) is not due to the inability of cells to divide on the host itself. I could show that bacteria growth rates of resistant *Duganella* and *Pelomonas* strains on *Hydra* did not differ significantly from those of the ancestral strain and the evolved control strain (***Duganella***: Kruskal-Wallis Test: chi-squared = 5.12, $dF = 3$; ancestral: $p_{T13} = 0.22$, $p_{T21} = 0.77$; evolved control: $p_{T13} = 0.28$, $p_{T21} = 0.77$; ***Pelomonas***: Kruskal-Wallis Test: chi-squared = 4.85, $dF = 3$, ancestral: $p_{T13} = 0.31$, $p_{T21} = 0.31$; evolved control: $p_{T13} = 0.45$, $p_{T21} = 0.36$) (Supplementary Fig. S2). Therefore, it is likely that the reduced recolonization ability was due to the bacteria failing to attach to the host rather than reduced bacterial replication on *Hydra*.

Since no resistance evolved in *Undibacterium*, all evolved strains (with and without the phage) displayed the same growth rate on *Hydra* as the corresponding ancestor (Kruskal-Wallis Test: chi-squared = 0.29, $dF = 3$; WT: $p_{T13} = 0.94$, $p_{T21} = 0.94$, T21 Control: $p_{T13} = 0.94$, $p_{T21} = 0.94$) (Supplementary Fig. S2).

Genomic changes

To elucidate which gene mutations might be driving the decreased recolonization success, I shotgun sequenced the community genomes of the evolved bacteria. I observed at least one reproducible gene mutation that occurred in all replicates of all phage treated *Duganella* and *Pelomonas* strains, while not in the control without phage.

In the evolved *Duganella* strains, I observed the most nonsynonymous mutations in genes that code for a Fibronectin III domain protein. The frequency of these mutations changed over the duration of the experiment in the bacterial population (Fig. 3 A), but all four replicates had one of these mutations by the end of the experiment.

The most abundant mutations in *Pelomonas* were found in genes that code for flagella-associated genes. Nonsynonymous mutations in the hook-length control protein *FliK* occurred in replicates 3 and 4 (Fig. 3 B). The frequencies of these mutations changed over time, but the mutations were still present at the end of the experiment. Other flagellum related nonsynonymous mutations could be found in the protein *FlgJ* (replicate 2) and in the flagellar motor rotation protein *MotB* (replicate 4) (Fig. 3 B, Supplementary Table S4).

In *Undibacterium* I did not find any mutations that were stable over time or occurred in all 4 replicates (Supplementary Table S2), consistent with the lack of a resistant phenotype in this bacterium.

Several other mutations occurred during the coevolution experiment, but these were either inconsistently present over time or not present in most of the replicates. All detected nonsynonymous SNP mutations are listed in Supplementary Tables S2-S4. Since *Curvibacter* phage TJ1 is a naturally occurring lysogenic phage, I checked for insertion of this phage in evolved bacteria by mapping the TJ1 genome against bacterial community sequences from all analyzed timepoints. However, I found no evidence of lysogenic conversion at any timepoint in any of the three bacteria, since none of the population reads aligned to the phage genome.

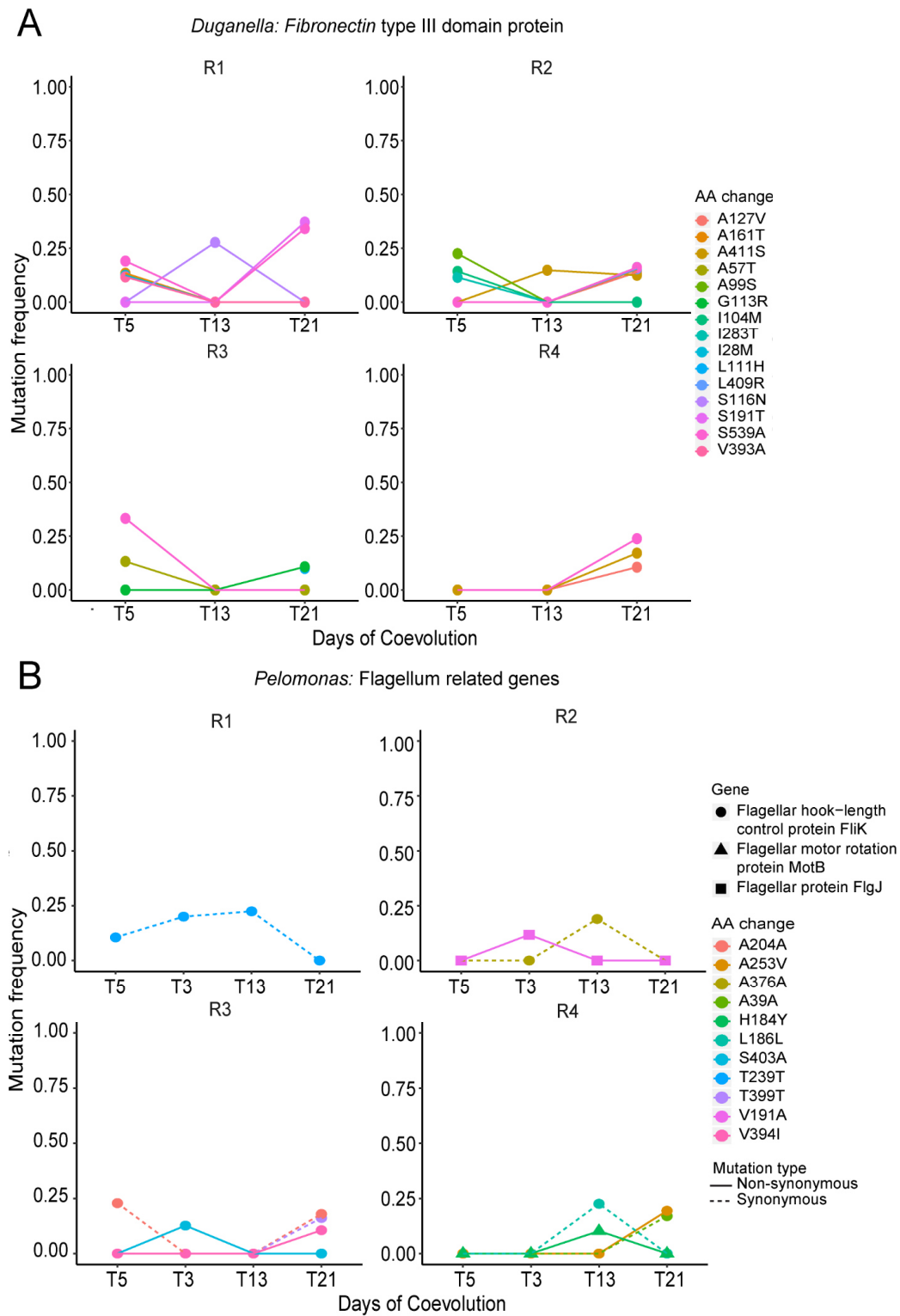


Figure 3: Frequency of most common bacterial mutations over time. **A:** Population frequency of mutations and amino acid (AA) changes in Fibronectin type III domain protein in all four replicates of evolved *Duganella*. **B:** Population frequency of mutations and amino acid (AA) changes in flagellum genes *FliK*, *MotB* and *FlgJ* in all four replicates of evolved *Pelomonas*.

Causes of decreased recolonization success of TJ1 resistant bacteria

The most frequent mutations detected in resistant *Duganella* were within genes that code for a Fibronectin III domain protein, which is known to support surface attachment and adhesion (50–53), which is important for initiating biofilm formation (54, 55). Nonsynonymous SNP mutations in this gene may impair biofilm formation in phage-resistant *Duganella*, which could reduce this bacteria’s ability to recolonize *Hydra*. Indeed, results of the Crystal violet assay indicated that the ability to build biofilms was significantly reduced in all resistant *Duganella* isolates (ONE-WAY-ANOVA: $F = 46.89$, $p_{T13} < 0.001$, $p_{T21} < 0.001$), suggesting that mutations in Fibronectin domain III interfered with biofilm formation (Fig 4 A).

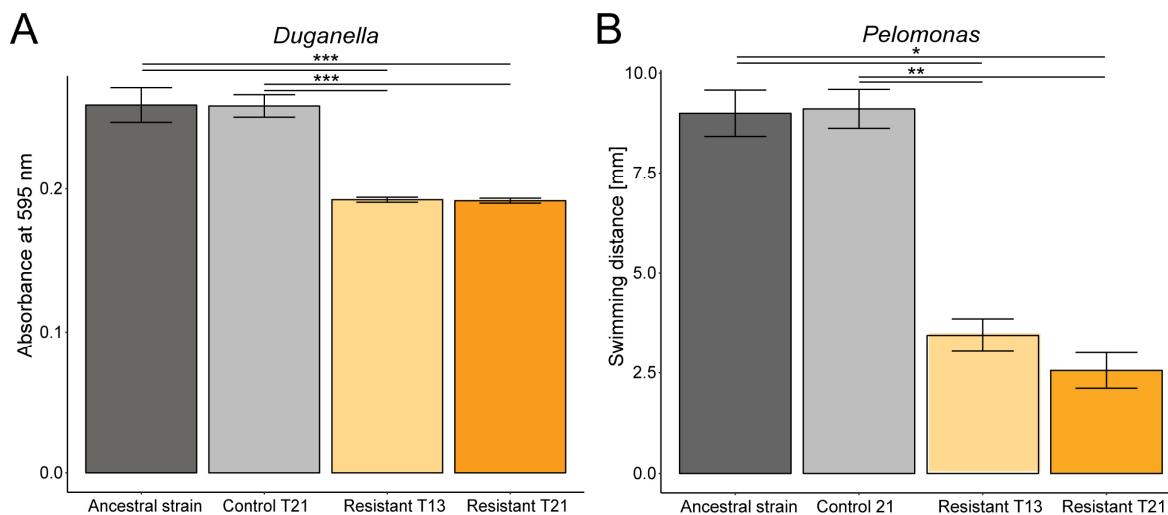


Figure 4: A: Biofilm assay: Absorbances (595 nm) measured for the Crystal violet assay as an indicator for biofilm formation in resistant, host control and ancestral strains of *Duganella* ($n=8$). Stars (***) indicate a significant difference between bacterial strains (ONE-WAY-ANOVA). **B:** Motility assay. Swimming distance (mm) as an indicator of motility of resistant, host control and ancestral strains of *Pelomonas* ($n=3$). Stars (*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$) indicate a significant difference between bacterial strains (Kruskal-Wallis-Test).

Flagella are important for bacterial motility (56). Mutations in flagella-related genes may have reduced or eliminated motility in phage-resistant *Pelomonas*. This may interfere with the capacity of *Pelomonas* to cross the surface laminar boundary layer and to reach the host epithelial surface, which would result in a lower recolonization efficiency. Results from a motility assay showed that the swimming diameters of all resistant *Pelomonas* were significantly reduced compared to ancestral and evolved

control strains (Kruskal-Wallis Test: chi-squared = 21.935, dF = 3, $p_{T13} = 0.037$, $p_{T21} = 0.009$), suggesting that bacterial motility was affected by phage resistance (Fig 4 B). Comparisons with control strains further showed that culture conditions did not influence biofilm formation (ONE-WAY-ANOVA: $F = 46.89$, $p = 0.99$) or motility (Kruskal-Wallis Test: chi-squared = 21.935, dF = 3; $p = 0.97$) (Fig. 4 A, B).

Discussion

My results showed that resistance to *Curvibacter* prophage TJ1 did not affect *Duganella*, *Undibacterium*, or *Pelomonas* bacterial fitness *in vitro* but did interfere with bacteria-*Hydra* interactions by reducing the ability of resistant strains to colonize their eukaryotic host.

This key finding shows that the environment is an important factor within bacteria-phage interactions and indicates whether mutation fixation is favoured or prevented in the bacterial population. Certain mutations, like changes in the flagellum or the bacterial surface structure, are favoured in liquid culture due to the benefit of resistance, but these mutations become rather costly in the *Hydra* host and are therefore selected against in the natural environment. Resistant bacteria lose the ability to colonize their eukaryotic host, while susceptible bacteria are able to quickly and efficiently colonize *Hydra*. Following the concept “the founder takes it all” (57), ancestral, susceptible bacteria reach higher densities and more efficiently colonize the host within *Hydra*’s limited carrying capacity (58, 59).

Phage resistance, acquired under broth conditions, has been shown before to be more costly in the natural environment than under laboratory conditions. Bacteriophage SBW25F2 resistant *Pseudomonas fluorescens* SBW25 and phage-resistant *Pseudomonas syringae pathovar tomato* showed a reduction in growth in natural environments, while there were no competitive costs associated with resistance in broth environments (1, 60). The higher cost of phage resistance in a natural environment has important implications for the potential of phages as antibacterial treatments for infection. However, in these studies resistances evolved during coevolution with strictly lytic phages. My study is one of the first studies to analyse dynamics between an originally temperate phage and their potential bacterial targets. Studies that analyzed temperate phages as potential weapons in competition between lysogenized and non-lysogenized bacteria showed that, after a few days of co-

existence, the phage was integrated as a prophage in most of the initially susceptible bacteria (lysogenization) (61, 62). Interestingly, I did not observe lysogenization in *Duganella*, *Undibacterium* or *Pelomonas*. This suggests that a phage can be temperate in one host but strictly lytic in other bacteria. Another explanation would be that my culture conditions did not select for lysogeny. Lysogeny is favoured at low frequencies of target bacteria to ensure the persistence of the phage population (63). In my study, enough susceptible bacteria were present at all given timepoints, which would allow phage TJ1 to continually lyse *Duganella*, *Undibacterium* and *Pelomonas* and spread rapidly, thereby favouring the lytic cycle (63).

Nonetheless, I could show that an originally temperate phage uses similar mechanisms to adsorb and infect bacteria target cells as strictly lytic phages. Flagella are a well-known structure for phages to infect host cells. Using protein–protein interactions between phage tail fibre proteins and bacterial flagella filaments, flagellatropic phages adsorb to the bacterial hosts via flagellin proteins (64, 65). One of those proteins is indeed the *FliK* protein, which is crucial for the infection of *Salmonella* by *Siphoviridae* phage iEPS5 (66) and is also mutated in resistant *Pelomonas* strains from this study. Furthermore, it has been shown that the rotation of the flagellum plays an important role in susceptibility and resistance against phages (66, 67). Given that *MotB* is an integral membrane protein and necessary for the rotation of the flagellum (68), it is not surprising that I also found a mutation in the gene that codes for the *MotB* protein in resistant *Pelomonas* strains. The third flagellum related mutation was found in the *FigJ* protein, which has not been described to be important for phage resistance so far. However, it is important for the complete formation and assembly of the flagellum (69–71) and thus could also play an important role in the resistance mechanism of *Pelomonas*. Nevertheless, phage resistance in *Pelomonas* came with the cost of reduced recolonization success, as a result of decreased motility. This is not surprising, since it has been shown that *FliK* mutants show reduced or no motility and that single amino acid changes in the *FliK* gene can have severe effects on motility (72). In addition, *MotB* is essential for bacterial motility because it plays an important role in flagellum rotation (73, 74), which is crucial for movement and swimming of bacteria (75).

Interestingly, my results indicate that phage TJ1 is able to use different mechanisms when infecting another bacterium. In resistant *Duganella* strains I observed several

mutations in the type III domain of Fibronectin (Fn), a protein ubiquitously found in the animal kingdom (76). It plays a crucial role in migration, development and cell adhesion and mediates the cellular interactions associated with the extracellular matrix (76). Acquired from an animal source (51), the Fn type III module has also been described to be present in several different bacteria (77–80). Due to its function, the type III domain is often part of the extracellular matrix (50, 52) or involved in extracellular enzymes (77–79, 81). The type III domain of Fibronectin was described to regulate protein-protein interaction and cell binding, and therefore, represents the perfect target for phage infection in *Duganella*. Phages often infect bacteria via attachment to host cell receptors at the outer cell wall and are dependent on protein-protein interactions (64). Phage TJ1 may bind to these Fibronectin type III domains of *Duganella* before injecting the phage DNA into the bacterial cell. Compared to ancestral or T21 control strains, resistant *Duganella* strains had a reduced recolonization success, which might be linked to a decreased ability to build biofilms. The fact that the FN III module is also known to support surface adhesion, attachment and spreading (50–53) and that a small, bacterial Fibronectin-mimicking protein induces cell spreading and focal adhesion formation (82), supports my thesis that a mutation in the Fibronectin III domain protein is the reason for the reduced biofilm formation and decreased recolonization success of resistant *Duganella*.

To date, phages are considered species and receptor specific (28, 83). However, recent studies indicate that phages from different environments can also possess broader host ranges by infecting several bacteria from the same but also different families than its original host bacterium (21, 84–87). Receptors on bacterial surfaces differ between families, and even between strains of the same species. Therefore, phages with broad host ranges are in need of a relaxed receptor specificity (88), like the *Escherichia coli* infecting, broad host range phage AR1. This phage is hypothesized to use both lipopolysaccharide (LPS) and an outer membrane porin (OmpC) as host receptors (84, 89, 90). My results suggest that the temperate *Curvibacter* phage TJ1 may be able to use more than just one mechanism to attach to and infect a target bacterial cell.

It is possible that phage TJ1 uses the same mechanism to enter the bacterial host cell but the bacteria are using different resistance mechanisms to withstand phage infection (91).

Interestingly, I did not observe *Undibacterium* with resistance against phage TJ1 at the end of the experiment. It might be that the phage titer was too low to trigger the evolution of phage resistance, or that the fitness cost was very high and therefore phage-resistant hosts were not a large part of the resulting population. Similar observations can be found in phage therapy experiments against *Staphylococcus aureus* where no resistant strains could be recovered (92, 93). Another reason might be that resistance is imparted by phenotypic rather than genetic changes. For instance, the fish pathogen *Vibrio anguillarum* demonstrates several non-mutational phage defence mechanisms against the lytic phage KVP40, including expressional downregulation of the phage receptor, putative abortive infection systems and secretion of extracellular proteases (94).

In conclusion, my results demonstrate that phage resistance acquired outside the host, can interfere with the ability of a host-associated bacterium to colonize the host. This observation may represent an important selection pressure influencing the bacterial colonization process of eukaryotic hosts. Phage resistance is favoured in the non-host-associated environment but causes high fitness costs in the host environment, thereby favouring phage-sensitive bacteria in the initial attachment phase.

Supplementary

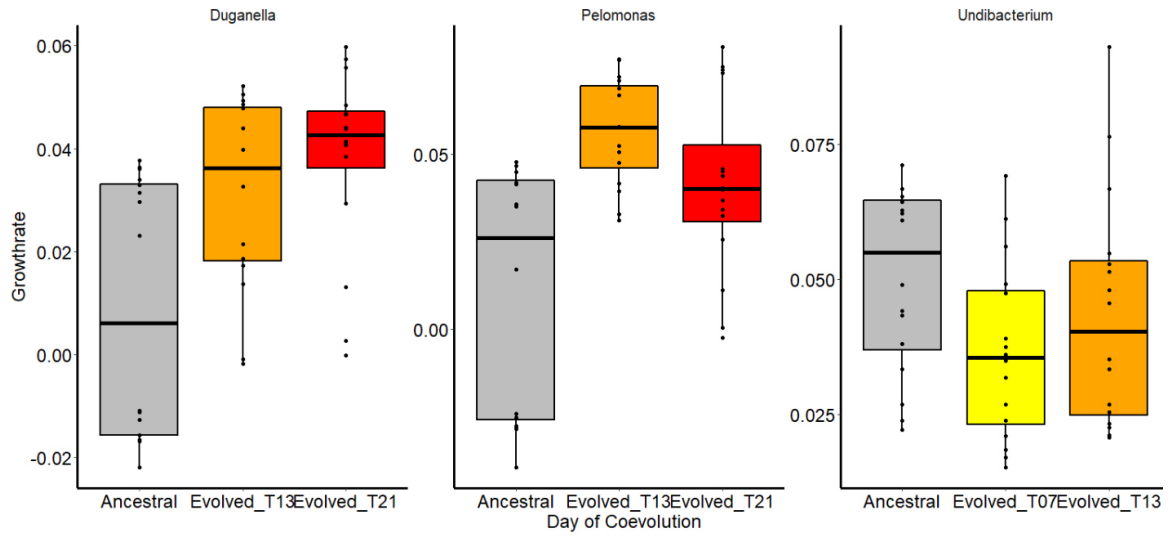


Figure S1: Boxplot of growth rate of phage-evolved and ancestral strains of *Duganella*, *Pelomonas* and *Undibacterium* *in vitro*.

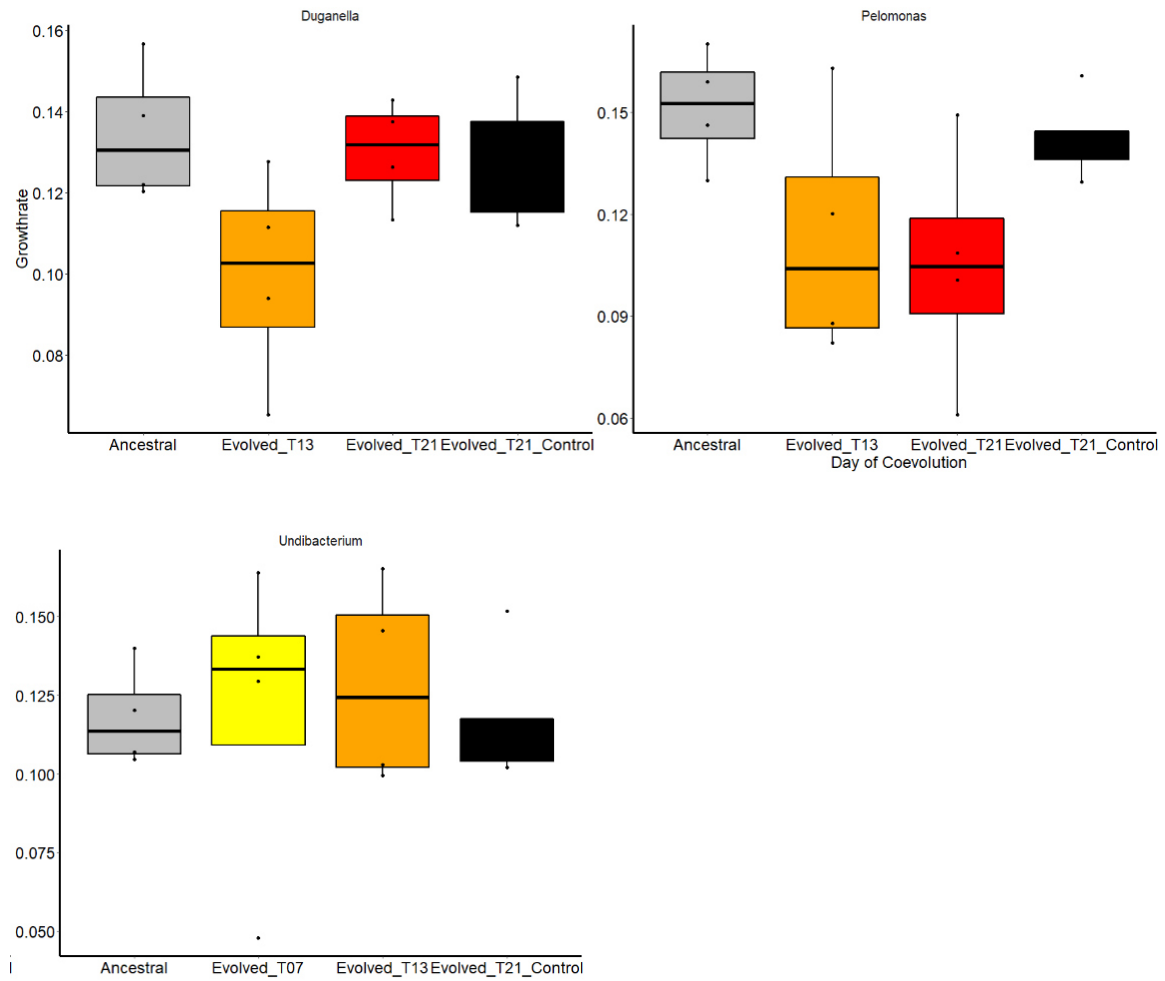


Figure S2: Boxplot of growth rate of phage-evolved, control and ancestral strains of *Duganella*, *Pelomonas* and *Undibacterium* on *Hydra* (*in vivo*).

| Chapter III |

Table S1: List of all nonsynonymous snps at all timepoints and all replicates of *Pelomonas*

AA_change	frequency	Replicate	Timepoint	gene_product	Genome Position
R141K	0.10777	R1	T1	3-ketoacyl-CoA thiolase (EC 2.3.1.16) @ Acetyl-CoA acetyltransferase (EC 2.3.1.9)	12184
V618F	0.111142	R1	T3	3'-to-5' exoribonuclease RNase R	45375
C618F	0.114327	R1	T3	3'-to-5' exoribonuclease RNase R	45376
D46G	0.107992	R3	T1	Acetylglutamate kinase (EC 2.7.2.8)	8219
P140L	0.120785	R1	T21	Alkyl hydroperoxide reductase protein F	10800
Y250H	0.110968	R1	T1	Anhydro-N-acetylmuramic acid kinase (EC 2.7.1.170)	15276
G49S	0.107686	R2	T21	Arginine decarboxylase (EC 4.1.1.19); Ornithine decarboxylase (EC 4.1.1.17); Lysine decarboxylase (EC 4.1.1.18)	12378
S394F	0.110687	R1	T3	Aspartyl-tRNA synthetase (EC 6.1.1.12) @ Aspartyl-tRNA(Asn) synthetase (EC 6.1.1.23)	7248
D17N	0.113791	R1	T1	Biotin carboxyl carrier protein of acetyl-CoA carboxylase	10738
S227A	0.115741	R4	T21	Branched-chain amino acid transport ATP-binding protein LivF (TC 3.A.1.4.1)	6419
P158R	0.102626	R1	T1	cd06127 DEDDh DNA polymerase III 3'-5' exonuclease domain	32603
L92F	0.111036	R1	T1	Copper tolerance protein	97314
S231G	0.10515	R2	T13	D-amino acid dehydrogenase (EC 1.4.99.6)	37676
M150L	0.113833	R2	T21	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)	50353

| Chapter III |

Y445D	0.120742	R3	T21	Dipeptide-binding ABC transporter, periplasmic substrate-binding component (TC 3.A.1.5.2)	45860
C130R	0.107957	R1	T3	DksA family protein PA5536 (no Zn-finger)	6368
D1014G	0.105111	R1	T21	DNA-directed RNA polymerase beta' subunit (EC 2.7.7.6)	9733
V106G	0.105882	R1	T1	DUF1854 domain-containing protein	44056
L523I	0.153908	R4	T21	Efflux ABC transporter, permease/ATP-binding protein Atu2242	14350
V346A	0.121078	R1	T13	Efflux ABC transporter, permease/ATP-binding protein Reut_A2584	45562
H184Y	0.102191	R4	T13	Flagellar motor rotation protein MotB	91
V191A	0.117503	R2	T3	Flagellar protein FigJ	64
V394I	0.105939	R3	T21	Flagellar hook-length control protein FliK	65
S403A	0.126825	R3	T3	Flagellar hook-length control protein FliK	2328
A253V	0.194089	R4	T21	Flagellar hook-length control protein FliK	24049
N164H	0.124564	R1	T21	Glutathione S-transferase (EC 2.5.1.18)	7061
P122Q	0.112413	R1	T3	Glycolate dehydrogenase (EC1.1.99.14), subunit GlcD	14703
I325M	0.105131	R2	T1	GMP reductase (EC 1.7.1.7)	723
W228G	0.113815	R1	T1	Histidine ABC transporter, permease protein HisQ (TC 3.A.1.3.1)	49949
G73A	0.118984	R1	T13	hypothetical protein	27959
S7N	0.134632	R1	T13	hypothetical protein	445
K112I	0.111027	R1	T13	hypothetical protein	33615
D427G	0.102438	R1	T1	hypothetical protein	52764
S16G	0.117519	R1	T1	hypothetical protein	4409

| Chapter III |

P15L	0.117315	R1	T1	hypothetical protein	65134
D498G	0.187408	R1	T21	hypothetical protein	65136
P499S	0.17601	R1	T21	hypothetical protein	208547
I107T	0.102414	R1	T21	hypothetical protein	208549
T662A	0.114144	R1	T21	hypothetical protein	12228
N50D	0.102416	R1	T21	hypothetical protein	38976
S154C	0.194387	R1	T21	hypothetical protein	36332
Q2P	0.129573	R1	T3	hypothetical protein	5541
G16E	0.324733	R1	T3	hypothetical protein	194031
D19E	0.342075	R1	T3	hypothetical protein	49
H20Y	0.15751	R1	T3	hypothetical protein	59
K82N	0.121853	R2	T21	hypothetical protein	60
V529I	0.1912	R2	T21	hypothetical protein	9901
N112D	0.121058	R2	T21	hypothetical protein	39375
D132G	0.117274	R2	T21	hypothetical protein	4375
S199N	0.105911	R2	T3	hypothetical protein	70976
V529I	0.326248	R2	T3	hypothetical protein	961
V76I	0.166248	R2	T3	hypothetical protein	39375
N95S	0.157795	R3	T13	hypothetical protein	33821
G725A	0.120507	R3	T21	hypothetical protein	33879
T152A	0.121062	R3	T21	hypothetical protein	18038
L141M	0.125118	R3	T3	hypothetical protein	1916
M208L	0.114198	R3	T3	hypothetical protein	72429
S381N	0.102203	R3	T3	hypothetical protein	431
S7N	0.158729	R3	T3	hypothetical protein	61262
N95S	0.170105	R3	T3	hypothetical protein	33615
S199N	0.127836	R4	T13	hypothetical protein	33879
S38R	0.102146	R4	T13	hypothetical protein	961
C241Y	0.110705	R4	T13	hypothetical protein	181600
G632E	0.104854	R4	T13	hypothetical protein	64458
Q18L	0.113172	R4	T1	hypothetical protein	27925
K38Q	0.157443	R4	T1	hypothetical protein	2523
S241N	0.113873	R4	T1	hypothetical protein	177728
N73K	0.114284	R4	T1	hypothetical protein	11908
I74M	0.114305	R4	T1	hypothetical protein	17655
Y75D	0.136093	R4	T1	hypothetical protein	17658
V75D	0.108289	R4	T1	hypothetical protein	17659
S84A	0.104822	R4	T1	hypothetical protein	17660
S371T	0.105201	R4	T21	hypothetical protein	17357
G8S	0.10216	R4	T21	hypothetical protein	47719
G40S	0.113853	R4	T21	hypothetical protein	9829
H306D	0.12167	R4	T3	hypothetical protein	15276
A280V	0.117326	R1	T1	Inner membrane protein, KefB/KefC family	12432
V244A	0.124857	R3	T3	Long-chain-fatty-acid-- CoA ligase (EC 6.2.1.3)F	1059

| Chapter III |

R29H	0.124558	R1	T3	Macrolide export ATP-binding/permease protein MacB (EC 3.6.3.-)	28310
N74I	0.102468	R2	T13	Methionine aminopeptidase (EC 3.4.11.18)	13045
E478D	0.11429	R1	T3	Methionyl-tRNA synthetase (EC 6.1.1.10)	22872
G427S	0.146298	R1	T1	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	42194
A240T	0.117288	R4	T1	Methyl-accepting chemotaxis sensor/transducer protein	4978
A543T	0.124582	R4	T3	Methyl-accepting chemotaxis sensor/transducer protein	287
E460K	0.107764	R2	T21	Methylcrotonyl-CoA carboxylase carboxyl transferase subunit (EC 6.4.1.4)	103041
D139G	0.120512	R4	T21	Methylmalonyl-CoA mutase (EC 5.4.99.2) / B12 binding domain of Methylmalonyl-CoA mutase (EC 5.4.99.2)	2091
R24Q	0.102211	R2	T21	NAD(P)H oxidoreductase YrkL @ Putative NADPH-quinone reductase (modulator of drug activity B) @ Flavodoxin 2	263580
T2A	0.114138	R1	T21	NAD-dependent formate dehydrogenase delta subunit	22831
S35T	0.100106	R1	T13	Oxidoreductase, Gfo/Idh/MocA family	22926
P182L	0.104904	R4	T21	Porphobilinogen synthase (EC 4.2.1.24)	33804
R369Q	0.11387	R3	T21	Response regulatory protein	546
V631I	0.117274	R4	T21	Rhs-family protein	76786
S582F	0.104917	R2	T21	Ribonucleotide reductase of class II (coenzyme B12-dependent) (EC1.17.4.1)	23460
E17Q	0.105188	R4	T1	SSU ribosomal protein S6p	15706
H159L	0.102458	R1	T21	Thiol:disulfide interchange protein DsbC	71673
D849E	0.108011	R1	T13	TonB-dependent receptor	194618

| Chapter III |

S774W	0.114277	R1	T21	TonB-dependent receptor	89496
T351A	0.110959	R2	T21	Transcriptional regulator	18749
L149F	0.113881	R4	T21	Tripartite tricarboxylate transporter TctC family	20238
K203Q	0.151016	R1	T3	Type IV fimbrial biogenesis protein PilW	59604
A132V	0.121572	R3	T3	Vibriolysin, extracellular zinc protease (EC 3.4.24.25) @ Pseudolysin, extracellular zinc protease (EC 3.4.24.26)	21030
M132V	0.121811	R3	T3	Vibriolysin, extracellular zinc protease (EC 3.4.24.25) @ Pseudolysin, extracellular zinc protease (EC 3.4.24.26)	9811
E314D	0.124472	R1	T21	YgfD: protein that forms a complex with the methylmalonyl-CoA mutase in a pathway for conversion of succinyl-CoA to propionyl-CoA	9812

| Chapter III |

Table S2: List of all nonsynonymous snps at all timepoints and all replicates of *Undibacterium*

AA_change	frequency	Replicate	Timepoint	gene_product	Genome position
D341E	0.170879	R2	T13	FIG032621: Hydrolase, alpha/beta hydrolase fold family	22711
D128N	0.166472	R2	T3	Transcriptional regulator, LysR family	161803
T12I	0.192127	R3	T3	hypothetical protein	1074
S19A	0.18709	R3	T3	hypothetical protein	2198
H358P	0.161814	R3	T3	hypothetical protein	151838
Y475D	0.166223	R4	T13	L-lactate permease	236404

Table S3: List of all nonsynonymous snps at all timepoints and all replicates of *Duganella*.

AA_change	frequency	Replicate	Timepoint	gene_product	Genome position
Y115H	0.117571	R1	T21	1-deoxy-D-xylulose 5-phosphate synthase (EC 2.2.1.7)	59239
M323V	0.102412	R2	T21	4-hydroxyphenylpyruvate dioxygenase (EC1.13.11.27)	22497
L602H	0.124927	R1	T21	5-methyltetrahydrofolate--homocysteine methyltransferase (EC 2.1.1.13)	37968
S1065G	0.110966	R4	T21	5-methyltetrahydrofolate--homocysteine methyltransferase (EC 2.1.1.13)	39356
H80R	0.104578	R1	T5	5'-nucleotidase (EC 3.1.3.5)	15484
R182K	0.102208	R3	T21	ABC-type amino acid transport/signal transduction systems, periplasmic component/domain	75397
I360M	0.110141	R1	T5	Alcohol dehydrogenase	55770
F347I	0.134207	R2	T21	Alcohol dehydrogenase	55811
D237Y	0.12824	R2	T21	AMP nucleosidase (EC 3.2.2.4)	12236
F237Y	0.128171	R2	T21	AMP nucleosidase (EC 3.2.2.4)	12237
I38N	0.10782	R3	T5	Bis-ABC ATPase Uup	15947
H366Q	0.10804	R1	T5	Cystathionine beta-lyase (EC 4.4.1.8)	113213
W633R	0.102411	R3	T5	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)	62560
W493S	0.114045	R3	T5	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)	54049
W209R	0.114135	R4	T21	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)	38861
C631W	0.104922	R1	T21	Dipeptidyl peptidase IV	135073
V219L	0.114212	R3	T5	DNA-binding heavy metal response regulator	30608

| Chapter III |

I246F	0.10527	R3	T5	DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	4765
C629G	0.110149	R3	T5	Ferrichrome-iron receptor	208078
S116N	0.277358	R1	T13	Fibronectin type III domain protein	347
S539A	0.341702	R1	T21	Fibronectin type III domain protein	69
S191T	0.371657	R1	T21	Fibronectin type III domain protein	56
V393A	0.117248	R1	T5	Fibronectin type III domain protein	1218
S539A	0.190733	R1	T5	Fibronectin type III domain protein	69
I28M	0.124346	R1	T5	Fibronectin type III domain protein	544
A161T	0.134173	R1	T5	Fibronectin type III domain protein	481
A411S	0.148219	R2	T13	Fibronectin type III domain protein	1233
S539A	0.160895	R2	T21	Fibronectin type III domain protein	69
T72A	0.394627	R2	T21	Fibronectin type III domain protein	216
I283T	0.161166	R2	T21	Fibronectin type III domain protein	850
L409R	0.155203	R2	T21	Fibronectin type III domain protein	1228
A411S	0.124873	R2	T21	Fibronectin type III domain protein	1233
A127V	0.139209	R2	T21	Fibronectin type III domain protein	380
A161T	0.151503	R2	T21	Fibronectin type III domain protein	481
A99S	0.225318	R2	T5	Fibronectin type III domain protein	297
I104M	0.142926	R2	T5	Fibronectin type III domain protein	314
I283T	0.115253	R2	T5	Fibronectin type III domain protein	850
L111H	0.100031	R3	T21	Fibronectin type III domain protein	334
G113R	0.108034	R3	T21	Fibronectin type III domain protein	339
S539A	0.332855	R3	T5	Fibronectin type III domain protein	69
A57T	0.132689	R3	T5	Fibronectin type III domain protein	169
S539A	0.238641	R4	T21	Fibronectin type III domain protein	69
A411S	0.171406	R4	T21	Fibronectin type III domain protein	1233
A127V	0.105965	R4	T21	Fibronectin type III domain protein	380
A411S	0.163057	R4	T5	Fibronectin type III domain protein	1233
T137A	0.270059	R1	T5	Flagellar hook-length control protein FliK	110
H134N	0.170444	R1	T5	Flagellar hook-length control protein FliK	119

| Chapter III |

A447T	0.11078	R4	T5	Glutamate--cysteine ligase (EC 6.3.2.2)	158889
I19T	0.190448	R3	T5	HNH endonuclease	51
T97P	0.124518	R1	T21	hypothetical protein	2337
G570A	0.108052	R1	T21	hypothetical protein	1711
I513T	0.113853	R1	T5	hypothetical protein	5368
A696S	0.142267	R1	T5	hypothetical protein	2088
M230L	0.12153	R1	T5	hypothetical protein	668
N201H	0.258781	R1	T5	hypothetical protein	755
D144E	0.144742	R1	T5	hypothetical protein	230
A144E	0.115221	R1	T5	hypothetical protein	231
S72T	0.122997	R1	T5	hypothetical protein	103
S95N	0.117311	R1	T5	hypothetical protein	33608
G19S	0.368166	R1	T5	hypothetical protein	55
R144K	0.104909	R1	T5	hypothetical protein	17756
E220V	0.114203	R1	T5	hypothetical protein	39865
S35P	0.107961	R2	T13	hypothetical protein	69
L103V	0.159766	R2	T13	hypothetical protein	355
V2976I	0.117169	R2	T13	hypothetical protein	47347
N12S	0.175735	R2	T13	hypothetical protein	51
G295V	0.21043	R2	T21	hypothetical protein	886
T343S	0.24901	R2	T21	hypothetical protein	1030
N201H	0.158696	R2	T21	hypothetical protein	755
L103V	0.155729	R2	T21	hypothetical protein	355
N93K	0.112321	R2	T21	hypothetical protein	383
M82L	0.134194	R2	T21	hypothetical protein	418
I610L	0.215821	R2	T21	hypothetical protein	1864
H45R	0.107427	R2	T21	hypothetical protein	43372
P46S	0.110782	R2	T21	hypothetical protein	51744
C15Y	0.117228	R2	T21	hypothetical protein	80147
N1177I	0.136365	R2	T21	hypothetical protein	23505
T475M	0.110724	R2	T21	hypothetical protein	22762
W767L	0.105244	R2	T21	hypothetical protein	23638
P769L	0.113454	R2	T21	hypothetical protein	23644
A10V	0.210104	R2	T5	hypothetical protein	644
H735R	0.121095	R2	T5	hypothetical protein	107549
P59L	0.102222	R3	T13	hypothetical protein	140750
M230L	0.180121	R3	T21	hypothetical protein	668
L103V	0.159519	R3	T21	hypothetical protein	355
G694R	0.142674	R3	T5	hypothetical protein	2082
P694R	0.142805	R3	T5	hypothetical protein	2083
A696S	0.176592	R3	T5	hypothetical protein	2088
M230L	0.123351	R3	T5	hypothetical protein	668
A10V	0.124397	R3	T5	hypothetical protein	644
L103V	0.15165	R3	T5	hypothetical protein	355
N93K	0.116196	R3	T5	hypothetical protein	383
T319A	0.102436	R3	T5	hypothetical protein	34911
D52A	0.117206	R3	T5	hypothetical protein	187400
S111P	0.114164	R3	T5	hypothetical protein	15445
L70P	0.108002	R3	T5	hypothetical protein	149306
M230L	0.142756	R4	T13	hypothetical protein	668
A176G	0.134686	R4	T13	hypothetical protein	135
V177A	0.124519	R4	T13	hypothetical protein	8019
T343S	0.16263	R4	T21	hypothetical protein	1030
M230L	0.118536	R4	T21	hypothetical protein	668
T36S	0.157647	R4	T21	hypothetical protein	109
R36S	0.149938	R4	T21	hypothetical protein	110
S99P	0.110953	R4	T21	hypothetical protein	178399
A10V	0.104921	R4	T5	hypothetical protein	644

| Chapter III |

R493G	0.114241	R4	T5	hypothetical protein	33677
S584N	0.104908	R4	T5	hypothetical protein	72230
R560C	0.113945	R4	T5	hypothetical protein	22759
L552M	0.124918	R4	T5	hypothetical protein	46338
L21P	0.11412	R4	T5	hypothetical protein	6297
V290I	0.117185	R4	T13	Hypothetical protein in Cyanoglobin locus	46469
G655E	0.107756	R1	T5	IncF plasmid conjugative transfer pilus assembly protein TraC	32673
V167M	0.110762	R1	T5	Inner membrane protein YpjD	10348
L378R	0.129862	R1	T5	Integrase	9446
E81K	0.124716	R2	T5	Integration host factor beta subunit	100636
G134D	0.120759	R2	T13	Isochorismatase (EC 3.3.2.1)	100932
L150M	0.10182	R1	T5	L-carnitine dehydratase/bile acid- inducible protein F	72584
S348P	0.102387	R1	T5	Lipoprotein releasing system transmembrane protein LolC/LolE	21055
L333*	0.18123	R2	T13	Lipoyl synthase (EC 2.8.1.8)	48592
L341F	0.124954	R3	T5	Lysophospholipid transporter LplT / 2- acylglycerophosphoethanolamine acyltransferase (EC 2.3.1.40)	6991
G220D	0.110735	R3	T21	Membrane-bound lytic murein transglycosylase A	72035
M306T	0.11753	R3	T5	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	95874
P114H	0.108154	R3	T5	Methyl-accepting chemotaxis sensor/transducer protein	6671
I258V	0.117526	R4	T21	Methyl-accepting chemotaxis sensor/transducer protein	128757
P272A	0.213943	R4	T21	Multimodular transpeptidase- transglycosylase (EC 2.4.1.129) (EC 3.4.-.-)	256244
T254S	0.110873	R3	T13	Murein hydrolase activator NlpD	53384
G1196R	0.135745	R2	T21	NAD-specific glutamate dehydrogenase (EC 1.4.1.2), large form	247513
Q509P	0.120775	R1	T5	O-antigen acetylase	32877
L25W	0.101599	R2	T21	predicted membrane protein	25598
V129D	0.10541	R4	T5	Probable GTPase related to EngC	73327
D167G	0.102416	R3	T5	putative homoserine/homoserine lactone efflux protein	58341
H283R	0.102175	R2	T21	Pyridoxamine 5'-phosphate oxidase (EC 1.4.3.5)	5394
A128V	0.205302	R3	T5	Short-chain dehydrogenase	1549
A128V	0.228279	R4	T21	Short-chain dehydrogenase	1549
R22W	0.104934	R4	T21	SSU ribosomal protein S3p (S3e)	293921
V70D	0.111043	R3	T5	Tellurium resistance protein TerD	103120
G196W	0.107127	R3	T5	Tol-Pal system beta propeller repeat protein TolB	19518
S623N	0.113932	R3	T21	TonB-dependent receptor	98166
D444G	0.105118	R3	T5	Transcriptional regulator, GntR family domain / Aspartate aminotransferase (EC 2.6.1.1)	65163
V1M	0.195441	R1	T5	Transposase	55431
R46G	0.216171	R3	T21	Transposase	57825

| Chapter III |

R46G	0.256366	R4	T13	Transposase	57825
R46G	0.187617	R4	T5	Transposase	57825
Y28F	0.108275	R1	T21	twitching motility protein PilG	22095
L81F	0.111882	R1	T5	twitching motility protein PilG	22255
T132M	0.162403	R2	T21	twitching motility protein PilJ	23861
N133H	0.16626	R2	T21	twitching motility protein PilJ	23863
A134S	0.162808	R2	T21	twitching motility protein PilJ	23866
N71D	0.114083	R1	T5	Type IV fimbrial biogenesis protein PilY1	59129
T20A	0.105136	R3	T5	UDP-galactose-lipid carrier transferase (EC 2.-.-.)	93150
F226Y	0.117847	R1	T5	UDP-glucose 4-epimerase (EC 5.1.3.2)	22317
N364I	0.12113	R1	T21	UDP-N-acetylmuramoylalanine--D-glutamate ligase (EC 6.3.2.9)	120010
C22G	0.113855	R2	T21	Uncharacterized chaperone protein YegD	16811
N125D	0.114161	R3	T5	Uncharacterized membrane anchored protein Mext_4159	195854
R458H	0.171125	R2	T21	Zinc protease	59144
V203A	0.107945	R4	T21	Zinc transporter, ZIP family	54157

References

1. Gómez P, Buckling A (2011) Bacteria-phage antagonistic coevolution in soil. *Science* (80-) 332(6025):106–109.
2. Bull JJ (1994) VIRULENCE. *Evolution (N Y)* 48(5):1423–1437.
3. Hamilton WD (1980) Sex versus Non-Sex versus Parasite. *Oikos* 35(2):282–290.
4. Pal C, Maciá MD, Oliver A, Schachar I, Buckling A (2007) Coevolution with viruses drives the evolution of bacterial mutation rates. *Nature* 450:1079.
5. Koskella B, Brockhurst MA (2014) Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS Microbiol Rev* 38(5):916–931.
6. Bohannan BJM, Lenski RE (2000) Linking genetic change to community evolution: Insights from studies of bacteria and bacteriophage. *Ecol Lett* 3(4):362–377.
7. Brüssow H, Canchaya C, Hardt W, Bru H (2004) Phages and the Evolution of Bacterial Pathogens : from Genomic Rearrangements to Lysogenic Conversion Phages and the Evolution of Bacterial Pathogens : from Genomic Rearrangements to Lysogenic Conversion. *Microbiol Mol Biol Rev* 68(3):560–602.
8. Obeng N, Pratama AA, Elsas JD Van (2016) The Significance of Mutualistic Phages for Bacterial Ecology and Evolution. *Trends Microbiol* 24(6):440–449.
9. De Paepe M, Leclerc M, Tinsley CR, Petit M-A (2014) Bacteriophages: an underestimated role in human and animal health? *Front Cell Infect Microbiol* 4(March):1–11.
10. Lenski RE (1988) Dynamics of Interactions between Bacteria and Virulent Bacteriophage. *Advances in Microbial Ecology*, ed Marshall KC (Springer US, Boston, MA), pp 1–44.
11. Garneau JE, et al. (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* 468:67.
12. Meeske AJ, Nakandakari-Higa S, Marraffini LA (2019) Cas13-induced cellular dormancy prevents the rise of CRISPR-resistant bacteriophage. *Nature*.
13. Rostøl JT, Marraffini L (2019) (Ph)ighting Phages: How Bacteria Resist Their Parasites. *Cell Host Microbe* 25(2):184–194.
14. Samson JE, Magadán AH, Sabri M, Moineau S (2013) Revenge of the phages: defeating bacterial defences. *Nat Rev Microbiol* 11(10):675.
15. Bosch TCG, McFall-Ngai MJ (2011) Metaorganisms as the new frontier. *Zoology* 114(4):185–190.
16. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* 5:355–362.
17. Zhang YJ, et al. (2015) Impacts of gut bacteria on human health and diseases. *Int J Mol Sci* 16(4):7493–7519.
18. Esser D, et al. (2018) Functions of the Microbiota for the Physiology of Animal Metaorganisms. *J Innate Immun*: 11:393-404.

| Chapter III |

19. Everard A, Cani PD (2013) Diabetes, obesity and gut microbiota. *Best Pract Res Clin Gastroenterol* 27(1):73–83.
20. Kelly JR, et al. (2016) Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *J Psychiatr Res* 82:109–118.
21. Lange J, Fraune S, Bosch TCG, Lachnit T (2019) The neglected part of the microbiome: Prophage TJ1 regulates the bacterial community of the metaorganism Hydra. *bioRxiv*.
22. Franzenburg S, et al. (2013) Distinct antimicrobial peptide expression determines host species-specific bacterial associations. *Proc Natl Acad Sci U S A* 110:E3730-8.
23. Grasis JA, et al. (2014) Species-specific viromes in the ancestral holobiont hydra. *PLoS One* 9(10):e109952.
24. Fraune S, Bosch TCG (2007) Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. *Pnas* 104(32):13146–13151.
25. Fraune S, et al. (2010) In an early branching metazoan, bacterial colonization of the embryo is controlled by maternal antimicrobial peptides. *Proc Natl Acad Sci U S A* 107(42):18067–72.
26. Augustin R, Fraune S, Bosch TCG (2010) How Hydra senses and destroys microbes. *Semin Immunol* 22(1):54–58.
27. Siebert S, Bosch TCG (2009) Identification of a kazal-type serine protease inhibitor with potent anti-staphylococcal activity as part of Hydra 's innate immune system. *Dev Comp Immunol* 33:830–837.
28. Koskella B, Meaden S (2013) Understanding bacteriophage specificity in natural microbial communities. *Viruses* 5(3):806–823.
29. Duplessis M, Moineau S (2001) Identification of a genetic determinant responsible for host specificity in *Streptococcus thermophilus* bacteriophages. *Mol Microbiol* 41(2):325–336.
30. Fraune S, et al. (2015) Bacteria-bacteria interactions within the microbiota of the ancestral metazoan Hydra contribute to fungal resistance. *ISME J* 9:1543–1556.
31. Buckling A, Wei Y, Massey RC, Brockhurst MA, Hochberg ME (2006) Antagonistic coevolution with parasites increases the cost of host deleterious mutations. *Proc R Soc B Biol Sci* 273(1582):45–49.
32. Sekiguchi M, Takagi Y (1960) Effect of mitomycin C on the synthesis of bacterial and viral deoxyribonucleic acid. *BBA - Biochim Biophys Acta* 41(3):434–443.
33. Adams M (1959) Assay of phage by agar layer method. *Bacteriophages Intersci Publ Inc, New York, NY*:450–454.
34. Baym M, et al. (2015) Inexpensive multiplexed library preparation for megabase-sized genomes. *PLoS One* 10(5):1–15.
35. Bankevich A, et al. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19(5):455–77.
36. Aziz RK, et al. (2008) The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 9:75.
37. Brettin T, et al. (2015) RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes.

- Sci Rep* 5:8365.
38. Overbeek R, et al. (2014) The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42(Database issue):D206–14.
 39. Deatherage DE, Barrick JE (2014) Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using breseq. *Methods Mol Biol* 1151:165–188.
 40. R Core Team (2018) R: A Language and Environment for Statistical Computing.
 41. Wickham H, Francois R, Henry L, Müller K (2019) dplyr: A Grammar of Data Manipulation.
 42. Wandro S, et al. (2019) Predictable molecular adaptation of coevolving *Enterococcus faecium* and lytic phage EfV12-phi1. *Front Microbiol* 10(JAN):1–11.
 43. Lenhoff HM, Brown RD (1970) Mass culture of hydra: an improved method and its application to other aquatic invertebrates. *Lab Anim* 4(1):139–154.
 44. Álvarez B, Secades P, Prieto M, McBride MJ, Guijarro JA (2006) A mutation in *Flavobacterium psychrophilum* tlpB inhibits gliding motility and induces biofilm formation. *Appl Environ Microbiol* 72(6):4044–4053.
 45. Castillo D, Christiansen RH, Dalsgaard I, Madsen L, Middelboe M (2015) Bacteriophage Resistance Mechanisms in the Fish Pathogen *Flavobacterium psychrophilum*: Linking Genomic Mutations to Changes in Bacterial Virulence Factors. *Appl Environ Microbiol* 81(3):1157–1167.
 46. Calvio C, et al. (2005) Swarming differentiation and swimming motility in *Bacillus subtilis* are controlled by swrA, a newly identified dicistronic operon. *J Bacteriol* 187(15):5356–66.
 47. Fox J, Weisberg S (2011) *An {R} Companion to Applied Regression* (Sage, Thousand Oaks {CA}). Second.
 48. Hothorn T, Bretz F, Westfall P (2008) Simultaneous Inference in General Parametric Models. *Biometrical J* 50(3):346–363.
 49. Signorell A (2019) DescTools: Tools for Descriptive Statistics.
 50. Potts JR, Campbell ID (1996) Structure and Function of Fibronectin Modules. *Matrix Biol* 15:313–320.
 51. Campbell ID, Spitzfaden C (1994) Building proteins with fibronectin type III modules. *Curr Biol* 2:333–337.
 52. Haas TA, Plow EF (1994) Integrin-ligand interactions : a year in review. *Curr Opin Cell Biol* 6:656–662.
 53. Carr PA, Erickson HP, Palmer AG (1997) Backbone dynamics of homologous fibronectin type III cell adhesion domains from fibronectin and tenascin. *Structure* 5(7):949–959.
 54. Armbruster CR, Parsek MR (2018) New insight into the early stages of biofilm formation. *Proc Natl Acad Sci* 115(17):4317–4319.
 55. Cramton SE, Gerke C, Schnell NF, Nichols WW, Götz F (1999) The Intercellular Adhesion (ica) Locus Is Present in *Staphylococcus aureus* and Is Required for Biofilm

- Formation. *Infect Immun* 67(10):5427–5433.
56. Terashima H, Kojima S, Homma M (2008) Chapter 2: Flagellar Motility in Bacteria. *Int Rev Cell Mol Biol* 270:39–85.
 57. Waters JM, Fraser CI, Hewitt GM (2013) Founder takes all: density-dependent processes structure biodiversity. *Trends Ecol Evol* 28(2):78–85.
 58. P. LD, J. AR (2015) Competition for space during bacterial colonization of a surface. *J R Soc Interface* 12(110):20150608.
 59. Wein T, et al. (2018) Carrying Capacity and Colonization Dynamics of *Curvibacter* in the Hydra Host Habitat. *Front Microbiol* 9:443.
 60. Meaden S, Paszkiewicz K, Koskella B (2015) The cost of phage resistance in a plant pathogenic bacterium is context-dependent. *Evolution (N Y)* 69(5):1321–1328.
 61. Gama JA, et al. (2013) Temperate Bacterial Viruses as Double-Edged Swords in Bacterial Warfare. *PLoS One* 8(3):e59043.
 62. Davies E V, et al. (2016) Temperate phages enhance pathogen fitness in chronic lung infection. *ISME J* 10(10):2553–2555.
 63. Li X-Y, et al. (2017) Temperate phages as self-replicating weapons in bacterial competition. *J R Soc Interface* 14(137):20170563.
 64. Bertozzi Silva J, Storms Z, Sauvageau D (2016) Host receptors for bacteriophage adsorption. *FEMS Microbiol Lett* 363(4):1–11.
 65. Chaturongakul S, Ounjai P (2014) Phage-host interplay: Examples from tailed phages and Gram-negative bacterial pathogens. *Front Microbiol* 5(AUG):1–8.
 66. Choi Y, Shin H, Lee J-HH, Ryua S, Ryu S (2013) Identification and Characterization of a Novel Flagellum-Dependent *Salmonella*-Infecting Bacteriophage, iEPS5. *Appl Environ Microbiol* 79(16):4829–4837.
 67. Zhang H, Li L, Zhao Z, Peng D, Zhou X (2016) Polar flagella rotation in *Vibrio parahaemolyticus* confers resistance to bacteriophage infection. *Sci Rep* 6(May):1–12.
 68. Apel D, Surette MG (2008) Bringing order to a complex molecular machine: The assembly of the bacterial flagella. *Biochim Biophys Acta - Biomembr* 1778(9):1851–1858.
 69. Nambu T, Minamino T, Macnab RM, Kutsukake K (1999) Peptidoglycan-Hydrolyzing Activity of the FlgJ Protein, Essential for Flagellar Rod Formation in *Salmonella typhimurium*. *J Bacteriol* 181(5):1555–1561.
 70. Macnab RM (2004) Type III flagellar protein export and flagellar assembly. *Biochim Biophys Acta - Mol Cell Res* 1694(1-3 SPEC.ISS.):207–217.
 71. Herlihey FA, Moynihan PJ, Clarke AJ (2014) The essential protein for bacterial flagella formation FlgJ functions as a β -N-acetylglucosaminidase. *J Biol Chem* 289(45):31029–31042.
 72. Waters RC, O'Toole PW, Ryan KA (2007) The FliK protein and flagellar hook-length control. *Protein Sci* 16(5):769–780.
 73. Blair DF (2003) Flagellar movement driven by proton translocation. *FEBS Lett* 545(1):86–95.

| Chapter III |

74. Doyle TB, Hawkins AC, McCarter LL (2004) The complex flagellar torque generator of *Pseudomonas aeruginosa*. *J Bacteriol* 186(19):6341–6350.
75. Howard C. Berg, Robert A. Anderson (1973) Bacteria Swim by Rotating their Flagellar Filaments. *Nature* 245(245):380–382.
76. Pankov R, Yamada KM (2002) Fibronectin at a glance. *J Cell Sci* 115(Pt 20):3861–3.
77. Little E, Bork P, Doolittle RF (1994) Tracing the spread of fibronectin type III domains in bacterial glycohydrolases. *J Mol Evol* 39(6):631–643.
78. Hansen CK (1992) Fibronectin type III-like sequences and a new domain type in prokaryotic depolymerases with insoluble substrates. *FEBS Lett* 305(2):91–96.
79. Valk V, Kaaij RM van der, Dijkhuizen L (2017) The evolutionary origin and possible functional roles of FNIII domains in two *Microbacterium aurum* B8.A granular starch degrading enzymes, and in other carbohydrate acting enzymes. *Amylase* 1(1):1–11.
80. Jee JG, et al. (2002) Solution structure of the fibronectin type III domain from *Bacillus circulans* WL-12 chitinase A1. *J Biol Chem* 277(2):1388–1397.
81. Kataeva IA, et al. (2002) The fibronectin type 3-like repeat from the *Clostridium thermocellum* cellobiohydrolase CbHa promotes hydrolysis of cellulose by modifying its surface. *Appl Environ Microbiol* 68(9):4292–4300.
82. Tegtmeyer N, et al. (2010) A small fibronectin-mimicking protein from bacteria induces cell spreading and focal adhesion formation. *J Biol Chem* 285(30):23515–23526.
83. Rakhuba D V, Kolomiets EI, Dey ES, Novik GI (2010) Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. *Pol J Microbiol* 59(3):145–155.
84. Goodridge L, Gallaccio A, Griffiths MW (2003) Morphological , Host Range , and Genetic Characterization of Two Coliphages. 69(9):5364–5371.
85. Malki K, et al. (2015) Bacteriophages isolated from Lake Michigan demonstrate broad host-range across several bacterial phyla. *Virology* 531:1–5.
86. Czajkowski R, Ozymko Z, Zwirowski S, Lojkowska E (2014) Complete genome sequence of a broad-host-range lytic *Dickeya*. 3153–3155.
87. Miller ES, et al. (2003) Complete Genome Sequence of the Broad-Host-Range Vibriophage KVP40: Comparative Genomics of a T4-Related Bacteriophage. *J Bacteriol* 185(17):5220–5233.
88. Jensen EC, et al. (1998) Prevalence of Broad-Host-Range Lytic Bacteriophages of. *Microbiology* 64(2):575–580.
89. Sabouri S, Sepehrizadeh Z, Amirpour-rostami S, Skurnik M (2017) International Journal of Food Microbiology A minireview on the in vitro and in vivo experiments with anti-*Escherichia coli* O157 : H7 phages as potential biocontrol and phage therapy agents. *Int J Food Microbiol* 243:52–57.
90. Yu S, Ko K, Chen C, Chang Y (2000) Characterization of the Distal Tail Fiber Locus and Determination of the Receptor for Phage AR1 , Which Specifically Infects *Escherichia coli* O157 : H7. 182(21):5962–5968.
91. Wright RCT, Friman VP, Smith MCM, Brockhurst MA (2018) Cross-resistance is modular in bacteria-phage interactions. *PLoS Biol* 16(10):e2006057.

| Chapter III |

92. Lerondelle C, Poutrel B (1980) Bacteriophage treatment trials on a staphylococcal udder infection in lactating cows. *Ann Rech Vet* 11(4):421–426.
93. Oechslin F (2018) Resistance Development to Bacteriophages Occurring during Bacteriophage Therapy. *Viruses* 10(7):351.
94. Castillo D, et al. (2019) Phage defense mechanisms and their genomic and phenotypic implications in the fish pathogen *Vibrio anguillarum*. *FEMS Microbiol Ecol* 95(3):1–13.

General discussion

Bacteriophages - the most abundant entity in the world

Phages are by far the most abundant biological entity (1) and can be found in all environments where their potential bacterial hosts are present (2). Most of the bacteria can be found in the ocean, soil and terrestrial sub-surfaces (2, 3). Therefore, it is not surprising that different phage species can be found in each of these environments. Phages outcompete bacteria by at least 10-fold in the oceans (1). Here, phages play a crucial role in the oceanic biogeochemistry, can alter the hosts genotype through horizontal gene transfer and can influence the metabolisms of infected bacteria (4). Similar observations were reported in terrestrial and soil environments. In this context, phages are also suggested to be a major driver in bacterial abundance and diversity (5). They have been shown to act as genetic mobile elements, transferring different characteristics to all kinds of terrestrial and soil bacteria (6–9).

Beside natural environments the focus of several studies lies on the natural associated microbial community of animals and humans, forming a metaorganism (10, 11). For instance, the colon harbours, with an estimated number of 3.8×10^{13} cells, the majority of bacteria in humans (12). Although clearly inferior to the number in aquatic and terrestrial environments, bacteria are considered symbionts in the metaorganism context and play an important role in health and diseases of their hosts (11, 13). Since bacteriophages are able to infect bacteria and therefore might be able to influence the microbial community (14, 15), appreciating bacteriophages in this scenario is from great importance (2).

Environmental stressors and their potential in bacterial community alteration

Most phages in environments such as the human gut, seem to be temperate phages or prophages integrated in the bacterial host (6, 16–19). Therefore, it is not surprising that I could find several bacteria in association with *Hydra*, containing complete, intact or partial prophage signatures, of which six could be induced.

Prophage induction takes place, when the bacterial host is situated in a stressful situation (20). Therefore, extrinsic factors play a major role in prophage induction (PI). As it was supported by my experiments, the factors which trigger PI can vary greatly

and depend on the respective prophage. Phages from *Curvibacter* Mag1.1, and *Pseudomonas* Mag2.2 were inducible by an increased pH, while the phages of *Undibacterium* C1.1 and *Pelomonas* AEP2.2 were not inducible by pH but reacted to UV radiation. Similarly, Gifsy prophages in *Salmonella* are induced by Reactive Oxygen Species (21), while in *Helicobacter pylori* phages a shift toward an acidic pH has a great effect on viral production (22). In humans, prophages, including the shiga-toxin converting prophage of *Escherichia coli*, can be induced by several dietary factors, such as Fructose, short-chain fatty acids and commonly used soft-drinks (23, 24). As it was also observed in my study, prophage induction can be triggered by more than just one environmental effect. Phages from *Curvibacter* Mag1.1, and *Pseudomonas* Mag2.2 were induced by UV-radiation but most effectively by an alkaline pH. Corresponding, in *Nitrosospora multiformis* phages an acidic pH has the most drastic effect on prophage induction, whereas increasing levels of chromium and the toxic compound potassium cyanide also lead to an increase in viral production (25). Fitting to the results from this study, elevated nutrition does not have any impact on prophage induction in phages of marine *Synechococcus* species (26). The results from the latter study even suggest that nutrient increase has an inhibitory effect on prophage induction and viral production (26).

Contradictory to my study, *N. multiformis* phage AOB (25), the phage of *Burkholderia thailandensis* (27) and *Lactobacillus* phage ϕ LC3 were induced by elevating the temperature (28), which could not be observed in phage TJ1. Interestingly, phage TJ1 gets induced by decreasing the temperature to 12° C (*in vivo*), which is contrary to *N. multiformis* phage AOB (25) and the phage of *B. thailandensis* (27), which remain dormant at low temperatures. These differences can be explained by different culture conditions, which is crucial for the intrinsic prophage stability (20). The TJ1 induction was detected on the *Hydra* host, while the other studies, analyzing phage AOB and the phage of *B. thailandensis*, are conducted in liquid culture (25, 27). Moreover, the level of prophage induction is highly dependent on the cultivation method (20). For instance, the induction of *E. coli* prophage λ is two orders of magnitudes higher in mouse gut, compared to liquid culture (29). Similarly, the prophage of enterohemorrhagic *E. coli* displayed a three logs greater induction level *in vivo* compared to *in vitro* conditions (30). This higher induction rate *in vivo* is either due to environment triggered bacterial DNA damage (29, 30) or might be influenced by

bacterial growth phase (31, 32). For instance, *Bartonella henselae* and other *Bartonella* species phages are induced when the bacterial culture enter the stationary phase, which is expected to be a possible explanation for the fast death of the cell culture (32). Another study shows that prophages are rather induced under exponential growth (31).

Nevertheless in all cases (29–32), prophages are probably induced because of the high expression of genes involved in the SOS response of the bacterium (29, 30, 33). This higher induction rate can easily result in a disadvantage for the lysogenized bacteria, since benefits of harbouring a prophage highly depend on the induction rate: the higher this factor, the lower the advantage (29). However, studies have shown that at low frequencies, phage-mediated lysis is in fact beneficial and lead to a higher competitiveness of the surviving lysogenized population, compared to the phage-free bacteria (14, 34, 35). Here, released phages might have a great impact on the bacterial community, by killing its host bacterium and infecting and killing other susceptible bacteria that colonize the same niche.

Bacteriophage host range

To shape bacterial communities, phages need to possess a suitable host range. So far, bacteriophages are still considered species specific (15), meaning they can only infect a small set of host bacteria. While some phages can only infect their original host (36) and are highly specific to one bacterial antigen (37, 38), some are specific to a certain species and are able to infect several bacteria within that species (15). For instance, *Pseudomonas* phages isolated from the rhizosphere, are able to infect several *Pseudomonas* strains, including *P. aeruginosa*, *P. chlororaphis* and *P. stutzeri*. Such a minor host range could also be observed in one phage of my study, in the phage of *Curvibacter* Mag1.1.

Even though the species specificity of phages is the prevailing opinion, several studies already provided contradicting results. For example, the *Myoviridae* coli phages AR1 and LG1 can infect many serogroups and clonal populations of *E. coli* as well as several other members of the *Enterobacteriaceae* species (39). Phages isolated from lake Michigan showed an even broader host range by infecting bacteria spread through different phyla of bacteria (40). Consistently, several broad host range phages have been found in this study, infecting bacteria throughout several bacteria species, families and even classes. Therewith, the results of my study support the hypothesis

that phages can be both, specialists and generalists. Therefore, host range might not be a stable feature, but rather change over coevolutionary time (41).

The functional role of temperate phages within metaorganisms

Even though, several studies already described the abundance and composition of phages in metaorganisms, such as seaweed, corals, humans and *Hydra* (17, 42–46), their functional role remains a fundamental question.

The functional question of temperate phages within microbial communities has been addressed before in the metaorganism *Ciona intestinalis*. Here, phages have been shown to be induced by the host's variable region-containing chitin-binding proteins (VCBP) (47). Those temperate phages also display lytic activity against other members of the microbiome *in vitro*. In some cases, they show cross-order and cross-family infections (47), comparable to the broad host range of *Hydra* phages. Here, phage TJ1 can infect several bacteria within its own microbiome, suggesting that this phage might be used as an advantage in competition between symbiotic bacteria. Moreover, several common food ingredients can induce several prophages within bacteria in the human gut, which in turn are hypothesized to regulate bacterial abundance in the human gut (48). The latter study also strongly emphasized the importance of *in vivo* investigations as the next logical step (48).

The second chapter of my study focussed on these important *in vivo* dynamics, analysing the interaction between phage TJ1 and other bacterial colonizers (Fig 1). My results could substantiate that, beside host factors like antimicrobial peptides (49, 50) and kazal 2-type protease inhibitors (50–52), induced prophages can indeed play an important role within the bacterial community of metaorganisms (Fig 1). In *Hydra* the phage of the main colonizer *Curvibacter* (TJ1) infects and downregulates three of the four most abundant colonizers after *Curvibacter* on *Hydra*, following the “Killing the winner” theory (53, 54) (Fig. 1 (3)). *Curvibacter* itself is resistant to phage TJ1 infection, since repressors that are expressed to maintain the lysogenic state of bacteriophages also act as superinfection exclusion genes (55). Therewith, I could support the hypothesis that phage TJ1 acts as a self-replicating weapon against susceptible bacterial colonizers (56).

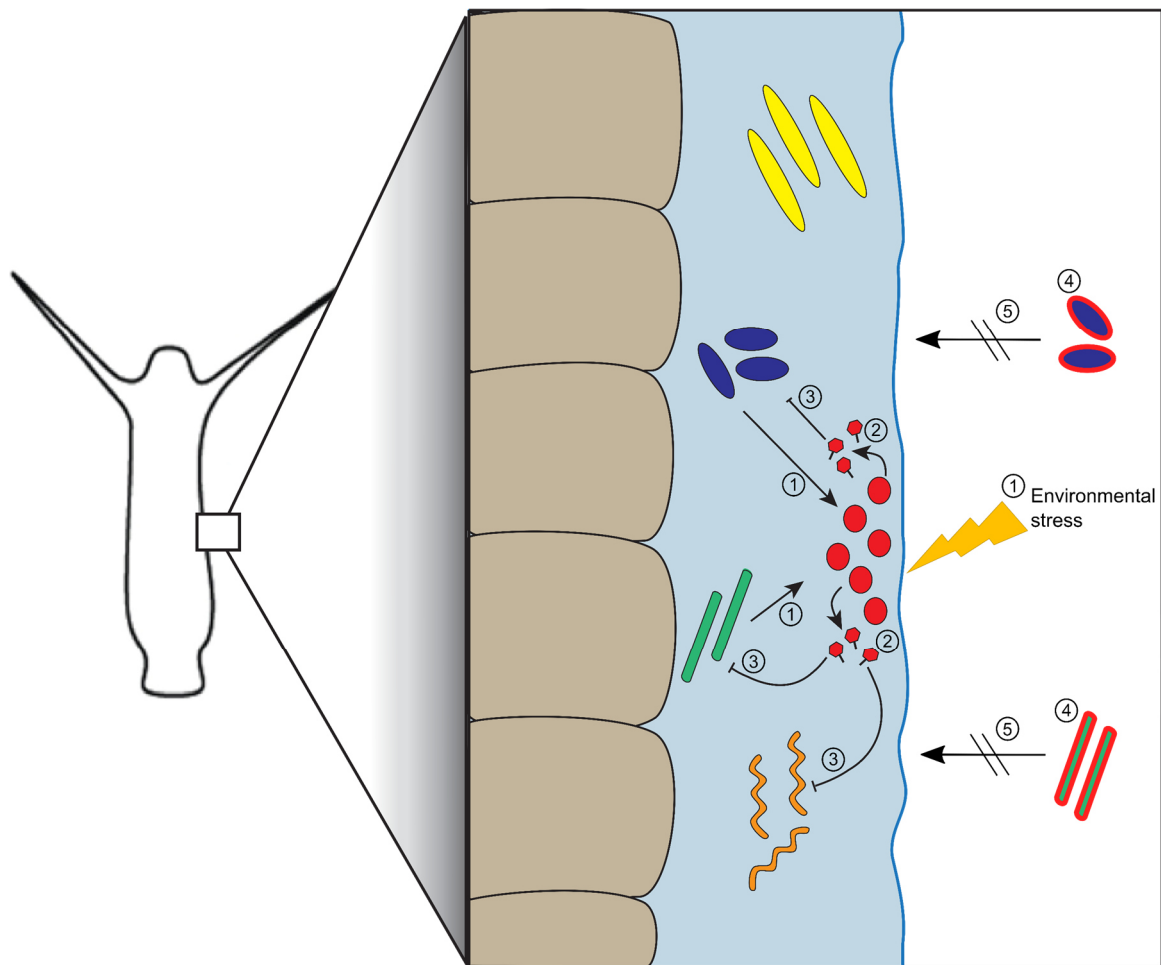


Figure 1: Schematic drawing of *Hydra*, with a magnified section of the ectodermal epithelial cells that are covered with a glycocalyx and the associated bacteria community. (1) Stressors from the environment or bacterial colonizer interact with *Curvibacter* (red), which results in (2) induction of prophage TJ1. (3) Released phage TJ1 is now able to kill and regulate the growth of other bacterial colonizers, like *Undibacterium*, *Duganella* and *Pelomonas*. (4) Susceptible bacteria may now evolve resistance against phage TJ1 (red outer line). (5) These resistant bacteria might lose their colonization ability and are outcompeted by susceptible bacteria.

Similar observations have been made in lysogens and phage sensitive *E. coli* strains (57) and *Pseudomonas aeruginosa* PAO-1 strains (35). In both cases temperate phages act as a weapon against susceptible bacteria and favour lysogens in competition with non-lysogens (35, 57).

In sponges, prophages are not shown to be weapons, but provide their bacterial host with another beneficial trait. Here, the Ankyphage provides its bacterial host with the ankyrin protein (ANKp), which modulates the eukaryotic host immune response against bacteria. Therewith, the Ankyphage-infected bacteria possesses a selective

advantage compared to their competitive bacteria, during the colonization of host tissue (58)

In summary, prophages play an important role within microbial communities, by shaping the composition of the microbiome. Here, temperate phages can function as weapons or provide their bacterial host with advantageous genes to outcompete competing bacteria. However, the effect of prophages is highly depending on the lysogenization rate. If competing bacteria get lysogenized, the new lysogens inherit either the same benefits as their former competitors, or get phage-resistant (35, 57). This results in the ceasing of the former benefit after a short period of time (35, 57). Interestingly, such an event could not be observed in this study, which eventuates in an ongoing benefit for the lysogen *Curvibacter*.

Bacteria-phage coevolution and the influence on metaorganisms

Phage-bacteria coevolution dynamics

Phage-bacteria coevolution is of great importance for microbial diversity, divergence and functionality in several ecosystems (59–62). There are several modes of ongoing coevolution dynamics (60). The first mode is termed “arms race dynamics” (ARD) and can be found in several bacteria-phage interactions, including the most intensively studied *Pseudomonas fluorescens* - podovirus Φ 2- system (59, 63) and *E.coli* – PP01 system (59, 64). Here, bacteria-phage interactions follow typical predator-prey dynamics (65, 66). Over time, bacteria evolve resistance (67–69) against bacteriophages, while phages counteract with increasing phage infectivity to overcome the initial resistance (70). The second mode is called “fluctuating selection dynamics” (FSD). Here, phage-mediated selection against common host resistance alleles drives changing allele frequencies in both, bacteria and phage populations (60). Moreover, early laboratory studies of bacteria-phage interactions, suggest that bacteria-phage coevolution is not indefinite but rather limited to a few cycles of evolutionary change (59, 65). After resistance emerges, the phage faces a resistant bacterial phenotype, that it is not able to overcome, due to its reduced genetic potential (59, 71). Here, resistance derives from *de novo* mutations that often cause changes in the bacterial surface structure and other typical phage targets (59, 72).

| General discussion |

In my study, the *in vitro* dynamics support the model of definite bacteria-phage dynamics. The bacteria rapidly evolve resistance against TJ1 via *de novo* mutations, with a maximum of two evolutionary cycles and a likely extinction of the phage at the end of the experiment. The fact that phage-susceptible bacteria fail to evolve resistance on *Hydra* aligns to studies in the mouse gut, which analyzed coevolutionary dynamics between the *E. coli* strain 55989 and three virulent bacteriophages. Comparable to my results, no resistance could be detected *in vivo*, while only two to six hours *in vitro* are sufficient for resistance development (73). These results can be interpreted two ways: Firstly, the metabolic state of the bacteria is crucial for bacteriophage infection and a principal barrier *in vivo*. Nutritional factors and stress responses differ between *in vitro* and *in vivo* conditions and can have severe impact on bacteria physiology, which is crucial for phage infection. Therefore, bacteria might have different opportunities for transient resistance to bacteriophages *in vivo*, without the need for evolving resistance, but with a susceptible phenotype *in vitro* (73).

Secondly, bacteriophage resistance can be costly for bacteria (Fig. 1 (4), (5)). Many of the resistance mechanisms that involve the alteration of the bacterial cell surface or appendages can come with significant fitness costs (74, 75). For instance, phage M^{SA}-resistant *Staphylococcus aureus* (76) and T4- and T7-resistant *Escherichia coli* (77) show a reduced growth rate compared to their respective ancestral strain and only display roughly 65 % of the ancestors fitness (77). In my study the growth rate of resistant bacteria was not affected, neither *in vitro*, nor *in vivo*. Nevertheless, the number of bacteria was significantly lower on polyps recolonized with resistant bacteria, compared to polyps recolonized with the respective ancestor (Fig. 1 (5)). Similar context-related resistance fitness costs can be observed in two more systems: Firstly, in phage-resistant opportunistic plant pathogen *Pseudomonas syringae pathovar tomato*, resistant strains show a reduced recolonization capacity in their natural environment, compared to the ancestor (78). Interestingly, no competitive costs in liquid culture conditions could be observed (78). Secondly, in the intracellular *Listeria monocytogenes*, phage resistance against phages A511 and A500, significantly reduces bacteria virulence by preventing the bacteria invasion and replication in human cells (79).

In both cases, the bacteria maintain their ability to invade the host at the cost of bacteriophage sensitivity. Notably, both studies were conducted on pathogenic

bacteria. To my best knowledge, this study is the first to report such a phenotype in symbiotic bacteria within a metaorganism. Here, resistance adversely affect the resistant bacteria *in vivo*, by interfering with the recolonization process (Fig. 1 (5)).

Phage-bacteria interactions as a possible explanation for colonization pattern

Understanding bacterial traits which are important for the bacterial colonization process within metaorganism is of great interest. Why can only some bacteria stably colonize the host environment while others fail to do so?

Physicochemical conditions, such as intestinal motility, pH value, redox conditions, nutrients and host secretions (e.g. antimicrobial peptides) vary between individuals and can severely impact the colonization success and composition of bacteria (80). Additionally, bacteria colonization can be influenced by other factors, like stress, antibiotic drugs, non-antibiotic drugs and diet (80–82). Due to the fact that eukaryotic organisms evolved in a microbial world, associated bacteria and their host have coevolved for a long time (83, 84). Commensals occupied several eukaryotic niches, including the well-described gastrointestinal tract (84). Therefore, bacterial adaptation is also an important factor in bacteria-host interactions and successful colonization (84, 85). For example, in zebrafish, adaptations during early colonization enhance immigration from the environment to the host. Intra-host-specific adaptations become traceable and more important at later stages of colonization (86).

Beside the factors mentioned above, prophages may also have a major influence on colonization. The prophage of the pathogen *Vibrio cholerae* encodes for an accessory colonization factor within the genomic pathogenicity island. The phage allows its host to adapt to and colonize the human intestinal tract (87–90). *Pseudomonas aeruginosa* prophage Pf4 suppresses host phagocytosis and thereby facilitates establishment of *P. aeruginosa* and infections in a mouse wound model (91). Similar events are also observable in non-pathogenic models. For instance, the ankyphage of a sponge symbiont provides its bacterial host with more efficient colonization capacity by evading the sponge-initiated phagocytosis (58). In the human gut two prophages (ϕ V1 and ϕ V7) of commensal *Enterococcus faecalis* can infect other closely related *Enterococcus faecalis* strains. It is proposed that these phages are used to maintain the dominance of their host and play an important role in the mammalian intestine ecosystem (92). My study also supports the hypothesis that phages influence

colonization: Initially susceptible bacteria that have evolved TJ1-resistance displayed a decreased colonization ability, compared to susceptible bacteria.

Carrying capacity describes the maximum number of individuals an environment can support and is defined by a number of factors, including food, macro and micronutrients and space (93). This is true for bacteria in natural environments (94), as well as on their respective hosts (95–98). *Hydra* has a limited carrying capacity, meaning that the polyp as a habitat is finite and only a restricted number of bacteria are able to colonize until the maximum microbial load is reached (95). Hence, the bacteria which colonize *Hydra* first and in a higher amount are more likely to dominate, following the “founder takes all” (FTA) hypothesis. The FTA thesis describes the evolutionary advantage of an organism that settles first in a particular environment, which leads to a rapid expansion of those founders and the effective blocking of the establishment of latecomers (99). This theory can explain various biological processes, like postglacial and island chain colonization, but also microbial sectoring (99). The latter is not necessarily dependent on selective forces but can rather be explained by density-dependent processes (99). Given the fact, that resistant strains in my study show a lower CFU per polyp in total, while showing the same growth rate, the FTA theory might apply here. Phage-sensitive *Duganella* and *Pelomonas* colonies expand rapidly on the *Hydra* host and hinder the establishment of the later, phage-resistant colonizers.

In summary, my results elucidate that phages can influence the colonization process of bacteria in microbial communities. Since carrying a phage-resistance is costly in terms of colonization efficiency, susceptibility seems to be favoured on *Hydra*. This fact in turn can enable coexistence of temperate phages and susceptible bacteria on the eukaryotic host.

References

1. Suttle C a (2007) Marine viruses--major players in the global ecosystem. *Nat Rev Microbiol* 5(10):801–812.
2. Clokie MRJ, Millard AD, Letarov A V., Heaphy S (2011) Phages in nature. *Bacteriophage* 1(1):31–45.
3. Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: The unseen majority. *Proc Natl Acad Sci U S A* 95(12):6578–6583.
4. Breitbart M, Bonnain C, Malki K, Sawaya NA (2018) Phage puppet masters of the marine microbial realm. *Nat Microbiol* 3(7):754–766.
5. Weinbauer MG (2004) Minireview Are viruses driving microbial diversification and diversity ? 6:1–11.
6. Armon R (2011) Soil Bacteria and Bacteriophages. *Biocommunication in Soil Microorganisms*, pp 403–412.
7. Ashelford KE, Day MJ, Bailey MJ, Lilley AK, Fry JC (1999) In situ population dynamics of bacterial viruses in a terrestrial environment. *Appl Environ Microbiol* 65(1):169–174.
8. Dröge M, Pühler A, Selbitschka W (1999) Horizontal gene transfer among bacteria in terrestrial and aquatic habitats as assessed by microcosm and field studies. *Biol Fertil Soils* 29(3):221–245.
9. Silander OK, et al. (2005) Widespread genetic exchange among terrestrial bacteriophages. *Proc Natl Acad Sci* 102(52):19009–19014.
10. Bosch TCG, McFall-Ngai MJ (2011) Metaorganisms as the new frontier. *Zoology* 114(4):185–190.
11. Esser D, et al. (2018) Functions of the Microbiota for the Physiology of Animal Metaorganisms. *J Innate Immun.* 11:393-404
12. Sender R, Fuchs S, Milo R (2016) Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol* 14(8):e1002533.
13. Sommer F, Backhed F (2013) The gut microbiota--masters of host development and physiology. *Nat Rev Microbiol* 11(4):227–238.
14. Harrison E, Brockhurst MA (2017) Ecological and Evolutionary Benefits of Temperate Phage : What Does or Doesn ' t Kill You Makes You Stronger. *BioEssays* 39.
15. Koskella B, Meaden S (2013) Understanding bacteriophage specificity in natural microbial communities. *Viruses* 5(3):806–823.
16. Stern A, Mick E, Tirosh I, Sagy O, Sorek R (2012) CRISPR targeting reveals a reservoir of common phages associated with the human gut microbiome. *Genome Res* 22(10):1985–1994.
17. Minot S, et al. (2011) The human gut virome: Inter-individual variation and dynamic response to diet. *Genome Res* 21:1616–1625.
18. Manrique P, Dills M, Young MJ (2017) The human gut phage community and its implications for health and disease. *Viruses* 9(6):9–11.
19. Furuse K, et al. (1983) Bacteriophage distribution in human faeces: Continuous survey of healthy subjects and patients with internal and leukaemic diseases. *J Gen Virol* 64(9):2039–2043.
20. Nanda AM, Thormann K, Frunzke J (2015) Impact of spontaneous prophage induction on the fitness of bacterial populations and host-microbe interactions. *J Bacteriol* 197(3):410–419.
21. Figueroa-Bossi N, Bossi L (1999) Inducible prophages contribute to Salmonella

| General discussion |

- virulence in mice. *Mol Microbiol* 33(1):167–176.
22. Alves de Matos AP, Lehours P, Timóteo A, Roxo-Rosa M, Vale FF (2013) Comparison of induction of B45 Helicobacter pylori prophage by acid and UV radiation. *Microsc Microanal* 19(S4):27–28.
 23. Pierzynowska K, et al. (2018) Effects of some commonly used drinks on induction of Shiga toxin-converting prophage in Escherichia coli. *J fur Verbraucherschutz und Leb* 13(2):125–129.
 24. Oh JH, et al. (2019) Dietary Fructose and Microbiota-Derived Short-Chain Fatty Acids Promote Bacteriophage Production in the Gut Symbiont Lactobacillus reuteri. *Cell Host Microbe* 25(2):273–284.e6.
 25. Choi J, Kotay SM, Goel R (2010) Various physico-chemical stress factors cause prophage induction in Nitrosospira multiformis 25196- an ammonia oxidizing bacteria. *Water Res* 44(15):4550–4558.
 26. L. M, J.H. P (2005) Effect of nutrient addition and environmental factors on prophage induction in natural populations of marine Synechococcus species. *Appl Environ Microbiol* 71(2):842–850.
 27. Shan J, et al. (2014) Temperature dependent bacteriophages of a tropical bacterial pathogen. *Front Microbiol* 5(NOV):1–7.
 28. Lunde M, Aastveit AH, Blatny JM, Nes IF (2005) Effects of diverse environmental conditions on ϕ LC3 prophage stability in Lactococcus lactis. *Appl Environ Microbiol* 71(2):721–727.
 29. De Paepe M, et al. (2016) Carriage of λ Latent Virus Is Costly for Its Bacterial Host due to Frequent Reactivation in Monoxenic Mouse Intestine. *PLoS Genet* 12(2):1–20.
 30. Tyler JS, et al. (2013) Prophage Induction Is Enhanced and Required for Renal Disease and Lethality in an EHEC Mouse Model. *PLoS Pathog* 9(3):e1003236.
 31. Madera C, García P, Rodríguez A, Suárez JE, Martínez B (2009) Prophage induction in Lactococcus lactis by the bacteriocin Lactococcin 972. *Int J Food Microbiol* 129(1):99–102.
 32. Chenoweth MR, Somerville GA, Krause DC, O'textquoterightReilly KL, Gherardini FC (2004) Growth Characteristics of Bartonella henselae in a Novel Liquid Medium: Primary Isolation, Growth-Phase-Dependent Phage Induction, and Metabolic Studies. *Appl Environ Microbiol* 70(2):656–663.
 33. Nanda AM, et al. (2014) Analysis of SOS-Induced Spontaneous Prophage Induction in Corynebacterium glutamicum at the Single-Cell Level. 196(1):180–188.
 34. Brown SP, Le Chat L, De Paepe M, Taddei F (2006) Ecology of Microbial Invasions: Amplification Allows Virus Carriers to Invade More Rapidly When Rare. *Curr Biol* 16(20):2048–2052.
 35. Burns N, James CE, Harrison E (2014) Polylysogeny magnifies competitiveness of a bacterial pathogen in vivo. *Evol Appl* 8(4):346–351.
 36. Wang K, Chen F (2008) Prevalence of highly host-specific cyanophages in the estuarine environment. *Environ Microbiol* 10(2):300–312.
 37. Kudva IT, Jelacic S, Tarr PI, Youderian P, Hovde CJ (1999) Biocontrol of Escherichia coli O157 with O157-Specific Bacteriophages. *Appl Environ Microbiol* 65(9):3767–3773.
 38. Gross RJ, Cheasty T, Rowe B (1977) Isolation of Bacteriophages Specific for the Ki Polysaccharide Antigen of Escherichia coli. *J Clin Microbiol* 6(6):548–550.
 39. Goodridge L, Gallaccio A, Griffiths MW (2003) Morphological , Host Range , and Genetic Characterization of Two Coliphages. 69(9):5364–5371.

| General discussion |

40. Malki K, et al. (2015) Bacteriophages isolated from Lake Michigan demonstrate broad host-range across several bacterial phyla. *Virol J*:1–5.
41. Ross A, Ward S, Hyman P (2016) More Is Better: Selecting for Broad Host Range Bacteriophages. *Front Microbiol* 7(September):1–6.
42. Lachnit T, Thomas T, Steinberg P (2016) Expanding our Understanding of the Seaweed Holobiont : RNA Viruses of the Red Alga *Delisea pulchra*. *Front Microbiol* 6(January):1–12.
43. Weynberg KD, Wood-Charlson EM, Suttle CA, van Oppen MJH, Oppen MJH van (2014) Generating viral metagenomes from the coral holobiont. *Front Microbiol* 5(MAY):1–11.
44. Rascovan N, Duraisamy R, Desnues C (2016) Metagenomics and the Human Virome in Asymptomatic Individuals. *Annu Rev Microbiol* 70:125–141.
45. Abeles SR, et al. (2014) Human oral viruses are personal , persistent and gender-consistent. *ISME J* 8(9):1753–1767.
46. Grasis JA, et al. (2014) Species-specific viromes in the ancestral holobiont hydra. *PLoS One* 9(10):e109952.
47. Leigh BA (2017) Role of viruses within metaorganisms: *Ciona intestinalis* as a model system.
48. Boling L, et al. (2020) Dietary prophage inducers and antimicrobials: toward landscaping the human gut microbiome. *Gut Microbes* 00(00):1–14.
49. Franzenburg S, et al. (2013) Distinct antimicrobial peptide expression determines host species-specific bacterial associations. *Proc Natl Acad Sci U S A* 110:E3730-8.
50. Augustin R, Fraune S, Bosch TCG (2010) How Hydra senses and destroys microbes. *Semin Immunol* 22(1):54–58.
51. Bosch TCG, et al. (2009) Uncovering the evolutionary history of innate immunity: The simple metazoan Hydra uses epithelial cells for host defence. *Dev Comp Immunol* 33(4):559–569.
52. Siebert S, Bosch TCG (2009) Identification of a kazal-type serine protease inhibitor with potent anti-staphylococcal activity as part of Hydra ' s innate immune system. *Dev Comp Immunol* 33:830–837.
53. Thingstad TF (2000) Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *45(6):1320–1328.*
54. Weinbauer MG (2004) Ecology of prokaryotic viruses. *FEMS Microbiol Rev* 28(2):127–181.
55. Bondy-Denomy J, Davidson AR (2014) When a Virus is not a Parasite : The Beneficial Effects of Prophages on Bacterial Fitness. *J Microbiol* 52(3):235–242.
56. Li X-Y, et al. (2017) Temperate phages as self-replicating weapons in bacterial competition. *J R Soc Interface* 14(137):20170563.
57. Gama JA, et al. (2013) Temperate Bacterial Viruses as Double-Edged Swords in Bacterial Warfare. *PLoS One* 8(3):e59043.
58. Jahn MT, et al. (2019) A Phage Protein Aids Bacterial Symbionts in Eukaryote Immune Evasion. *Cell Host Microbe* 26(4):542–550.
59. Koskella B, Brockhurst MA (2014) Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS Microbiol Rev* 38(5):916–931.
60. Brockhurst MA, Koskella B (2013) Experimental coevolution of species interactions.

Trends Ecol Evol 28(6):367–375.

61. Scanlan PD (2017) Bacteria–Bacteriophage Coevolution in the Human Gut: Implications for Microbial Diversity and Functionality. *Trends Microbiol* 25(8):614–623.
62. Scanlan PD, et al. (2015) Coevolution with bacteriophages drives genome-wide host evolution and constrains the acquisition of abiotic-beneficial mutations. *Mol Biol Evol* 32(6):1425–1435.
63. Buckling A, Rainey PB (2002) Antagonistic coevolution between a bacterium and a bacteriophage. *Proc R Soc L* 269:931–936.
64. Woolhouse MEJ, Webster JP, Domingo E, Charlesworth B, Levin BR (2002) Biological and biomedical implications of the co-evolution of pathogens and. *Nat Genet* 32:569–577.
65. Chao L, Levin BR, Stewart FM (1977) A Complex Community in a Simple Habitat: An Experimental Study with Bacteria and Phage. *Ecology* 58(2):369–378.
66. Levin BR, Stewart FM, Chao L (1977) Resource-Limited Growth, Competition, and Predation: A Model and Experimental Studies with Bacteria and Bacteriophage. *Am Nat* 111(977):3–24.
67. Garneau JE, et al. (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* 468:67.
68. Meeske AJ, Nakandakari-Higa S, Marraffini LA (2019) Cas13-induced cellular dormancy prevents the rise of CRISPR-resistant bacteriophage. *Nature*.
69. Rostøl JT, Marraffini L (2019) (Ph)ighting Phages: How Bacteria Resist Their Parasites. *Cell Host Microbe* 25(2):184–194.
70. Samson JE, Magadán AH, Sabri M, Moineau S (2013) Revenge of the phages: defeating bacterial defences. *Nat Rev Microbiol* 11(10):675.
71. Dennehy JJ (2012) What Can Phages Tell Us about Host-Pathogen Coevolution? *Int J Evol Biol* 2012:1–12.
72. Lenski RE, Levin BR (1985) Constraints on the Coevolution of Bacteria and Virulent Phage: A Model, Some Experiments, and Predictions for Natural Communities. *Am Nat* 125(4):585–602.
73. De Sordi L, Lourenço M, Debarbieux L (2019) “I will survive”: A tale of bacteriophage-bacteria coevolution in the gut. *Gut Microbes* 10(1):92–99.
74. Brockhurst MA, Buckling A, Rainey PB (2005) The effect of a bacteriophage on diversification of the opportunistic bacterial pathogen, *Pseudomonas aeruginosa*. *Proc R Soc B Biol Sci* 272(1570):1385–1391.
75. Oechslin F (2018) Resistance Development to Bacteriophages Occurring during Bacteriophage Therapy. *Viruses* 10(7):351.
76. Capparelli R, et al. (2010) Bacteriophage-Resistant *Staphylococcus aureus* Mutant Confers Broad Immunity against Staphylococcal Infection in Mice. *PLoS One* 5(7):1–13.
77. Lenski RE (1988) EXPERIMENTAL STUDIES OF PLEIOTROPY AND EPISTASIS IN *ESCHERICHIA COLI*. 1. VARIATION IN COMPETITIVE FITNESS AMONG MUTANTS RESISTANT TO VIRUS. *Evolution (N Y)* 42(3):425–432.
78. Meaden S, Paszkiewicz K, Koskella B (2015) The cost of phage resistance in a plant pathogenic bacterium is context-dependent. *Evolution (N Y)* 69(5):1321–1328.
79. Sumrall ET, et al. (2019) Phage resistance at the cost of virulence: *Listeria monocytogenes* serovar 4b requires galactosylated teichoic acids for InIB-mediated invasion. *PLoS Pathog* 15(10):1–29.

| General discussion |

80. Zhang YJ, et al. (2015) Impacts of gut bacteria on human health and diseases. *Int J Mol Sci* 16(4):7493–7519.
81. David LA, et al. (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505(7484):559–563.
82. Maier L, et al. (2018) Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* 555(7698):623–628.
83. Deines P, Hammerschmidt K, Bosch TC (2019) The effect of symbiosis on symbiont fitness – interactions within a simple metaorganism. *bioRxiv*:609271.
84. Gerritsen J, Smidt H, Rijkers GT, de Vos WM (2011) Intestinal microbiota in human health and disease: the impact of probiotics. *Genes Nutr* 6(3):209–240.
85. Rezzonico E, et al. (2011) Bacterial adaptation to the gut environment favors successful colonization: microbial and metagenomic characterization of a simplified microbiota mouse model. *Gut Microbes* 2(6):307–318.
86. Robinson CD, et al. (2018) Experimental bacterial adaptation to the zebrafish gut reveals a primary role for immigration. *PLoS Biol* 16(12):1–26.
87. Hou Y-M (1999) Transfer RNAs and pathogenicity islands. *Trend Biochem Sci* 24(8):295–298.
88. Kovach ME, Shaffer MD, Peterson KM (1996) A putative integrase gene defines the distal end of a large cluster of ToxR-regulated colonization genes in *Vibrio cholerae*. *Microbiology* 142(8):2165–2174.
89. KARAOLIS DKR, et al. (1998) A *Vibrio cholerae* pathogenicity island associated with epidemic. *Proc Nat Acad Sci USA* 95(March):3134–3139.
90. Karaolis DKR, Somara S, Maneval DR, Johnson JA, Kaper JB (1999) A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. *Nature* 399(6734):375–379.
91. Sweere JM, et al. (2019) Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. *Science* (80-) 363(6434).
92. Duerkop BA, Clements C V., Rollins D, Rodrigues JLM, Hooper L V. (2012) A composite bacteriophage alters colonization by an intestinal commensal bacterium. *Proc Natl Acad Sci U S A* 109(43):17621–17626.
93. McArthur J. (2006) *Microbial Ecology: An Evolutionary Approach* (Academic Press, Oxford, UK).
94. Patra P, Klumpp S (2013) Population Dynamics of Bacterial Persistence. *PLoS One* 8(5):e62814.
95. Wein T, et al. (2018) Carrying Capacity and Colonization Dynamics of *Curvibacter* in the Hydra Host Habitat. *Front Microbiol* 9:443.
96. Pais IS, Valente RS, Sporniak M, Teixeira L (2018) *Drosophila melanogaster* establishes a species-specific mutualistic interaction with stable gut-colonizing bacteria. *PLOS Biol* 16(7):1–45.
97. Jemielita M, et al. (2014) Spatial and Temporal Features of the Growth of a Bacterial Species Colonizing the Zebrafish Gut. *MBio* 5(6).
98. Remus-Emsermann MNP, Tecon R, Kowalchuk GA, Leveau JHJ (2012) Variation in local carrying capacity and the individual fate of bacterial colonizers in the phyllosphere. *ISME J* 6(4):756–765.
99. Waters JM, Fraser CI, Hewitt GM (2013) Founder takes all: density-dependent processes structure biodiversity. *Trends Ecol Evol* 28(2):78–85.

Conclusion

In this study I highlight the abundance and diversity of different temperate bacteriophages within the metaorganism *Hydra*. Moreover, I could demonstrate the importance and functional role of the dominant phage TJ1 in the bacterial community of the metaorganism *Hydra*.

The first chapter confirmed the presence of several prophages in *Hydra*-associated bacteria. These temperate phages demonstrate a wide variety in terms of phenotype, sensitivity to environmental stress and host range. All these factors are key elements to determine their potential benefits or disadvantage for their bacterial hosts and the whole metaorganism.

After describing prophages of *Hydra*-associated bacteria, the following two chapters show the importance of the dominant *Curvibacter* phage TJ1 within *Hydra*'s microbial community. I could show that phage TJ1 is important for the maintenance of the bacterial community composition, by regulating the growth and colonization of other bacteria on *Hydra* in mono-colonization and in complex bacterial communities.

Susceptible bacteria seem to not evolve resistance against phage TJ1, since no resistant strains could be recovered from *Hydra* polyps. This phenomenon can be explained by the fact that resistance, acquired under liquid culture conditions, interferes with bacteria-*Hydra* interactions, resulting in a reduced colonization efficiency. These results emphasize the importance of bacteria-phage interactions for colonization and shed light on how bacteria and bacteriophages are able to coexist on a eukaryotic host: Susceptibility might be favoured in some environments, due to the high cost of resistance.

Overall, my results show that prophages play an important role within microbial communities and can indeed act as a non-host derived factor that contributes to shaping and maintaining the bacterial community composition within metaorganisms.

List of publications

Janina Lange, Sebastian Fraune, Thomas C.G. Bosch, Tim Lachnit: The neglected part of the microbiome: Prophage TJ1 regulates the bacterial community of the metaorganism *Hydra*. bioRxiv, 2019, doi: <https://doi.org/10.1101/607325>

Janina Lange, Stephen Wandro, Stefan Riedel, Thomas C.G. Bosch, Katrine Whiteson, Tim Lachnit: Evolution of temperate phage resistance interferes with bacterial colonisation on *Hydra*. 2020

Daniel Castillo, Nanna Rørbo, Jóhanna Jørgensen, **Janina Lange**, Demeng Tan, Panos G Kalatzis, Sine Lo Svenningsen, Mathias Middelboe, Phage defense mechanisms and their genomic and phenotypic implications in the fish pathogen *Vibrio anguillarum*, *FEMS Microbiology Ecology*, Volume 95, Issue 3, March 2019, fiz004, <https://doi.org/10.1093/femsec/fiz004>

Esser D, **Lange J**, Marinos G, Sieber M, Best L, Prasse D, Bathia J, Rühlemann M, C, Boersch K, Jaspers C, Sommer F: Functions of the Microbiota for the Physiology of Animal Metaorganisms. *J Innate Immun* 2019; 11:393-404. doi:0.1159/000495115

Jinru He, **Janina Lange**, Georgios Marinos, Jay Bathia, Danielle Harris, Ryszard Soluch, Vaibhvi, Peter Deines, M. Amine Hassani, Kim-Sara Wagner, Roman Zapien-Campos, Cornelia Jaspers, Felix Sommer: Advancing our functional understanding of host-microbiota interactions: A need for new types of studies; *BioEssays* 2019, 1900211. <https://doi.org/10.1002/bies.201900211>

Acknowledgements

I would like to extend my sincere thanks to Professor Dr. Thomas Bosch, who gave me the opportunity to do my PhD in his group. Thank you for your critical feedback and all your thoughts and advices for my thesis and papers. I always enjoyed working in the Bosch lab, a time, I will be always grateful for.

I'm extremely grateful to Dr. Tim Lachnit. I'd like to thank you for the trust you put in me by making me your first PhD student. Thank you for the extraordinary supervision, motivating discussions and your help and support at all times. You have left a formative influence on me and shaped me not only as a scientist but also as a person.

Special appreciation to the members of my thesis committee, Prof. Dr. Thomas Roeder, Prof. Dr. Unterweger and Prof. Dr. Gorb for the time they dedicated for the evaluation of this work.

Many thanks to Professor Dr. Katrine Whiteson and the whole Whiteson lab. Part of this work would have not been possible without your support, input and commitment. Thanks for putting your faith in me and giving me the opportunity to continue my science journey with you.

I would also like to extend my deepest gratitude to Anika Hintz. You did not only organize my life as a graduate student in a bureaucratic world but became one of my closest friends. The PhD journey was not easy, but I knew I can always count on you. Thank you for listening whenever I was struggling, thanks for always being there for me and thank you for your friendship.

I want to thank Dr. Sebastian Fraune, Dr. Peter Deines and Dr. Alexander Klimovich for always having a sympathetic ear for me. Thank you for all the fruitful discussions about my newest results and the helpful hints about where to go from there.

I would like to thank Jay Bathia. You were my colleague first, but soon became a close friend. Thank you for sharing my nerdiness and the extensive discussions about Marvel, DC, Harry Potter and science of course. Thanks for being the best movie buddy ever!

| Acknowledgements |

I am glad to call Kai Rathje and Katja Schröder not only colleagues but also my friends. Thank you for all your support throughout the years, your sympathy, your input and for a wellness weekend, I will never forget.

The completion of my dissertation would not have been possible without Jörg Wittlieb, Doris Willoweit-Ohl, Eva Herbst and Maria Franck. You bring order into the lab chaos and always took care that I had all the protocols, equipment and transgenic *Hydra* I needed. Thank you for the support and the interesting discussions at the coffee table.

My deepest thanks go to all my friends and my husband Timo. Thanks for bearing me when I had a hard day in the lab, was devastated by another rejection or was just tired and hungry. Thanks for being my shoulder to lean on and all your encouraging pep talks.

Finally, I want to thank my parents. Thanks for your support, your faith and all the love you gave me throughout my whole life. Nothing of this would have been possible without you! Thank you!

Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation, nach Regeln guter wissenschaftlicher Praxis, eigenständig verfasst und keine anderen als die angegebenen Hilfsmittel und Quellen benutzt habe. Dabei habe ich keine Hilfe, außer der wissenschaftlichen Beratung durch meinen Doktorvater Prof. Dr. Dr. h.c. Thomas C. G. Bosch in Anspruch genommen. Des Weiteren erkläre ich, dass ich noch keinen Promotionsversuch unternommen habe, diese Arbeit noch nicht im Rahmen eines Prüfungsverfahrens vorgelegt wurde und Teile dieser Arbeit bereits zur Publikation eingereicht wurden. Darüber hinaus wurde mir noch kein akademischer Grad entzogen.

Kiel, den

Janina Lange