

**Inter-kingdom communication:  
quorum sensing and quorum quenching  
in the metaorganism Hydra**

**Dissertation**

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## Summary

Every metaorganism consisting of a host and its associated microbes needs to maintain its homeostasis. Bacteria are controlling their community wide behavior, as virulence and biofilm formation, by their communication system, called quorum sensing (QS). As a host is interested in suppressing such processes, the interference with QS systems (quorum quenching, QQ) could be an instrument for a host to regulate the behavior of its bacteria.

In this thesis, it could be proven that six commensal bacteria of *Hydra vulgaris* (AEP) and six bacteria isolated from the Hydra environment are producing *N*-acyl-homoserine-lactones (AHLs), a class of QS signaling molecules. By analyzing the QS system of *Curvibacter* sp., the main colonizer of *Hydra vulgaris* (AEP), it could be shown that it can detect long-chain 3-hydroxy-HSLs (3OH-HSLs) as well as long-chain 3-oxo-HSLs (3O-HSLs) and is able to produce 3OHC12-HSLs. In contrast, a new host mechanism could be identified, which enables Hydra to modify specifically long-chain 3O-HSLs via an oxidoreductase activity to the 3OH-HSL counterpart. Consequently, Hydra's QQ activity promotes *Curvibacter*'s 3OH-HSL regulated processes. Transcriptional expression data revealed that the *Curvibacter* sp. possess a differential response to 3OHC12-HSLs compared to 3OC12-HSLs. While genes involved in flagella biosynthesis were significantly induced by 3OC12-HSLs, the 3OHC12-HSLs promote the expression of a trans-AT polyketide synthase cluster potentially exhibiting antibacterial or antifungal activity. Therefore, host QQ represses bacterial motility and promotes the production of bioactive compounds of its commensal bacteria.

Interestingly, investigating the impact of AHL modification on metaorganism assembly *in vivo* revealed, that 3OHC12-HSLs promote, while 3OC12-HSLs repress colonization of the host by its main colonizer *Curvibacter* sp. Similar to 3O-HSLs, also flagellin led to a strong reduction of the bacterial load. These findings indicate that 3O-HSLs induce the production of flagella in *Curvibacter* sp., which cause a response in Hydra, finally resulting in a decreased bacterial community within the metaorganism Hydra. These insights show for the first time, that a host organism is manipulating bacterial QS signals *in vivo* and thereby maintain symbiotic function of its bacterial colonizers, which contribute to the homeostasis of the metaorganism.

In cooperation with Xiang-Yi Li the interaction between the two main colonizers of *Hydra vulgaris* (AEP) was investigated by a biological and a theoretical approach. In a double culture experiment it could be shown that the two bacteria are interacting with each other during their growth. Such cooperations are necessary to get an overall understanding of the complex regulatory network within metaorganisms.

## Zusammenfassung

Jeder Metaorganismus, bestehend aus einem Wirt und seinen assoziierten Mikroben, muss seine Homöostase erhalten. Bakterien kontrollieren ihr Gemeinschaftsverhalten, wie beispielsweise die Regulation von Virulenz und Biofilmbildung, mittels ihres Kommunikationssystems, dem so genannten Quorum sensing (QS). Da ein Wirt daran interessiert ist solche Prozesse zu unterdrücken, könnte das Eingreifen in QS-Systeme (Quorum quenching, QQ) eine Möglichkeit darstellen um das Verhalten seiner Bakterien zu regulieren.

In dieser Arbeit konnte gezeigt werden, dass sechs commensale Bakterien von *Hydra vulgaris* (AEP) und sechs Bakterien, die aus der Umgebung von Hydra isoliert wurden, *N*-Acyl-Homoserin-Lactone (AHLs) produzieren, eine Klasse von QS-Signalmolekülen. Durch Analyse des QS-Systems von *Curvibacter* sp., der Hauptkolonisierer von *Hydra vulgaris* (AEP), konnte gezeigt werden, dass er sowohl langkettige 3-hydroxy-HSLs (3OH-HSLs) als auch langkettige 3-oxo-HSLs (3O-HSLs) erkennen kann und in der Lage ist 3OHC12-HSLs zu produzieren. Des Weiteren, wurde ein neuer Wirtsmechanismus entdeckt, wodurch Hydra in der Lage ist spezifisch langkettige 3O-HSLs mittels einer Oxidoreduktase-Aktivität zu ihrem 3OH-HSL Gegenstück zu modifizieren. Folglich fördert Hydras QQ-Aktivität *Curvibacter* 3OH-HSL regulierte Prozesse. Transkriptionsdaten ergaben, dass *Curvibacter* sp. eine unterschiedliche Reaktion gegenüber 3OHC12-HSLs im Vergleich zu 3OC12-HSLs aufweist. Während Gene, die an der Flagellenbiosynthese beteiligt sind, durch 3OC12-HSLs signifikant induziert werden, fördern 3OHC12-HSLs die Expression des trans-AT-Polyketid Clusters, welches potentiell eine antibakterielle oder antifungale Aktivität aufweist. Somit unterdrückt das QQ durch den Wirt die bakterielle Motilität und fördert die Produktion von bioaktiven Substanzen seiner kommensalen Bakterien.

Interessanterweise hat die Untersuchung des Einflusses dieser AHL-Modifizierung auf den Metaorganismus *in vivo* gezeigt, dass 3OH-HSLs die Kolonisierung durch den Hauptkolonisierer *Curvibacter* sp. fördern, während 3O-HSLs inhibierend wirken. Ferner führt die Zugabe von 3O-HSLs oder Flagellin zum Metaorganismus Hydra zur starken Reduktion des Bakteriengehalts. Diese Ergebnisse deuten an, dass 3O-HSLs die Flagellenproduktion von *Curvibacter* sp. induzieren, wodurch eine Antwort in Hydra ausgelöst wird, dass schließlich zu einer Reduktion der bakteriellen Gemeinschaft des Metaorganismus Hydra führt. Diese Erkenntnisse zeigen zum ersten Mal, dass ein Wirtsorganismus *in vivo* bakterielle QS-Signale manipuliert und dadurch die

symbiotischen Eigenschaften seiner bakteriellen Kolonisierer erhält, wodurch er zu Homöostase des Metaorganismus beiträgt.

In Zusammenarbeit mit Xiang-Yi Li wurde die Interaktion zwischen den beiden Hauptkolonisieren von *Hydra vulgaris* (AEP) mittels eines theoretischen und eines biologischen Ansatzes untersucht. In einem Kulturexperiment mit diesen beiden Bakterien konnte gezeigt werden, dass Sie während des Wachstums miteinander interagieren. Solch eine Kooperation ist notwendig um ein allumfassendes Verständnis des komplexen Regulationsnetzwerkes innerhalb eines Metaorganismus zu erlangen.

## Abbreviations

°C	degree Celsius
µg	microgram
µl	microliter
µM	micromolar
% (v/v)	volume percent (volume/volume)
% (w/v)	gram substance in 100 ml solution (weight/volume)
3OC6-HSL	<i>N</i> -(β-Ketocaproyl)-L-homoserine lactone
3OC8-HSL	<i>N</i> -(3-Oxooctanoyl)-L-homoserine lactone
3OC10-HSL	<i>N</i> -(3-Oxodecanoyl)-L-homoserine lactone
3OC12-HSL	<i>N</i> -(3-Oxododecanoyl)-L-homoserine lactone
3OC14-HSL	<i>N</i> -(3-Oxotetradecanoyl)-L-homoserine lactone
3OHC8-HSL	<i>N</i> -(3-Hydroxyoctanoyl)-L-homoserine lactone
3OHC10-HSL	<i>N</i> -(3-Hydroxydecanoyl)-L-homoserine lactone
3OHC12-HSL	<i>N</i> -(3-Hydroxydodecanoyl)-L-homoserine lactone
3OHC14-HSL	<i>N</i> -(3-Hydroxytetradecanoyl)-L-homoserine lactone
A	adenine
AHL	<i>N</i> -acyl-homoserine lactone
AMP	antimicrobial peptide
Amp	ampicillin
AP	alkaline phosphatase
BCIP	5-bromo-4-chloro-3-indolyl phosphate
BLAST	Basic Local Alignment Search Tool
bp	base pair
BSA	bovine serum albumin
C	cytosine or carbon
C4-HSL	<i>N</i> -Butyryl-L-homoserine lactone
C6-HSL	<i>N</i> -Hexanoyl-L-homoserine lactone
C8-HSL	<i>N</i> -Octanoyl-L-homoserine lactone
C10-HSL	<i>N</i> -Decanoyl-L-homoserine lactone
C12-HSL	<i>N</i> -Dodecanoyl-L-homoserine lactone
cDNA	complementary DNA

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Cam	chloramphenicol
cm	centimeter
Da	dalton
DNA	deoxyribonucleic acid
DNAse	deoxyribonuclease
dNTP	deoxynucleoside triphosphate
ds	double stranded
eGFP	enhanced green fluorescent protein
G	guanine
g	gram or gravitational acceleration
h	hour
H.	Hydra
hF	hectofarad
HSL	homoserine lactone
HPLC	high-performance liquid chromatography
Kan	kanamycin
kb	kilo base pairs
KEGG	Kyoto Encyclopedia of Genes and Genomes
kV	kilovolt
l	liter
LB	Lauria Bertani
LC-ESI-MS	Liquid chromatography coupled to electrospray ionization mass spectrometry
LPS	lipopolysaccharide
M	molar
mA	milliampere
MAMP	microbe-associated molecular pattern
MALDI-TOF	Matrix-assisted laser desorption/ionization- time of flight
mg	milligram
min	minute(s)
ml	milliliter
mM	millimolar
mRNA	messenger-RNA
NBT	nitro blue tetrazolium chloride
NCBI	National Center for Biotechnology Information
ng	nanogram

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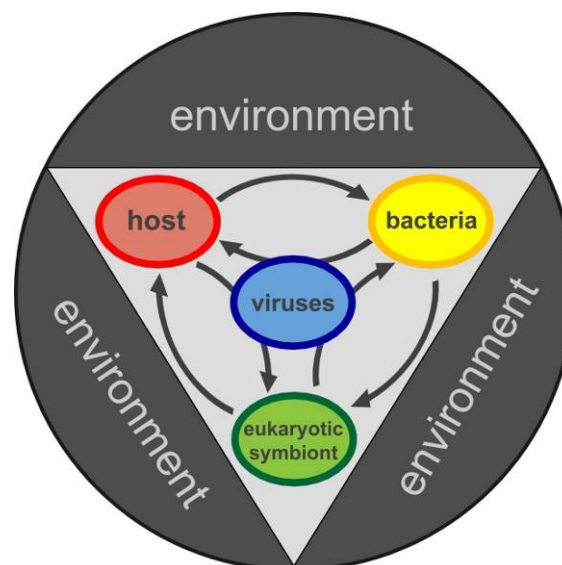
NLR	NOD-like receptor
NOD	nucleotide-binding oligomerization domain
OD	optical density
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
qRT-PCR	quantitative Real-Time-PCR
RNA	ribonucleic acid
RNAse	ribonuclease
rpm	rounds per minute
rRNA	ribosomal RNA
RT	room-temperature or reverse transcription
RT-PCR	reverse transcription PCR
SDS	sodium dodecyl sulfate
sp.	species
ss	single stranded
T	thymine
TAE	tris-acetate-EDTA-buffer
<i>Taq</i>	<i>Thermophilus aquaticus</i>
TBE	tris-boric acid-EDTA-buffer
TEMED	<i>N,N',N',N'</i> -tetraethylenediamine
Tet	tetracycline
$T_m$	melting temperature
tris	Tris (hydroxymethyl) aminomethane hydrochloride
U	units or uracil
UV	ultraviolet light
V	volt
X-gal	5-bromo-4-chloro-3-indolyl- $\beta$ -D- galactopyranoside

Furthermore, general abbreviations and units and prefix of the International System of Units (SI) were used.

# 1 Introduction

## 1.1 The concept of a metaorganism

Every multicellular organism has to be considered as a metaorganism, consisting of a host with its associated microorganisms, which can range from bacteria to archaea, viruses and fungi. This metaorganism is interacting with its environment as a unit of selection in evolution (Figure 1) (McFall-Ngai et al., 2013; Rosenberg et al., 2009; Zilber-Rosenberg and Rosenberg, 2008). In contrast to the concepts “holobiont”, which is originally used for cnidarians (Rohwer et al., 2002; Vega Thurber et al., 2009), and “superorganism”, which describe social insects like ants (Behmer, 2009) and bees (Seeley, 1989), the idea of a “metaorganism” can be applied to every taxonomical group. The term metaorganism was first used by Bell, who applied it to organisms, which are between two levels of organization (Bell, 1998). Meanwhile the term is used for the entity of a multicellular organism and its coevolved associated microorganisms. Also humans with their symbiotic relationship to their gut microbiota has to be assigned as metaorganism (Biagi et al., 2012; Bosch, 2012; Bosch and McFall-Ngai, 2011).



**Figure 1: Interactions in a metaorganism**

Multicellular organisms are metaorganisms, which consist of a host and its synergistic interactions with bacteria, archaea, fungi, viruses and symbiotic microbes or other eukaryotes. This consortium is interacting with its environment as a unit of selection in evolution (Bosch and McFall-Ngai, 2011; Fraune et al., 2011; Zilber-Rosenberg and Rosenberg, 2008) (the figure was provided by T. C. G. Bosch).

If one has a closer look at the host-microbe interaction, it is becoming clear that bacteria have a strong effect on the host phenotype, by influencing its physiological and ecological features (Bäckhed et al., 2005; Fraune and Bosch, 2010). For example, the intestinal



bacteria fulfill a metabolic function, i.e. they are responsible for the synthesis of essential amino acids (Gustafsson, 1959; Smith et al., 2007). For instance, termites are only able to use cellulose as nutrient, because of their synergistic interaction with different microorganisms (Scharf et al., 2011). Additionally, by altering their population of symbiotic bacteria, corals can adapt rapidly to changing environmental conditions (Reshef et al., 2006). These microorganisms confer benefits to their coral host by various mechanisms, including photosynthesis, nitrogen fixation, the provision of nutrients and infection prevention (Rosenberg et al., 2007). Furthermore, it seems that the commensal bacteria are also involved in speciation. The severe hybrid lethality in larvae of different *Nasonia* species occurs because of gene-microbe interactions with beneficial members of the gut microbiome (Brucker and Bordenstein, 2013). The mating choice in *Drosophila melanogaster* is dependent on genotype but also on infection status by its symbiotic bacteria *Wolbachia* (Markov et al., 2009). Thus, the interactions between host and symbionts affect the fitness of the metaorganism within its environment. Furthermore, under environmental stress, the bacterial community can change rapidly compared to the host, thereby allow fast adaptation by the metaorganism. Consequently, the unit of host and its microorganisms are selected in evolution (Zilber-Rosenberg and Rosenberg, 2008).

As the commensal bacteria play a fundamental role in the host physiology, the host has to strictly regulate the bacterial composition. It has to fulfill the challenging task that on one hand the host has to attract beneficial and repel potential pathogenic bacteria, while on the other hand it has to prevent beneficial bacteria, from becoming harmful. How this regulation network is functioning is not completely understood, but some regulation mechanisms are already known.

An important mechanism for the bacterial recognition takes place by Toll-like-receptors (TLRs). These are evolutionary conserved receptors, which are present from basal metazoan, as cnidarians, to mammals (Beutler, 2004; Miller et al., 2007; Poltorak et al., 1998). In contrast to their structure, the function of the TLRs evolutionary is not conserved. The Toll-receptor was identified in *Drosophila melanogaster*, where it possess mainly a developmental function (Anderson et al., 1985a, 1985b), but it was also shown that it is involved in the immune response to the fungus *Aspergillus fumigatus* (Lemaitre et al., 1996). However the vertebrate TLRs are classical pattern recognition receptors (PRRs) and play an important role in the innate immune system, among others in the bacterial recognition (Athman and Philpott, 2004; Vandewalle, 2008). In mice, the TLR-signaling pathway is used to induce the expression of c-type lectins (Brandl et al., 2007). The human c-lectin REG3 $\alpha$  is mainly located in the paneth cells, which are secretory cells in the small intestinal epithelium. The orthologues in mice, lectin REG3 $\alpha$  and REG3 $\gamma$ , are

able to bind peptidoglycan, a component of the bacterial cell wall, and possess a bactericidal effect on Gram-positive bacteria (Bevins and Salzman, 2011a; Cash et al., 2006). Furthermore, it could be shown that the lectin REG3 $\gamma$  promotes the spatial separation of host and microbiota in the mice gut (Vaishnava et al., 2011).

The role of nucleotide-binding oligomerization domain (NOD) like receptors (NLRs), especially in the bacterial interaction, is not completely understood. So far it could be shown that the human NOD2 receptor is able to recognize muramyl dipeptide, which is a component of the bacterial cell wall (Girardin et al., 2003; Inohara et al., 2003). The knockout of the NOD2 receptor in mice leads to a diminished ability to control the colonization of commensals and pathogens, resulting in a breakdown of the homeostasis (Petnicki-Ocwieja et al., 2009; Rehman et al., 2011). Furthermore, genetic variations in the NLRs are associated with inflammation diseases (Fukata et al., 2009; Rosenstiel et al., 2008).

The regulation of the bacteria composition by production of antimicrobial peptides (AMPs) is widely distributed and can be found in all three kingdoms of the tree of life. But because of their high diversity they cannot be classified based on their primary structure. The only common feature is their amphipathic structure (Zasloff, 2002). The antimicrobial activity is mostly based on their strong cationic charge, promoting the interaction with the negatively charged phospholipids of the bacterial cell membrane (Shai, 1999; Zasloff, 2002). Multicellular organism use AMPs not only for protection but also for the regulation of its commensal bacteria (Nakatsuji and Gallo, 2012). For example, Salzman et al. could show that human  $\alpha$ -defensin regulates the bacterial composition in the mice gut (Bevins and Salzman, 2011b; Salzman et al., 2010) and the Arminin peptide family is involved in selecting the species-specific bacterial community of different *Hydra* species (Franzenburg et al., 2013a).

The coevolution between host and its bacteria can lead to such a strong adaptation, that the bacterial composition of a metaorganism resembles the phylogenic tree of the host species, what is known as phylosymbiosis (Brucker and Bordenstein, 2012). So far this could be shown for termites (Hongoh et al., 2005), hominids (Ochman et al., 2010), the parasitic wasp *Nasonia* (Brucker and Bordenstein, 2012) and the freshwater polyp *Hydra* (Franzenburg et al., 2013a).

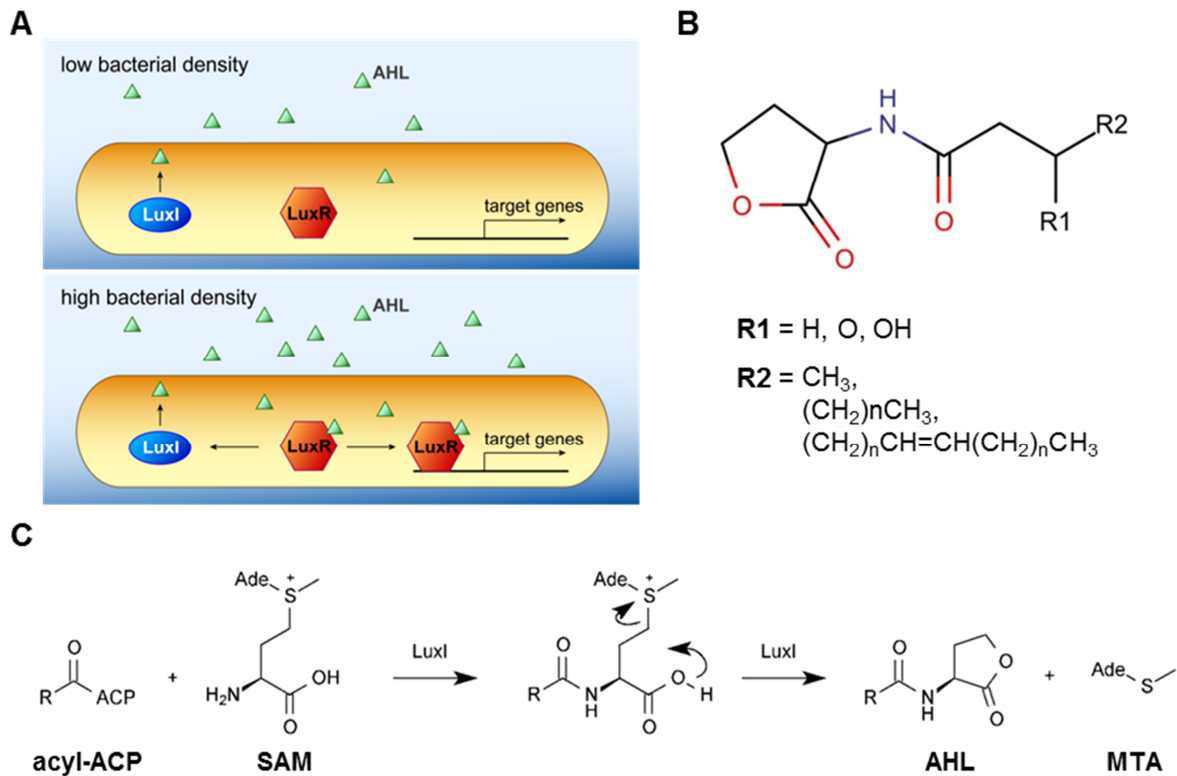
## 1.2 Quorum sensing – the bacterial communication system

As described in chapter 1.1, some mechanisms, which enable a host to recognize its bacteria and to regulate the composition of its bacterial community, are already known, but for the specific regulation by a eukaryote of bacterial community wide behavior, like formation of biofilms and secretion of virulence factors, only few is known. Bacteria use their communication system, called quorum sensing (QS), for the coordination of processes, like biofilm formation, virulence, sporulation and take-up of DNA from the environment, which are crucial for the survival of bacteria. For an effective utilization of these mechanisms, they have to be coordinated. Quorum sensing enables bacteria to communicate with each other. Due to the production and detection of signaling molecules, called autoinducers (AIs), they are able to sense the cell density of their bacterial population. In a density dependent manner they can synchronize their gene expression, allowing them to act as a unit, like a multicellular organism (Schauder and Bassler, 2001). The basal steps, which are necessary for the detection and the response to a changed cell density, are analogue in all known QS systems. In the beginning AIs are synthesized intracellularly, which either diffuse passively into the environment or are actively secreted. Consequently, an increasing cell density leads to an accumulation of extracellular AIs. At a critical threshold concentration of signaling molecules, a receptor binds to the molecules and induces a signal transduction cascade, which causes a population wide change in gene expression (Ng and Bassler, 2009).

### 1.2.1 Quorum sensing in Gram-negative bacteria

Gram-negative bacteria use homologues of the LuxI/LuxR quorum sensing system for their communication (Figure 2A) (Ng and Bassler, 2009). Thereby *N*-acyl homoserine lactones (AHLs) serve as signaling molecules. They consist of a homoserine lactone ring (HSL) and an acylated fatty acid (Figure 2B). The specificity of this system is based on the variance in the acyl-side chain, their length differs from four to eighteen carbon molecules and the nature of the substituent on the third carbon is varying from hydrogen to an oxo or a hydroxy group, and in addition the acyl-side chain can be unsaturated (Figure 2B) (Fuqua et al., 2001).

The first AHL autoinducer and its corresponding regulation mechanisms were characterized in the marine bacterium *Vibrio fischeri* (Ruby, 1996). This bacterium colonizes the light organ of the Hawaiian bobtail squid *Euprymna scolopes* and provides bioluminescence for its host. By this the squid can mask its shadow and gets an advantage during hunting.



### Figure 2: Quorum sensing in Gram-negative bacteria

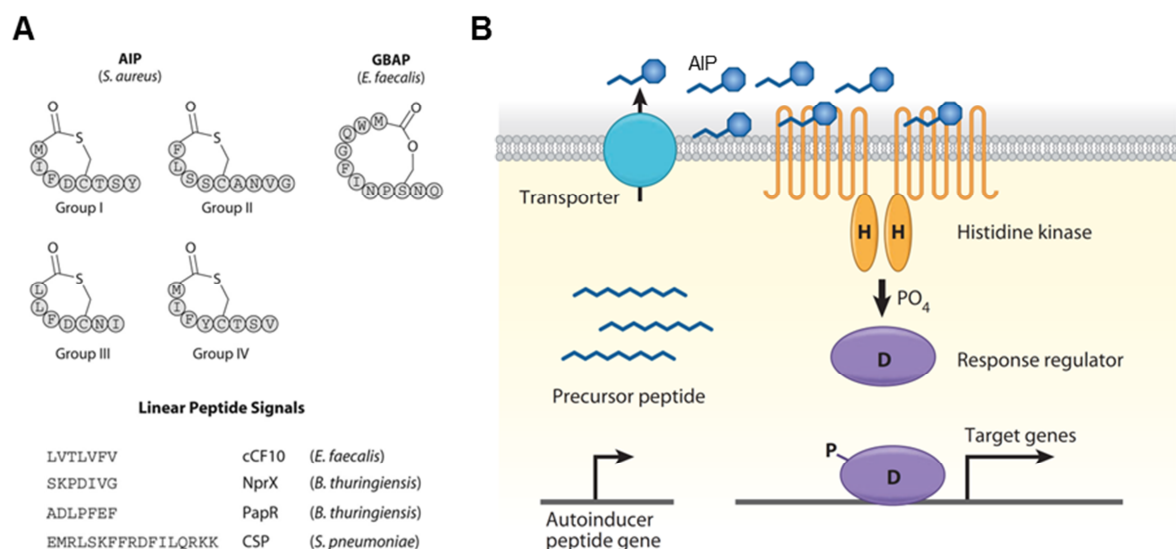
(A) Gram-negative bacteria communicate with each other via the LuxI/LuxR QS system. The LuxI-synthase produces the AHLs, which can diffuse through the membrane. The extracellular AHL concentration increases with an increase in bacterial density. At an AHL threshold concentration the LuxR-receptor binds to the molecules and activates the transcription of target genes, including *luxI*. (B) The specificity of a QS system is based on the variance in its signaling molecules. AHLs are composed of a homoserine lactone ring and an acyl-side chain of 4 to 18 carbons. The third carbon can be modified by an oxo- or a hydroxy-group and the acyl-side chain can be unsaturated (Federle and Bassler, 2003; Ng and Bassler, 2009). (C) Synthesis of the QS signaling molecules of Gram-negative bacteria AHLs by LuxI. The AHLs are synthesized from the acyl-ACP and SAM by LuxI-type synthases. This catalytic reaction is performed by a two-step mechanism, first the acyl-ACP transfers its acyl-group to the amino group of SAM, followed by lactonization of the methionine part, which leads to the formation of an AHL and the release of MTA (taken from Dickschat, 2010 and modified). AHLs, *N*-acyl-homoserine lactones; LuxI, AHL-synthase; LuxR, AHL-receptor; ACP, acyl-carrier-protein; SAM, *S*-adenosyl-L-methionine; Ade, adenosine; MTA, methylthioadenosine.

The two proteins LuxI and LuxR are critical for this system. LuxI is the synthase of the AHLs. It was shown that LuxI of *Vibrio fischeri* catalyze the acylation and lactonization reactions between the substrates *S*-adenosylmethionine (SAM) and hexanoyl-acyl carrier protein (ACP) (Figure 2C) (Schaefer et al., 1996a). There is a huge variety of acyl-ACPs, originating from the fatty acid biosynthesis, but the LuxI homologues often show a preference for a specific acyl-ACP. The AHL-synthases mostly except also similar chain lengths and/or oxidation states in the 3-position, leading to one dominant AHL and small proportions of similar AHL species (Dickschat, 2010; Hoang et al., 2002). After the synthesis, the AHLs can diffuse through the cell membrane (Kaplan and Greenberg, 1985; Williams, 2007). An increasing cell density entails an increase in the extracellular AHL concentration. At a critical threshold concentration of the signaling molecules, the

cytoplasmic AHL-receptor LuxR binds to the molecules and the resulting complex activates the transcription of target genes. In addition this complex induces the expression of the *luxI* gene, which creates a positive feedback loop in the system (Figure 2A) (Engelbrecht et al., 1983). In the absence of AHLs, the LuxR protein is unstable and degrades relatively fast (Koch et al., 2005; Zhu and Winans, 1999, 2001).

### 1.2.2 Quorum sensing in Gram-positive bacteria

For communication, Gram-positive bacteria use modified oligopeptides, so called autoinducing peptides (AIPs). So far the mechanisms for the peptide modification, which determines the specificity of the system, are poorly understood (Figure 3A).



**Figure 3: Quorum sensing (QS) system of Gram-positive bacteria**

(A) Gram-positive bacteria are using autoinducing oligopeptides (AIPs) as QS signaling molecules. So far different types of AIPs were identified, but their modification is only basically understood (LaSarre and Federle, 2013) (B) The precursor peptides of the autoinducing oligopeptides (AIPs) get processed before they can be transported out of the cell. The AIP-concentration increases in a cell-density dependent manner. Peptide recognition takes place by a two-component system. The extracellular oligopeptides get recognized by a membrane bound histidine kinase receptor. At a AIP-threshold concentration the intrinsic autophosphorylation activity of the receptor leads to the phosphorylation of the response regulator, which activates the transcription of target genes (taken from Ng and Bassler, 2009 and modified).

Like in Gram-negative bacteria, the extracellular autoinducer concentration is proportional to the bacterial cell-density. Because peptides cannot diffuse through biological membranes they have to be secreted by transporters (Solomon et al., 1996). Another difference to the LuxI/LuxR system is the localization of the autoinducer receptor. The detection of the oligopeptides takes place by a two-component system (Skerker et al., 2005; Stock et al., 1989), which typically consists of a membrane bound histidine kinase receptor and a cytoplasmic response regulator, which acts as a transcriptional regulator (Figure 3B). Binding of the peptides activates the intrinsic autophosphorylation activity of the histidine kinase receptors, which leads to the phosphorylation, under ATP

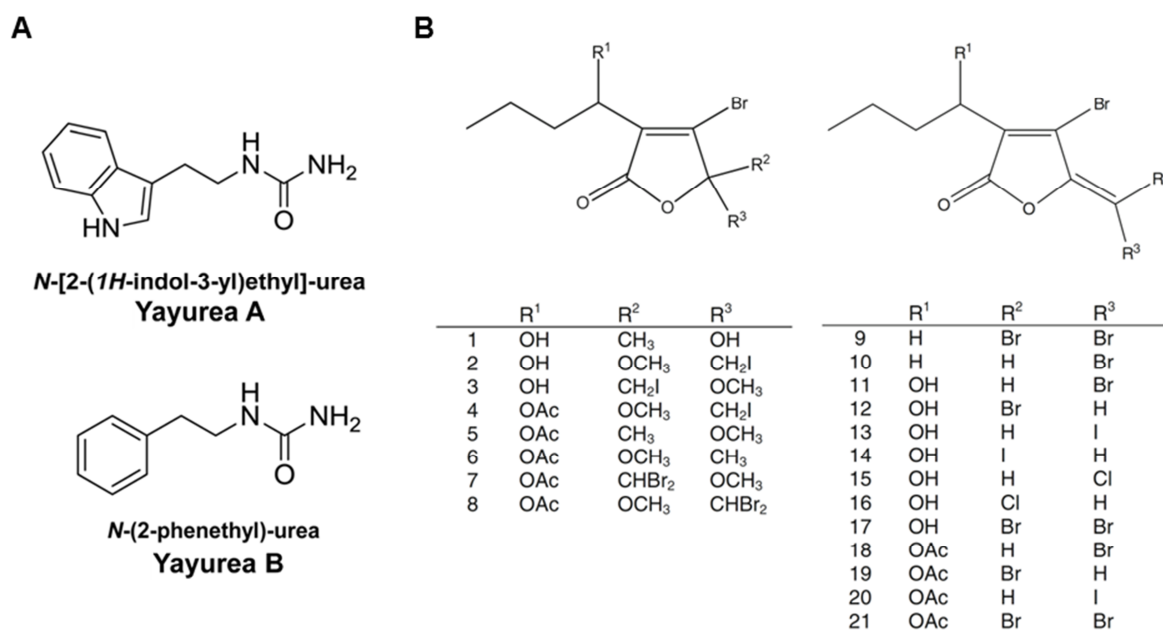
consumption, of the conserved histidine residue of the cytoplasmic part of the receptor. The phosphate group gets immediately transferred to the conserved aspartate residue of the response regulator. The phosphorylation activates the response regulator, which is now able to act as DNA binding transcription factor to modulate the expression of target genes (Figure 3B) (Ng and Bassler, 2009).

### **1.3 Mechanisms to disturb the bacterial communication (quorum quenching)**

As quorum sensing (QS) is a key regulator of bacterial behavior, including virulence and motility (chapter 1.2), it is interesting for bacteria and eukaryotes to interfere with bacterial QS. In theory any interference with a key process of QS can be used to disturb bacterial communication, these processes are called quorum quenching (QQ). Different aspects make interference with the bacterial QS system attractive for bacteria and eukaryotic hosts. As QS regulate many bacterial behaviors, it is linked to high costs. Therefore, bacteria need mechanisms to regulate their own QS system. For example, *Agrobacterium tumefaciens* degrades its signaling molecule 3OC8-homoserine lactone (HSL) in a growth dependent manner, thereby regulating the tumor-inducing (Ti) plasmid conjugation transfer, which is necessary to become virulent for the host plant (Piper et al., 1993; Zhang et al., 2002). *Pseudomonas aeruginosa* specifically degrades at the early stationary phase its 3OC12-HSL, which regulates many virulent factors, but not its communication molecule C4-HSL, responsible for a broad range of processes (Huang et al., 2003).

But bacteria can also get an advantage if they interfere with the QS system of their competitors. *Variovorax paradoxus* use *N*-acyl-homoserine lactones (AHLs) as a carbon source and by this indirectly inhibits bacteria in the same niche (Leadbetter and Greenberg, 2000). The pathogenesis of *Staphylococcus aureus* is regulated by the accessory gene regulator (*agr*) quorum sensing system. The unique feature of this system is the huge variance of autoinducing peptides (AIPs), resulting in at least four specificity groups within *S. aureus*. All strains within a group produce the same AIP. Those AIPs have two biological functions; they can activate the virulence response within its own specificity group and inhibit the virulence response in other groups (Ji et al., 1997; Lyon et al., 2002).

A nice example for an inter-phyllum interference between Firmicutes (Gram-positive) and Beta-/Gammaproteobacteria (Gram-negative) were described by Chu et al. (Chu et al., 2013). The two compounds yayurea A and B, produced by members of the *Staphylococcus* “intermedius group” (Firmicutes), including *Staphylococcus delphini*, inhibit the expression of QS controlled toxins and other compounds as well as the growth in Gram-negative bacteria. This suggests that QQ plays an important role, in natural environments shared with Gram-negative bacteria, in self-protection and competitiveness (Figure 4A) (Chu et al., 2013).

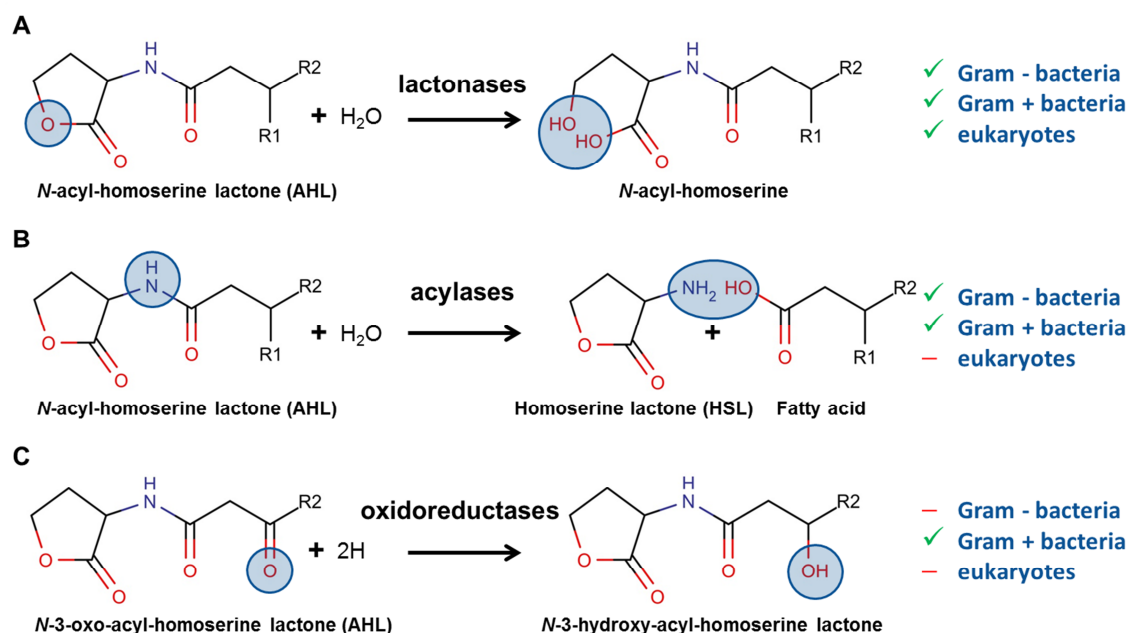


**Figure 4: Quorum quenching molecules**

(A) QS-inhibitor *N*-[2-(1H-indol-3-yl)ethyl]-urea (yayurea A) and *N*-(2-phenethyl)-urea (yayurea B) isolated from *Staphylococcus delphini* (Chu et al., 2013). (B) Halogenated furanones produced by the red alga *Delisia pulchra* (taken from Harder et al., 2012 and modified).

For eukaryotes it is crucial to suppress the QS regulated behavior virulence, motility and biofilm formation (Cullender et al., 2013; Cvitkovitch et al., 2003; Smith and Iglewski, 2003; Yang and Defoirdt, 2015) of colonizing bacteria, as this would lead to dysbiosis. QQ could be an instrument for a eukaryotic host to control these behaviors of its commensal bacteria, but so far there are only two well-characterized examples, halogenated furanones of the red algae *Delisia pulchra* and the mammalian paraoxonases. *D. pulchra* is able to interfere with QS by the production of a broad range of halogenated furanones (Figure 4B) (Harder et al., 2012; Hartmann and Schikora, 2012). It could be shown that the addition of halogenated furanones in a natural concentration to the surfaces of algal thalli deter the settlement of ecologically relevant fouling organisms and have strong effects on the species composition of the bacterial community on the alga's surface (Dworjanyn et al., 2006; Maximilian et al., 1998). This ability of halogenated furanones to affect the bacterial composition is a strong hind that the furanones play an important role

in the eukaryote-bacterial interactions (Teplitski et al., 2011). Furthermore, it could be shown that the produced furanones enable *D. pulchra* to control virulence genes and corresponding behaviors of *Pseudomonas aeruginosa* (Hentzer et al., 2002). It was shown that these effects of furanones on bacteria are based on their possibility to destabilize the AHL-receptor LuxR (Manefield et al., 2002). Many other compounds were identified, which have the potential to interfere with the bacterial QS system, but the *in vivo* relevance is not proven so far (reviewed in Teplitski et al., 2011; LaSarre and Federle, 2013). Beside these QQ molecules, three classes of enzymes, lactonases, acylases and oxidoreductases, are known to inactivate AHLs (Figure 5).



### Figure 5: Quorum quenching (QQ) enzymes

(A) AHL-lactonases inactivate AHLs by hydrolyzing the lactone ring, which leads to the formation of an *N*-acyl-homoserine. They are widely distributed in the bacterial kingdom, while only one example, the paraoxonases, is known in eukaryotes (Chun et al., 2004; Dong et al., 2000; Draganov et al., 2005; Koch et al., 2014; Simanski et al., 2012). (B) AHLs get degraded by AHL-acylases by cleavage of the amine bond of the AHLs. They were only found in bacteria (Leadbetter and Greenberg, 2000; Shepherd and Lindow, 2009). (C) Few is known about QQ by AHL-oxidoreductases. They modify the acyl-side chain of the AHLs by converting an oxo-group into a hydroxy-group. So far they were only reported in the Gram-positive bacteria *Rhodococcus erythropolis* and *Bacillus megaterium* and one was found in a soil metagenome (Bijtenhoorn et al., 2011; Chowdhary et al., 2007; Uroz et al., 2005) (reviewed in LaSarre and Federle, 2013).

The first QQ enzyme was found in a *Bacillus* sp. Cloning of the identified AHL-lactonase AiiA into plants reduce the pathogenicity of *Erwinia carotovora* (Dong et al., 2002, 2000, 2001). AHL-lactonases inactivate AHLs by hydrolyzing their lactone ring, which leads to the formation of the corresponding *N*-acyl-homoserine (Figure 5A). This hydrolysis occurs at alkaline pH, while an acidic pH can restore it (Yates et al., 2002). AHL-lactonases have relatively broad substrate specificity, based on the fact that they target the conserved homoserine lactone ring. They are widely distributed over the bacterial clade and can be found in Gram-positive and -negative bacteria (Carrier et al., 2003; Huang et al., 2012;



Koch et al., 2014; Krysciak et al., 2011; Mei et al., 2010; Park et al., 2003; Uroz et al., 2008; Wang et al., 2010, 2012). For the two AHL-lactonases DlhR and QsdR1, identified in the nitrogen fixing symbiont *Rhizobium* sp. strain NGR234, it is suggested that they are involved in the colonization of roots in the rhizospheres of cowpea plants (Krysciak et al., 2011).

Furthermore, the only identified eukaryotic QQ enzyme is a lactonase and belongs to the paraoxonase (PON) family. PON like proteins are evolutionary conserved and can be found in many metazoans (Draganov and La Du, 2004) and were recently identified in prokaryotes (Bar-Rogovsky et al., 2013). Chun et al. could show that different mammalian cells have the ability to inactivate 3OC12-HSL. It seems that cells, which have contact with the environment and potentially see bacteria, show a higher inactivation rate of 3OC12-HSL. The authors claimed that the human PONs are responsible for this activity (Chun et al., 2004; Draganov et al., 2005). In the meantime it could be shown that the human PON2 is also responsible for the QQ activity of human keratinocytes (Simanski et al., 2012).

Also acylases could be identified as common bacterial QQ enzymes. They degrade AHLs by cleaving their amine bond, which leads to the formation of a homoserine lactone (HSL) and the corresponding fatty acid (Figure 5B). The first AHL-acylase activity was identified in the Betaproteobacteria *Variovorax paradoxus*, which uses different AHLs as a source for energy and nitrogen (Leadbetter and Greenberg, 2000). Homologues to the AHL-acylase AiiD, characterized in *Ralstonia* strain XJ12B (Lin et al., 2003), were found in many other bacteria, where the QQ activity could be confirmed (Chen et al., 2009; Huang et al., 2003, 2006; Koch et al., 2014; Shepherd and Lindow, 2009; Sio et al., 2006; Wahjudi et al., 2011).

So far, only four examples for quorum quenching by use of an oxidoreductase activity are known. In contrast to AHL-lactonases and -acylases, AHL-oxidoreductases do not degrade the AHLs; they modify the acyl-side chain by reduction or oxidation (Figure 5C). The first AHL-oxidoreductase activity was described in *Rhodococcus erythropolis*. This bacterium is able to use a broad spectrum of AHLs as carbon source and it could be shown by high-performance liquid chromatography (HPLC) analysis that *R. erythropolis* is reducing the 3OC14-HSL *in vivo* to the corresponding derivate 3OHC14-HSL (Uroz et al., 2005). The responsible enzyme has not yet been identified. Burkholderia strain GG4, isolated from the ginger rhizosphere, also reduced 3-oxo-HSLs to the corresponding 3-hydroxy-HSLs, however the enzyme of the strain was not identified (Chan et al., 2011). The enzyme CYP102A1 from *Bacillus megaterium*, a P450 monooxygenase, was identified to efficiently oxidize *in vitro* AHLs at the  $\omega$ -1,  $\omega$ -2 and  $\omega$ -3 carbons of the acyl-

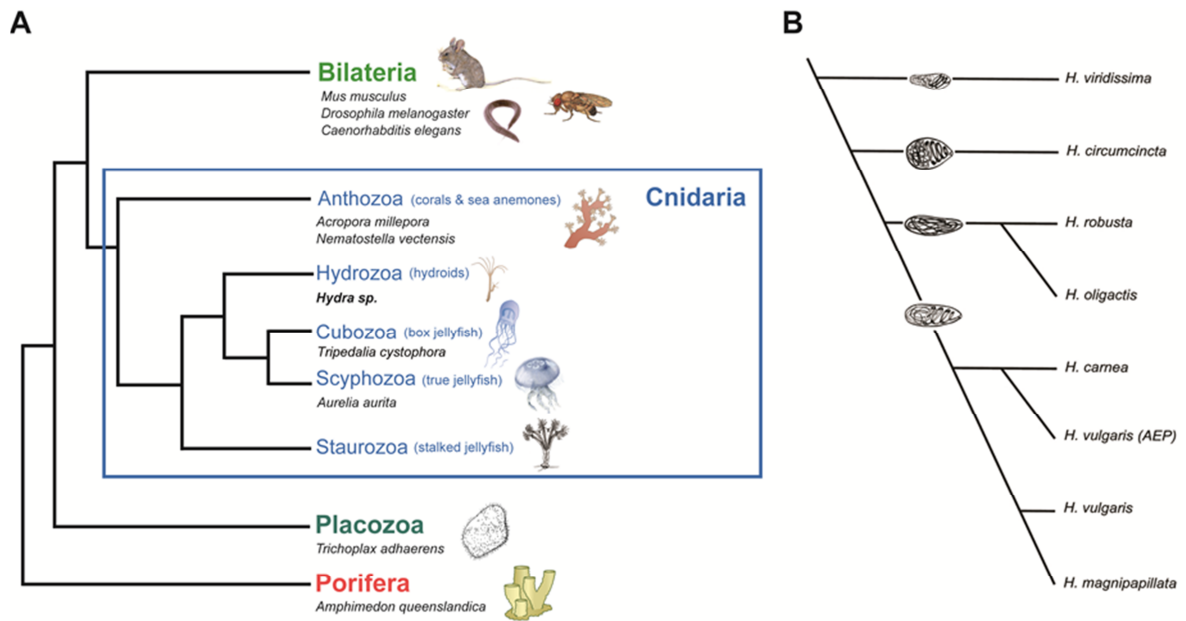
side chain (Chowdhary et al., 2007), but the *in vivo* relevance has not been proven so far. The fourth AHL-oxidoreductase was identified in a soil metagenome. The NADH-dependent enzyme BpiB09 was able to modify 3OC12-HSL by reducing the 3-oxo-moiety to the corresponding hydroxy-AHL (Bijtenhoorn et al., 2011).

## 1.4 The freshwater polyp Hydra as model organism

The Swiss zoologist Abraham Trembley was the first who scientifically studied and described Hydra (Trembley, 1744). Since then this polyp was established as model organism for different biological questions. Because of its size and transparency it is suitable for observing developmental processes. Hydra possesses a remarkable regeneration capacity, which makes it interesting to study fundamental properties of stem cells and developmental process, for example axis formation (Gierer and Meinhardt, 1972; Meinhardt and Gierer, 1974, 2000). Hydra is an attractive model organism, because beside the extensive knowledge about its cytology, numerous methods such as in-situ-hybridization and the generation of transgenic animals are already established. Furthermore, since 2010 the genome of *Hydra magnipapillata* is available (Chapman et al., 2010) and transcriptomes of several species, *Hydra oligactis* (Domazet-Lošo et al., 2014), *Hydra viridissima* (unpublished data) and *Hydra vulgaris* (AEP) (Hemmrich et al., 2012) were sequenced. Additionally, it is easy and cheap to cultivate and has a high reproduction rate.

### 1.4.1 Phylogeny of Hydra

The freshwater polyp Hydra belongs to the phylum Cnidaria, which is named after their stinging cells, so called cnidocytes, which they used for prey capture. Cnidarians own a true tissue structure and possess nerve and muscle cells, which they distinguishes from Parazoa (Porifera and Placozoa) and assigns them to the Eumetazoa. In contrast to the Bilaterians, the Cnidarians are diploblastic, possessing only an endo- and an ectoderm. The phylum Cnidaria is divided into five classes: Anthozoa, Staurozoa, Hydrozoa, Cubozoa and Scyphozoa (Figure 6A). The Anthozoa form a sister group to the Medusozoa, which consist of the four other classes of the Cnidarians. Within the Medusozoa the Staurozoa forms a sister group to the others, within this group the Hydrozoa build a sister group to the sister group of Scyphozoa and Cubozoa (Collins *et al.* 2006; Van Iten *et al.* 2006).



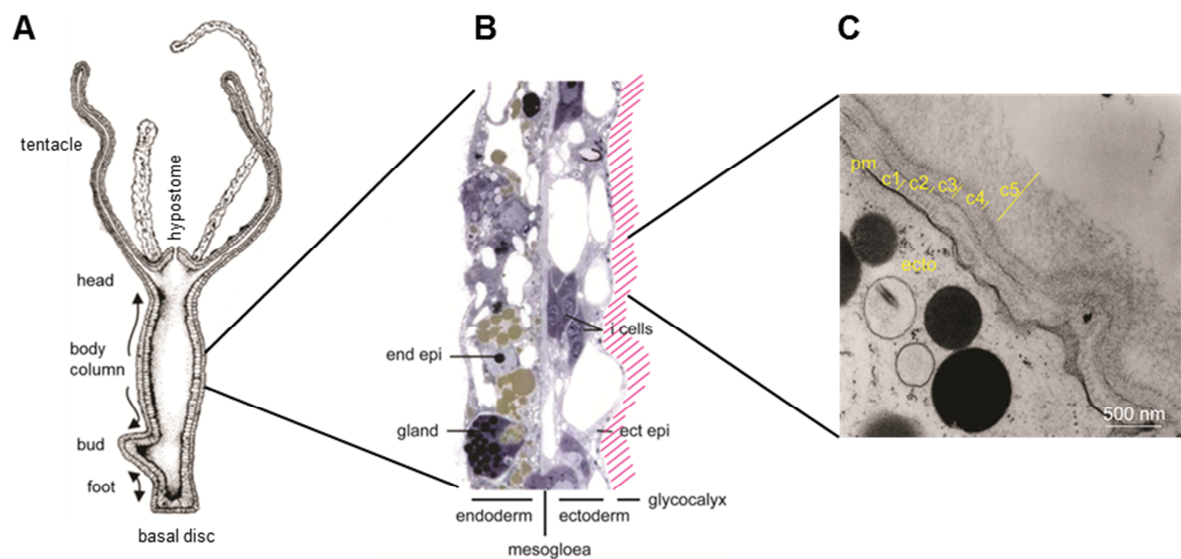
### Figure 6: Phylogeny of Hydra

(A) Schematic phylogenetic tree of Metazoan, where the position of the Cnidarians is highlighted (taken from Collins et al., 2006; Van Iten et al., 2006; Khalturin et al., 2009 and modified). (B) Phylogenetic relations of eight *Hydra* species, based on molecular and morphological data. In the branches holotrichous isorhizas of the different groups are schematically depicted (taken from Hemmrich et al., 2007 and modified).

At the beginning of the 20<sup>th</sup> century the genus *Hydra*, which belongs to the Hydrozoa, was divided, based on their morphology, into four groups: the *viridissima* group (“green hydra”), the *braueri* group (“gracile hydra”), the *oligactis* group (“stalked hydra”) and the *vulgaris* group (“common hydra”) (Campbell, 1987; Schulze, 1917). Molecular analyses of the phylogenetic relationships within the *Hydra* species, based on mitochondrial and nuclear DNA sequences, have shown that *Hydra viridissima* forms a sister group to the other *Hydra* species (Martínez et al., 2010; Schwentner and Bosch, 2015). This species is the only one, who lives in endosymbiosis with algae, belonging to the genus *Chlorella*. Furthermore, molecular analyses have shown that the species *Hydra vulgaris* (AEP), which is used for the production of transgenic animals, is closer related to *Hydra carnea* than to its eponymous species *Hydra vulgaris* (Figure 6B) (Hemmrich et al., 2007).

### 1.4.2 Morphology and histology of Hydra

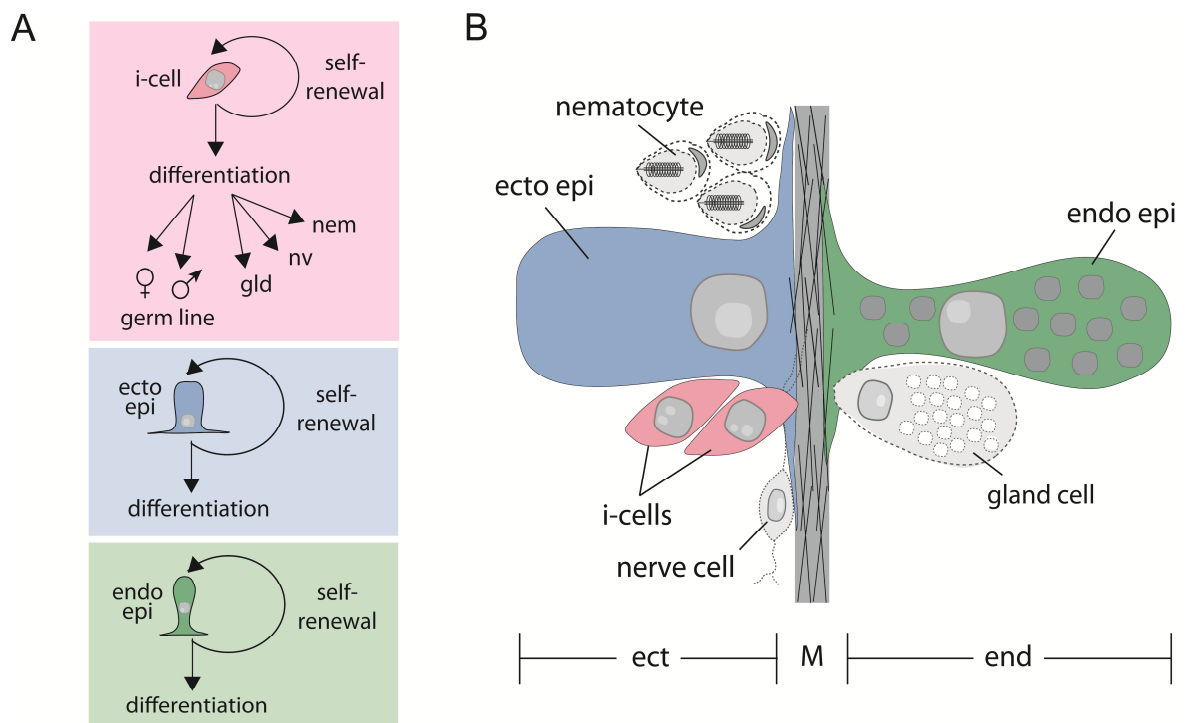
The 0.5 to 1.0 cm tall freshwater polyp Hydra has a radial symmetric body plan with an oral-aboral axis. The body is structured into three parts: head, body column and foot (Figure 7A). The head is characterized by its tentacle zone with four to twelve tentacles and the hypostome. Because the hypostome is the only orifice of Hydra, it is used for nutrition uptake as well as for the excretion of digestion products. The body column is constructed as a tube, the inside consists of a gastrovascular cavity and outside gonads can be formed (Shimizu et al., 2007). In the lower third of the body column buds can be formed for asexual reproduction. The foot possesses a basal disc, which enables the Hydra polyp to attach to substrate. Hydra is a diploblastic organism, consequently it possesses only two epithelial cell linages, the endo- and the ectoderm. Both cell layers are separated by a noncellular layer, called mesoglea. The mesoderm, known from bilateria, is missing (Figure 7B). The ectoderm is covered by a layer made of polysaccharides, which is called glycocalyx. It is structured into five layers, while the inner layers are firm and the outer, fifth layer is loose (Figure 7C) (Böttger et al., 2012; Fraune et al., 2014; Holstein et al., 2010).



**Figure 7: The bodyplan of Hydra**

(A) Schematic longitudinal cross section of an adult Hydra polyp. The oral-aboral body axis is structured into three parts, head, body column and foot. The head is characterized by its tentacle zone and the hypostome. In the lower region of the body column a bud is formed. At the end of the foot is a basal disc located, which is used for the attachment to the substrate. Arrows indicate the cell flow to the extremities. (B) Cross section of the diploblastic epithelium of Hydra, where the endoderm and ectoderm are separated by the mesoglea, a noncellular layer. On top of the ectoderm a layer out of glycoproteins, called glycocalyx, is located (illustrated with pink strips). endo epi: endodermal epithelia cells, gland: gland cell, i-cells: interstitial cells, ect epi: ectodermal epithelial cells. (C) The glycocalyx, a layer of glycoproteins, is located on the ectoderm (ecto). The four inner layers (c1-4) are firmly structured, while the outer layer (c5) is looser. pm, plasma membrane (taken from Bosch, 2007a; Fraune et al., 2014 and modified).

The Hydra polyp consists of 20 cell types, which originate from three stem cell lineages. Besides the unipotent ectodermal and endodermal epithelial stem cells, Hydra also possess multipotent interstitial stem cells (i-cells) (Figure 8A) (Hemmrich et al., 2012). Both epithelial stem cells give rise to epithelial muscle cells, which form the framework of the epithelial layers. The epithelial muscle cells are particular, as they combine features of epithelial cells and muscle cells. In contrast, i-cells can differentiate into different somatic cells, as nerve cells, gland cells and different types of nematocytes, but also into germ cells (Figure 8A) (Bode et al., 1987; David and Murphy, 1977). Consequently, Hydra polyps do not have a separate germline (Bosch, 2007b; Bosch and David, 1987). Most cells originating from i-cell lineage are embedded into the ectoderm. Only gland cells and some nerve cell types are located in the endoderm (Figure 8B).



**Figure 8: Histology of Hydra**

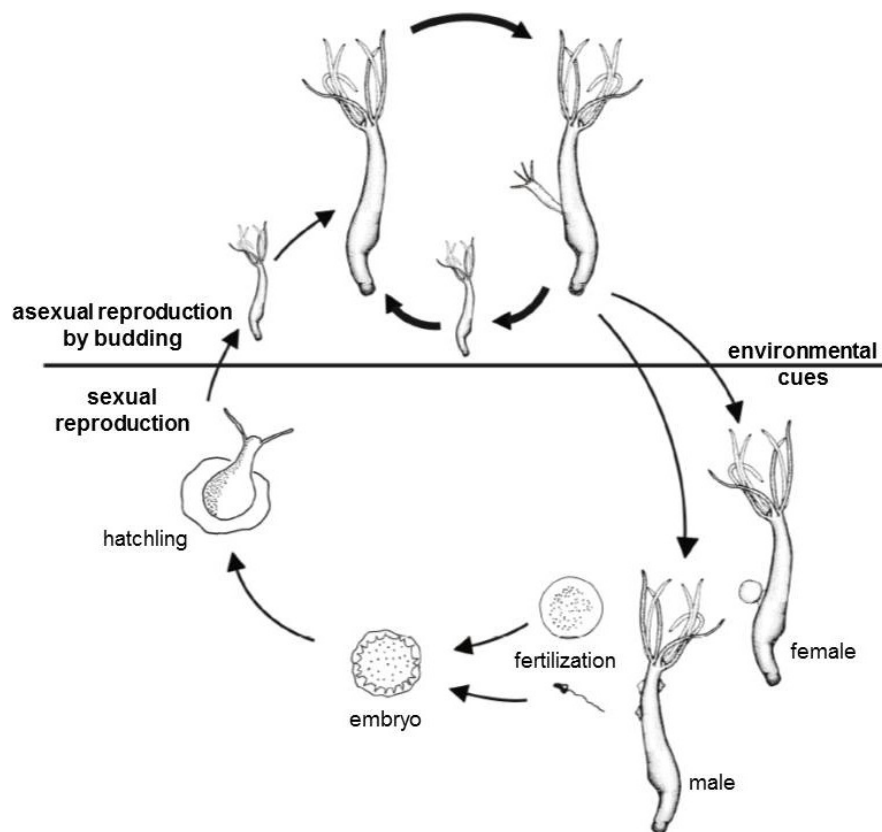
(A) The three stem cell lineages in Hydra give rise to 20 cell types. Besides the unipotent ectodermal (ecto epi) and endodermal epithelial stem cells (endo epi), which differentiate into epithelial muscle cells, Hydra also possesses multipotent interstitial stem cells (i-cells). I-cells can differentiate into different somatic cells, as nerve cells (nv), gland cells (gld) and different types of nematocytes (nem), but also into germ cells. (B) Schematic diploblastic organization of the Hydra epithelium. The endo- (end) and the ectoderm (ect) are separated by a extracellular matrix, called mesoglea (M) Stem cell lineages are colored; derivatives of the interstitial cell lineage are shown in gray (taken from Hemmrich et al., 2012 and modified).

The permanent proliferation of the stem cell lineages creates a continuous cell flow from the body column to the bud and the extremities. Excessive cells get released at the basal disc, the hypostome and the ends of the tentacles (Figure 7A) (Bosch, 2003; Campbell, 1967). Consequently, mature nematocytes are located in the tentacles. There, always 8 to 24 cnidocytes are integrated into a so called battery cell, which differentiate out of an

ectodermal epithelial muscle cell. This is characteristic for Hydra's cell system: the stem cells are located in the body column, whereas the progenies can be found in foot and head.

### 1.4.3 Feeding, reproduction and life cycle of Hydra

In contrast to most Cnidarians, Hydra polyps can exclusively be found in freshwater and are sensitive to pollution and salt. They are widely distributed in the temperate and tropical regions in the Northern and in the Southern Hemisphere (Martínez et al., 2010). By use of its tentacles, which harbors nematocytes, they are able to capture small Crustacean like Daphnia or Copepods. Reproduction occurs mainly through budding, but Hydra is also able to reproduce sexually via the production of eggs and sperms (Figure 9).



**Figure 9: Life cycle of Hydra**

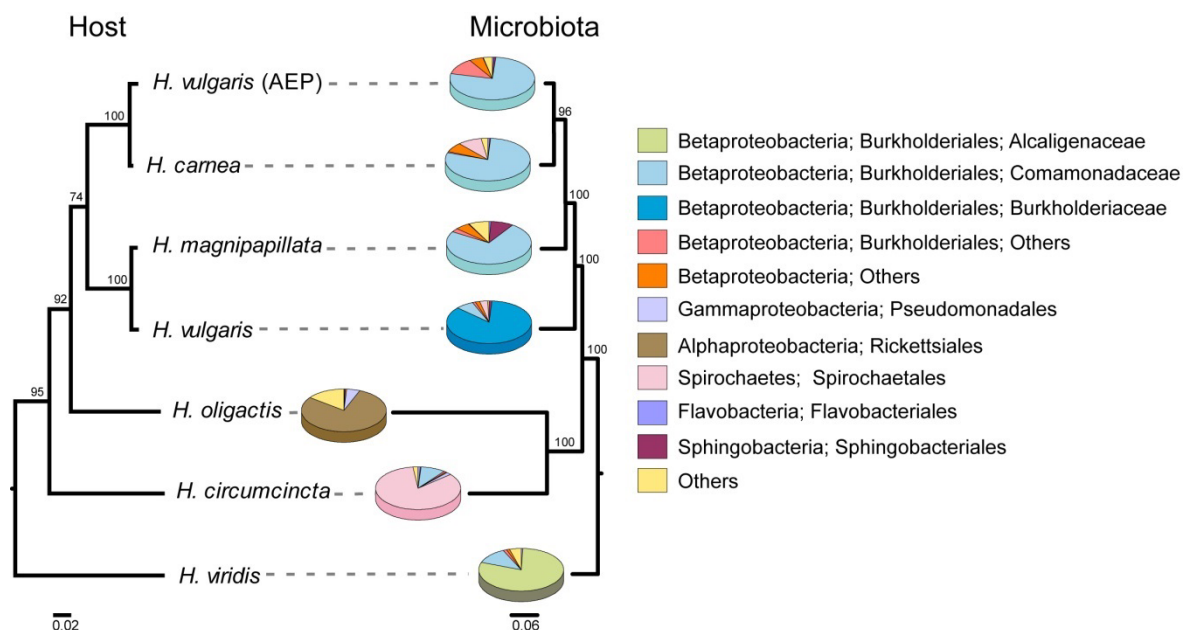
Hydra mainly reproduces asexual by budding. Environmental cues induce the sexual reproduction. Approximately four weeks after fertilization a polyp hatches (taken from Bosch, 2009; Fraune, 2008 and modified).

The alteration of generations (metagenesis), from polyp to medusa, is typical for Cnidarians, but does not occur in the genus Hydra (Holstein and Emschermann, 1995). Sexual reproduction is induced by various environmental factors. Lowering the temperature induces *Hydra oligactis* to reproduce sexually, while *Hydra vulgaris* (AEP) and *Hydra viridissima* need a longer starvation period. *Hydra* species have different reproductive strategies; species like *Hydra viridissima* are hermaphrodites, which develop

at the same time testes and an egg patch, while other species as *Hydra oligactis* are gonochorous, meaning that a polyp has only one sex. The species *Hydra vulgaris* and *Hydra magnipapillata* spontaneously change their sexes, they are designated as unstable gonochorous (Bosch and David, 1986; Holstein and Emschermann, 1995). The eggs, attached to the female polyps, get fertilized by free swimming spermatozoa, which were released from the testes into the water. In the early phase of embryogenesis the embryo stays at the egg cup of the mother polyp, but it drops down before the gastrulation is completed. By the development of a thick cuticle (the embryotheca) the embryo can stay in a period of dormancy up to 52 weeks before the polyp finally hatches (Figure 9) (Martin et al., 1997).

#### 1.4.4 Host-microbe interactions in the metaorganism Hydra

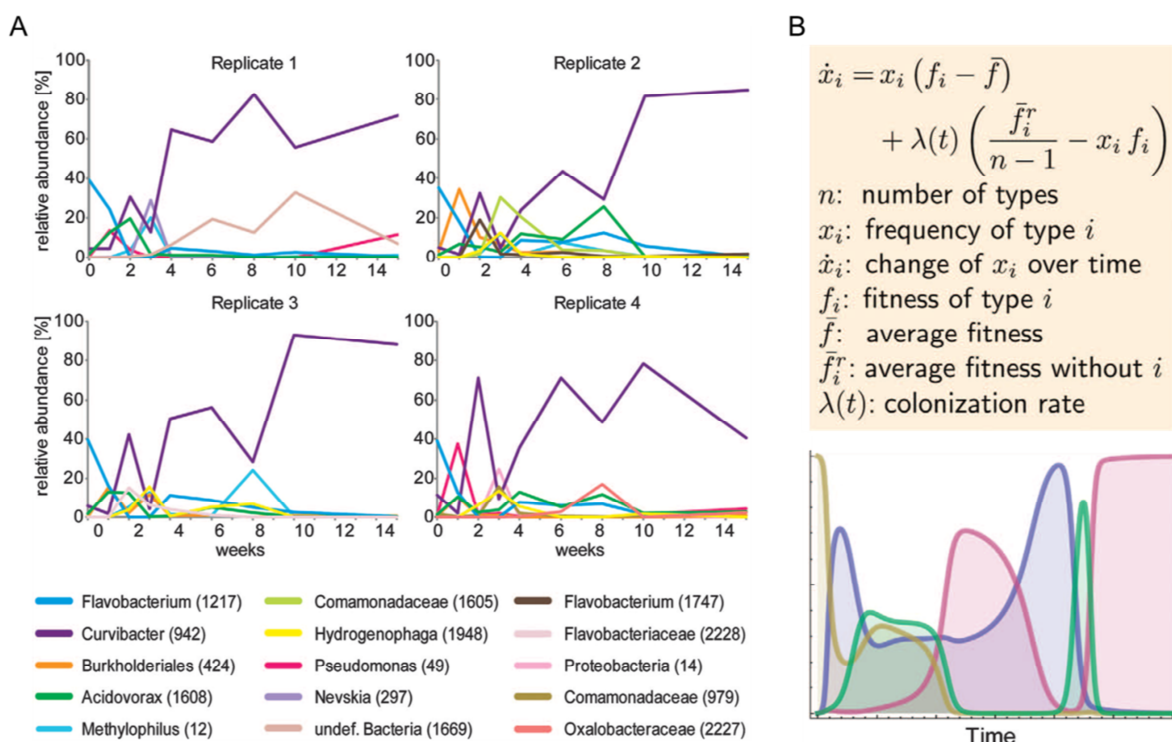
As, with every multicellular organism, Hydra also has to be considered as a metaorganism (Bosch and McFall-Ngai, 2011). In the last years Hydra has been established as a model organism for host-microbe interactions. It could be shown that different Hydra species possess a species specific microbiota, even though they were cultivated independently for over 30 years under constant environmental conditions (Figure 10) (Franzenburg et al., 2013a; Fraune and Bosch, 2007). In general they possess a simple bacterial composition with around ten bacterial species and are interestingly dominated by Gram-negative bacteria (Franzenburg et al., 2013a).



**Figure 10: Species specific bacterial community of seven Hydra species**

Comparison of the phylogenetic tree of different Hydra species and their bacterial communities. The species specific bacterial communities of Hydra are dominated by Gram-negative bacteria (taken from Franzenburg et al., 2013a and modified).

90% of the bacterial community associated with *Hydra vulgaris* (AEP), which is used for the generation of transgenic animals, is represented by six cultivated bacteria species, *Curvibacter* sp., *Acidovorax* sp., *Pelomonas* sp., *Undibacterium* sp., *Duganella* sp. and *Pseudomonas* sp. (Fraune et al., 2014). *Curvibacter* sp. is the main colonizer of *Hydra vulgaris* (AEP); it makes up to 75% of the bacterial community. After hatching, the bacterial composition of a Hydra polyp starts to form, resulting after four weeks in a bacterial community dominated by *Curvibacter* sp. (Figure 11A) (Franzenburg et al., 2013b). Using an modeling approach, it could be shown that the colonization pattern during ontogeny of Hydra is only explainable, if one considers a host factor and a frequency dependent fitness for the bacteria (Figure 11B) (Franzenburg et al., 2013b). The mutual dependency of the host and its bacteria can be seen, once Hydra's epithelial homeostasis is disturbed, as it causes a drastic change in the bacterial composition of Hydra (Fraune et al., 2009). This indicates that there have to be many regulatory mechanisms that keep the metaorganism Hydra in homeostasis.



**Figure 11: Dynamics in the microbiota composition.**

(A) Relative abundances of bacterial OTUs on a *Hydra vulgaris* (AEP) polyp over the first 15 weeks post hatching. After week four *Curvibacter* sp. is dominating the bacterial community (Franzenburg et al., 2013b). (B) Mathematical modeling of the bacterial colonization process in Hydra. Frequency-dependent growth rates of the bacteria are not enough to explain the dynamics, an external modulation of the colonization process, e.g. by the host, is additionally required (taken from Franzenburg et al., 2013b and modified).



It could be shown that the presence of bacteria is sensed via the Toll signaling pathway. In *Hydra magnipapillata*, a functional Toll-like receptor (TLR) was identified, which is composed of two components, HyTRR-1 and HyLRR-2. Hydra's TLR could be stimulated by flagellin (Bosch et al., 2009). Also, the other components of the vertebrate TLR signaling cascade could be identified in Hydra. Through a knockdown of the key adapter protein myeloid differentiation primary response gene 88 (MyD88), it could be shown that Hydras TLR is responsible for the recognition of bacteria (Franzenburg et al., 2012). Furthermore, nucleotide-binding oligomerization domain (NOD) like receptors (NLRs) could be identified in Hydra, which are known from mammals to be involved in the cytosolic bacteria recognition (Philpott and Girardin, 2004). In Hydra the NLRs are upregulated in the presence of LPS and flagellin, but a direct bacteria interaction has not been shown so far (Lange et al., 2011).

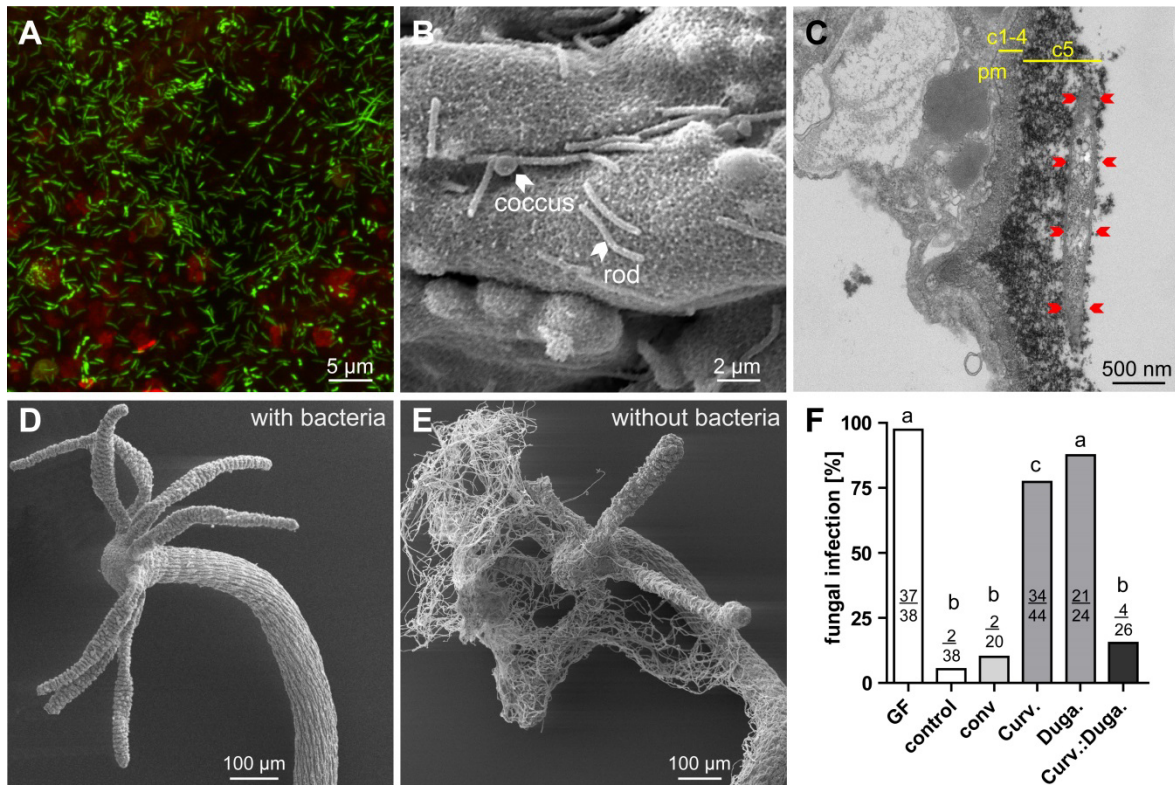
In the selection of beneficial microbes for the metaorganism Hydra, antimicrobial peptides (AMPs) are playing a major role. So far three AMP peptide families could be identified in Hydra: Hydramacins, Periculins and Arminins (Augustin et al., 2009; Bosch et al., 2009). Hydramacin-1 is a 60 amino acid long secretory peptide with a cationic charge, which is upregulated by microbe-associated molecular patterns (MAMPs) and LPS in a concentration dependent manner (Bosch et al., 2009; Jung et al., 2009). It is expressed in the endoderm.

Periculin-1 expression gets stimulated by a broad range of signals; flagellin, LPS and double stranded RNA. In contrast to Hydramacin-1, Periculin-1 is not only regulated by exogenous signals, also an elevated apoptosis rate influence the Periculin-1 expression (Bosch et al., 2009). Analysis of the amino acid sequence revealed that Periculin-1 is structured into an anionic N-terminal and a cationic C-terminal part and possess a signal peptide (Bosch et al., 2009). After proteolytic cleavage of the cationic C-terminal part from the pro-peptide, the cationic fragment possesses bactericidal activity (0.2  $\mu$ M to 0.4  $\mu$ M). The ectopic expression of the Periculin-1a has drastic quantitative and qualitative effects on the Hydra associated bacterial community (Fraune et al., 2010). Furthermore, it seems that Periculin-1a plays a role in the pathogen defense in the early Hydra embryo, as the expression of the AMP strongly increases during oogenesis in the female polyp (Fraune et al., 2010).

The AMPs from the Arminin peptide family are secreted peptides that also exhibit an anionic N-terminal and a cationic C-terminal part. The C-terminal fragment of Arminin-1a develops antimicrobial activity after the proteolytic cleavage of the N-terminal part, and possess strong bactericidal activity in nanomolar range (Augustin et al., 2009). Franzenburg et al. could show that different Hydra species possess a species specific set of Arminins. In *Hydra vulgaris* (AEP) almost all Arminins were expressed in the endoderm, except Arminin6560, which is located in the ectoderm (Franzenburg et al., 2013a). Furthermore, they could show that Arminin-deficient polyps are less able to select the native bacterial composition than the control polyps. This indicates that the AMP peptide family Arminin is involved in the selection of the Hydra species specific microbial communities (Franzenburg et al., 2013a). These findings show that the antimicrobial peptides are mainly expressed in the endoderm or during embryogenesis and are playing an important role in the selection and regulation of the total bacterial community of Hydra.

The metaorganism Hydra possess different potential niches for the colonization of bacteria, the surface of the endoderm, the mesoglea and the surface of the ectoderm (Figure 7). In the gastric cavity so far no bacteria could be localized, most probably they are regulated by the endodermal expressed AMPs and can get taken up by phagocytosis (Bosch et al., 2009). In the mesoglea spirochaetes were reported, but because they are only rarely present they can be considered as tourist of the Hydra bacterial community (Davis and Haynes, 1968; Hufnagel and Myhal, 1977). The surface of the ectoderm is covered by five layers out of glycoproteins, the so called glycocalyx (Figure 7). The outer, loose layer of the glycocalyx serves as a habitat for the commensal bacteria of Hydra (Figure 12A-C) (Fraune et al., 2014).

Hydra is investing high metabolic costs in the bacterial habitat and in their regulation but in exchange it gains an antifungal protection provided by its commensal (Figure 12D-F). Interestingly the two main colonizer *Curvibacter* sp. and *Duganella* sp. alone cannot protect Hydra from infection by *Fusarium* sp., but a di-association of these bacteria leads to almost full antifungal protection (Figure 12F) (Fraune et al., 2014). These results show that bacteria-bacteria interactions are essential for the metaorganism homeostasis. Furthermore, it could be shown that the Hydra species possess also a species specific virome. These viromes consist of eukaryotic viruses but is dominated by bacteriophages, indicating that Hydras microbial community get also regulated by phages, revealing another weal of the metaorganism machinery (Grasis et al., 2014).



**Figure 12: Bacterial colonization of the Hydra polyp**

(A-C) Hydra's ectodermal glycocalyx is colonized by a complex bacterial community. (A) Staining of bacteria with SYBR gold. (B) Raster electron micrograph (REM) of bacterial cells located on the surface of ectodermal cells. (C) Transmission electron micrograph (TEM) of a rod-shaped bacterium (red arrows) located within the outer loose layer (c5) of the glycocalyx; c1-4, four firm layer of the glycocalyx; pm, plasma membrane (modified after Fraune et al., 2014). (D-F) Hydra's commensals provide antifungal protection. (D) Raster electron micrograph (REM) of a control polyp showing no fungal infection. (E) Raster electron micrograph (REM) of a germfree (GF) Hydra polyp infected by *Fusarium* sp. (F) *In vivo* infection rates with *Fusarium* sp. of Hydra polyps recolonized by different bacterial isolates: conv, conventionalized; Curv., mono-association with *Curvibacter* sp.; Duga., mono-association with *Duganella* sp.; Curv.:Duga., di-association with *Curvibacter* sp. and *Duganella* sp. Different lowercase letters indicate significant differences between treatments: 'a' indicates significantly different from control ( $P < 0.01$ ), 'b' indicates significantly different from GF ( $P < 0.01$ ), 'c' indicates significantly different from control and GF ( $P < 0.01$ ) (taken from Fraune et al., 2014 and modified).

## 1.5 Aims of the thesis

A metaorganism, consisting of a host and a multitude of associated microbes, needs a fine regulated network to keep this system in homeostasis. It is fundamental to understand the underlying regulatory mechanisms, as an unbalanced system is the cause of diseases. On one hand there have to be mechanisms to regulate the interactions within one group of a metaorganism, while on the other hand the different groups within the system are influencing each other, which also have to be regulated. In this thesis only the host-bacteria interaction is investigated.

In previous studies, it could be shown that bacteria are colonizing Hydra's glycocalyx (Fraune et al., 2014) and that these bacteria get selected by the species specific secretion of antimicrobial peptides by the host (Franzenburg et al., 2013a). The presence of the bacteria is sensed by a TLR-pathway (Bosch et al., 2009; Franzenburg et al., 2012). Additionally, it could be shown that the commensal bacteria provide an antifungal protection for their host (Fraune et al., 2014). But a host does not only has to decide which bacteria it allows to colonize, even more important is that it suppresses potentially harmful behavior of its commensal bacteria, as biofilm formation, motility and virulence, but so far no mechanism are known how Hydra could influence such bacterial behavior.

Bacteria are regulating their community wide behaviors, as biofilm formation, by their communication system, called quorum sensing (QS), consequently interfering with the bacterial QS (quorum quenching, QQ) could be a starting-point for Hydra to control its commensal bacteria.

The aim of this thesis was to investigate, whether Hydra possesses a quorum quenching (QQ) mechanism, which enables Hydra to influence the behavior of colonizing bacteria and if this mechanism is involved in the maintenance of homeostasis within the metaorganism. Therefore, the involved QQ mechanism was investigated, and the bacterial QS in the neighborhood of Hydra was analysed. Especially the QS system of *Curvibacter* sp., the main colonizer of *Hydra vulgaris* (AEP), was studied. Finally, the consequences of the QQ for the metaorganism Hydra were investigated. Additionally, the interaction between *Curvibacter* sp. and *Duganella* sp., the two main colonizers of *Hydra vulgaris* (AEP), was investigated as their interactions result in an antifungal activity.

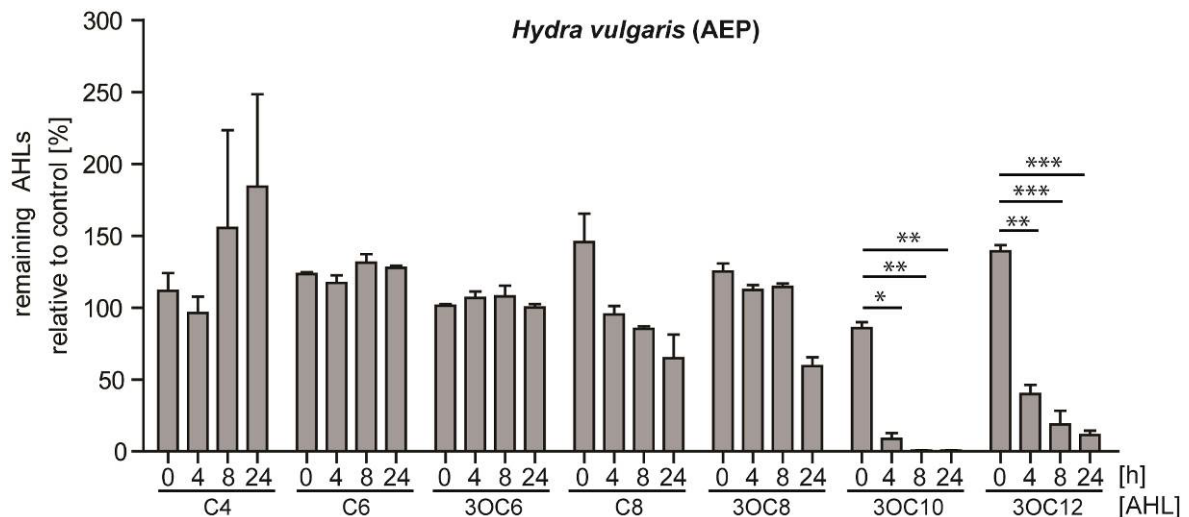
## 2 Results

### 2.1 Quorum quenching in Hydra

Bacteria from very different habitats, in soil (Dessaux et al., 2010), fresh water (Feng et al., 2013), marine ecosystem (Miller et al., 2005) and even on eukaryotic hosts (Alagely et al., 2011; Golberg et al., 2011; Hartmann and Schikora, 2012; Ransome et al., 2014), are communicating via quorum sensing (QS). Many bacteria developed mechanisms to interfere with their own QS system, but also with that of their competitors (quorum quenching, QQ). If one considers that a eukaryotic host and its bacteria evolved together, it is likely, that also the host developed mechanisms to interfere with the bacterial communication system. But so far, little is known about eukaryotic QQ mechanisms (chapter 1.3).

#### 2.1.1 *Hydra* sp. possess a general quorum quenching activity against long-chain *N*-acyl-homoserine lactones

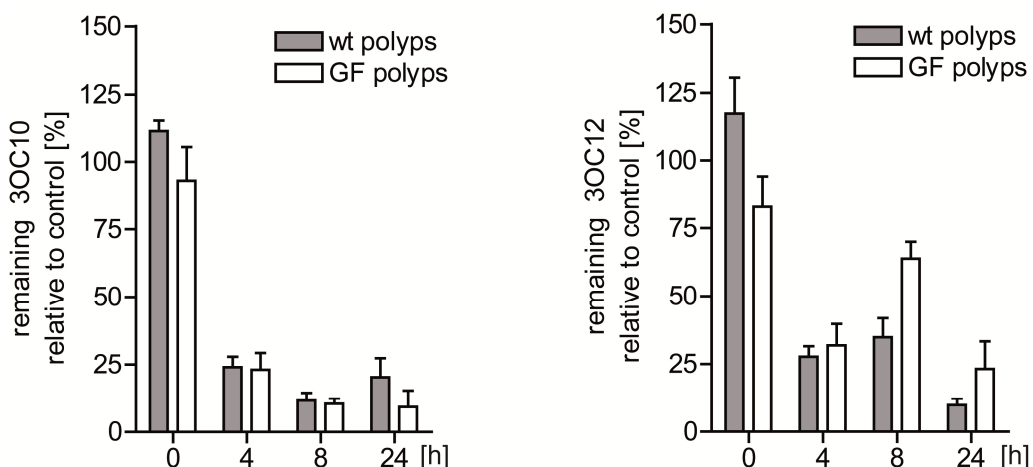
To investigate whether or not Hydra is able to interfere with the bacterial communication system, quorum sensing (QS), *Hydra vulgaris* (AEP) polyps were incubated for 24 hours with different, commercially available *N*-acyl-homoserine lactone species (AHLs), the QS signaling molecules of Gram-negative bacteria. The remaining AHLs were analysed with a bacterial reporter system (chapter 5.30.1). The statistical analysis was performed with a Two-Way ANOVA and a Bonferroni post-test. *Hydra vulgaris* (AEP) is able to specifically modify long-chain 3-oxo-homoserine lactones, i.e. 3OC10- and 3OC12-HSLs, which get inactivated within four hours, while 3OC6- and 3OC8-HSLs are not affected by Hydra's QQ activity (Figure 13). The unmodified short-chain C4-, C6- and C8-HSLs, as their 3-oxo-counterparts, get also not modified by Hydra. Because of the low sensitivity of the AHL-bioreporters used for unmodified long-chain AHLs, it could not be investigated if Hydra is able to modify these kinds of AHLs. Also 3-hydroxy-HSLs, another class of signaling molecules of Gram-negative bacteria, cannot be detected with the employed bacterial AHL-reporter strains (appendix 8.1). The measured QQ activity within the first four hours was used to calculate the reaction rate of the AHL modification by Hydra; accordingly one *Hydra vulgaris* (AEP) polyp is able to modify within one hour 23 pmol 3OC10-HSLs or 18 pmol 3OC12-HSLs.



**Figure 13: Quorum quenching activity of *Hydra vulgaris* (AEP)**

*Hydra vulgaris* (AEP) polyps were incubated with 10  $\mu$ M of seven different AHL species (C4-, C6-, 3OC6-, C8-, 3OC8-, 3OC10-, and 3OC12-HSL) for 24 h at 18°C. The remaining AHLs were extracted with ethyl acetate and semi-quantification was performed with bacterial reporter strains. For statistical analysis a Two-Way ANOVA with a Bonferroni post-test was used (\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ,  $n = 3$ ).

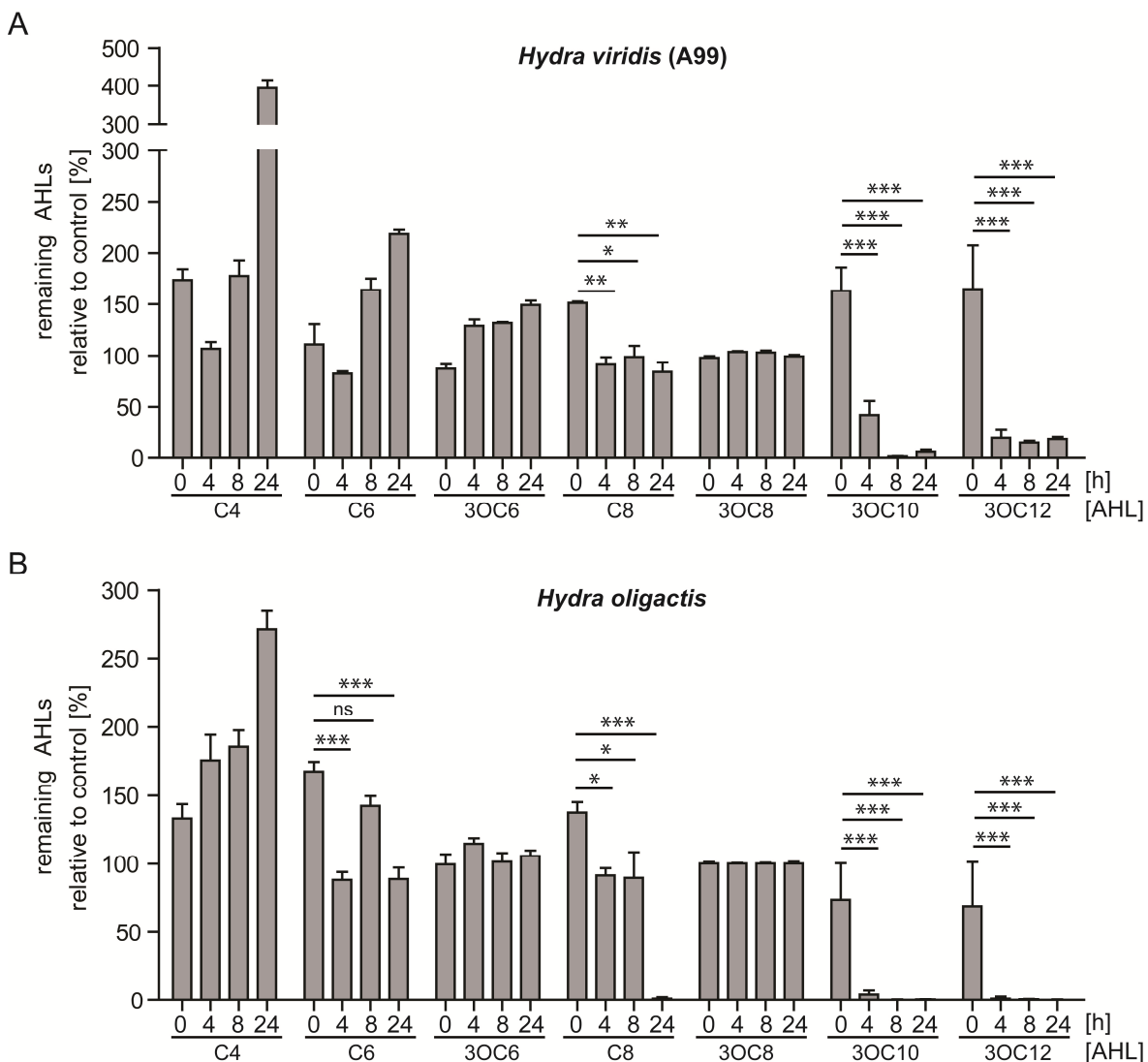
Because many bacteria have QQ activity reported, it had to be clarified, if the AHL modification by the Hydra metaorganism is based on a QQ activity of its commensal bacteria or of the host. Therefore, the inactivation of the two long-chain AHLs 3OC10- and 3OC12-HSL by wild type and germfree (chapter 5.1.3) *Hydra vulgaris* (AEP) polyps was compared (chapter 5.30.1). The QQ activities, shown in Figure 14, do not significantly differ (Two-Way ANOVA with Bonferroni post-test) between control and germfree polyp, showing that the Hydra polyp, and not its bacterial community, is responsible for the AHL inactivation.



**Figure 14: Quorum quenching activity of germfree *Hydra vulgaris* (AEP)**

Wild type (wt) or germfree (GF) *Hydra vulgaris* (AEP) polyps were incubated for 24 h at 18°C with 10  $\mu$ M of the long-chain AHLs 3OC10-, or 3OC12-HSL. The remaining AHLs were extracted with ethyl acetate and the semi-quantification was performed with bacterial reporter strains. The differences between QQ by wild type and germfree Hydra polyps were calculated with a Two-Way ANOVA with Bonferroni post-test ( $P > 0.05$ ,  $n = 3$ ).

To investigate if the QQ activity of Hydra is species-specific, the experiment was repeated with the phylogenetic distant related Hydra species *Hydra viridis* (strain A99) and *Hydra oligactis* (strain St. Petersburg). Interestingly, the QQ activity toward long-chain 3-oxo-HSLs is a general phenomenon of the three distantly related Hydra species (Figure 15).



**Figure 15: Quorum quenching activity of different Hydra species**

*Hydra* polyps of the two species *Hydra viridis* (strain A99) and *Hydra oligactis* (strain St. Petersburg) were incubated with 10  $\mu$ M of seven different AHL species (C4-, C6-, 3OC6-, C8-, 3OC8-, 3OC10-, and 3OC12-HSL) for 24 h at 18°C. The remaining AHLs were extracted with ethyl acetate and the semi-quantification was performed with bacterial reporter strains. For statistical analysis a Two-Way ANOVA with a Bonferroni post-test was used (\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ,  $n = 3$ ).

*H. viridis* and *H. oligactis*, like *H. vulgaris* (AEP) exhibit the highest reaction rate for 3OC10-HSLs (12 pmol/hour/polyp *H. viridis*, 23 pmol/hour/polyp *H. oligactis*) and 3OC12-HSLs (15 pmol/hour/polyp *H. viridis*, 24 pmol/h/polyp *H. oligactis*). In contrast to *H. vulgaris* (AEP), *H. viridis* and *H. oligactis* are also able to alter unmodified short-chain AHLs, even though their reaction rate is much lower. Both modify 6 pmol (*H. viridis*) or rather 8 pmol (*H. oligactis*) C8-HSLs within one hour per polyp. Furthermore, *H. oligactis* is able to

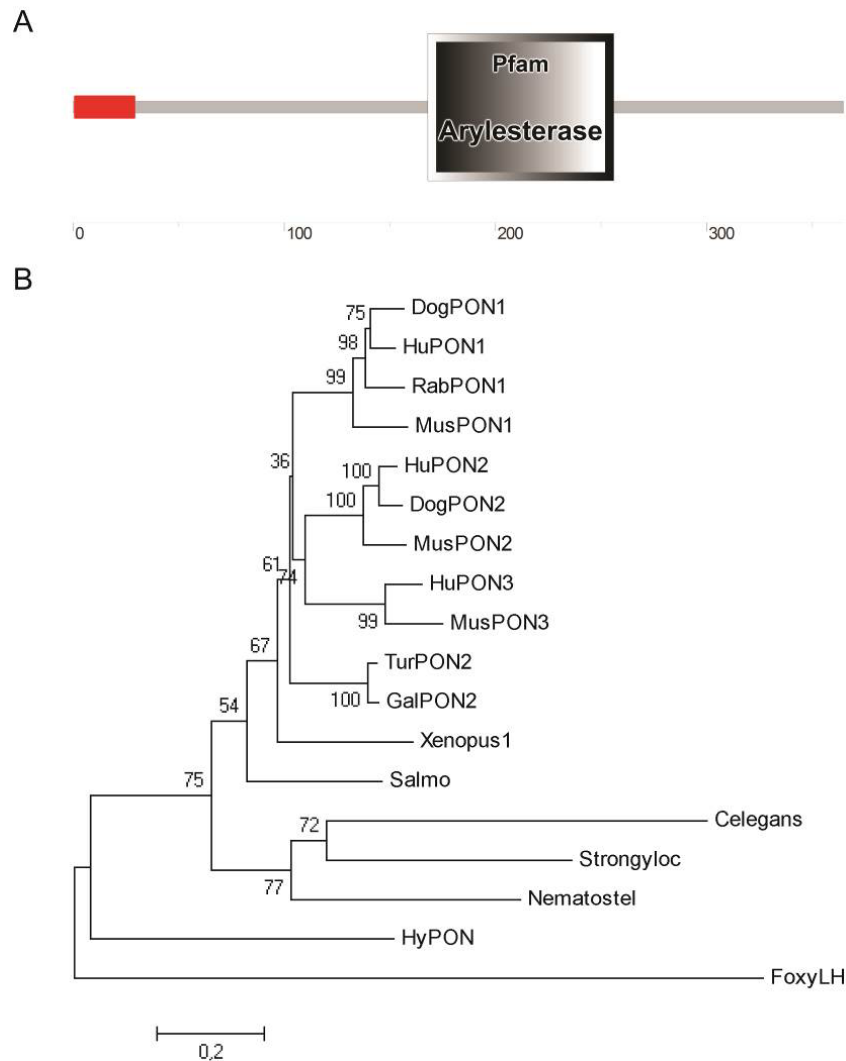
modify C6-HSL (12 pmol/hour/polyp). If the alteration of unmodified short-chain AHLs is caused by the Hydra polyps or by their microbiota remains to be investigated.

### **2.1.2 The paraoxonase of *Hydra vulgaris* (AEP) has no quorum quenching activity, as its homologues in human**

It was shown that Hydra possesses quorum quenching (QQ) activity. Consequently, it was the question, which kind of mechanism enables Hydra to modify *N*-acyl-homoserine lactones (AHLs). In eukaryotes only paraoxonases were described to possess a QQ activity (chapter 1.3). Therefore it was analysed if the QQ activity in Hydra is also possessed by a paraoxonase. At first an *in silico* approach was used to investigate, if Hydra possesses a paraoxonase. The amino acid protein sequence of the human paraoxonase PON1 (GenBank: EAL24133.1) were BLASTed against the transcriptome of *Hydra vulgaris* (AEP) (db454\_haep\_celera\_v02). Thereby the contig 6197 could be identified to be similar to the human paraoxonases, which will be named in the following as HyPON. The cDNA sequence of the HyPON (appendix 8.2.1), was translated into the amino acid sequence (appendix 8.2.2). The protein domain prediction was performed by the program SMART. As shown in Figure 16, an arylesterase/paraoxonase domain, as known from the human paraoxonases PON1-3 (Primo-Parmo et al., 1996), was identified on the HyPON sequence. Finally a BLASTp search led to the identification of contig 6197 in Hydra's transcriptome as a homologue to the human paraoxonases.

A phylogenetic tree of metazoan paraoxonases was constructed to analyse the relationship of the HyPON to the other metazoan PONs (Figure 16). The human paraoxonases cluster together with other mammalian PONs, while PONs of *Caenorhabditis elegans*, *Strongylocentrotus purpuratus* and *Nematostella vectensis* form a sister clade to the other metazoan PONs. The HyPON is basal to the metazoan PON clade, but compared to the outgroup it cluster together with the other PONs. In total, the phylogenetic tree of the metazoan PONs recapitulates phylogenetic trees of other phylogenetic markers.

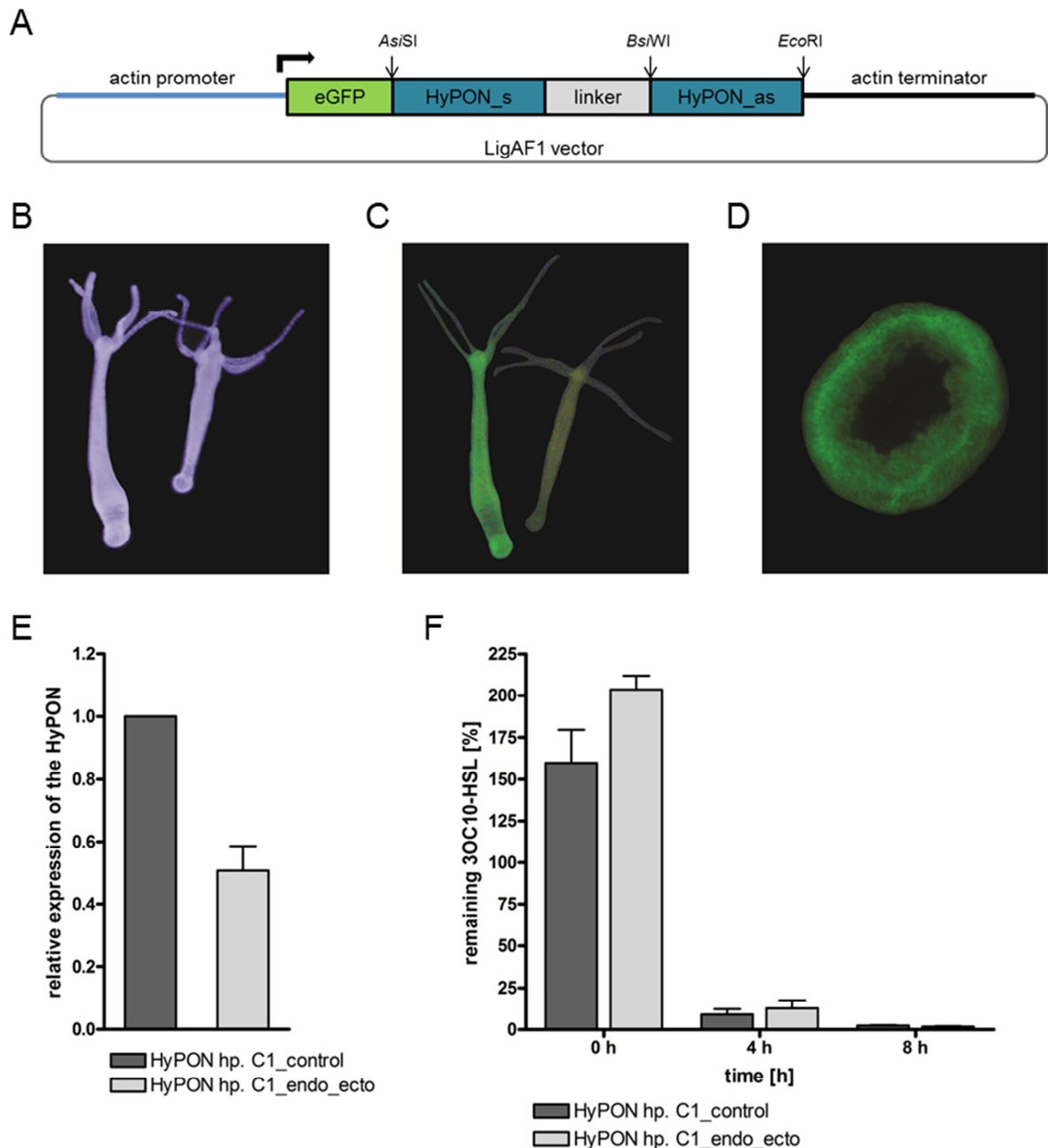




### Figure 16: Classification of Hydra's paraoxonase HyPON

(A) The SMART program identified an arylesterase/paraoxonase domain (PF01731) in the HyPON amino acid sequence. As indicated in red, the HyPON possesses a signal peptide, which was confirmed by a SignalP analysis. (B) Phylogenetic tree of metazoan paraoxonases (PON). The program MEGA was used to construct the phylogenetic tree (neighbor-joining algorithm, bootstrap 1000). The following PON nucleotide sequences were used: human (HuPON1, M63012 (Adkins et al., 1993; Hassett et al., 1991); HuPON2, L48513 (\*); HuPON3, L48516 (\*)), mice (MusPON1, L42938 (Cole et al., 2011); MusPON2 (\*), L48514; MusPON3 (\*)), rabbit (RabPON1, M63011 (Hassett et al., 1991)), dog (DogPON1, XM\_845126; DogPON2, L48515 (\*)), turkey (TurPON2, L47572 (\*)), chicken (GalPON2, L47573 (\*)), frog (Xenopus1, NM\_001087180.1), salmon (Salmo, NM\_001141506.1), sea urchin (Strongyloc, XM\_782720.2), worm (Celegans, AF295927.1), sea anemone (Nematostel, XM\_001630782.1), *Hydra vulgaris* (AEP) (HyPON, (\*\*)), lactonohydrolase from fungi *Fusarium oxysporum* AKU 3702 (FoxyLH, AB010465 (Kobayashi et al., 1998)). \* Sequences are taken from the following reference (Primo-Parmo et al., 1996). \*\* The sequence of the HyPON can be found in appendix 8.2.

To analyse if the HyPON possess, as its human homologous, a quorum quenching (QQ) activity, its *in vivo* activity was analysed by a loss of function experiment. Transgenic *Hydra vulgaris* (AEP) with a knockdown of the HyPON were generated. Therefore a HyPON hairpin construct was built (Figure 17A) and introduced into embryonic *Hydra vulgaris* (AEP) via microinjection (chapter 5.22).



**Figure 17: *In vivo* quorum quenching activity of Hydra's paraoxonase HyPON**

(A) Plasmid for the generation of transgenic Hydra, which express the hairpin construct for the paraoxonase of Hydra (HyPON). After the transcription of the fusion gene the complementary parts HyPON<sub>s</sub> (s = sense) and HyPON<sub>as</sub> (as = antisense) form a hairpin structure. Both pieces are connected by the linker. The transgene is under the control of the Hydra actin promoter. For the selection of positive transgenic polyps the eGFP gene was put upstream of the hairpin. Arrows indicates which restriction enzymes (*AsiSI*, *BsiWI*, *EcoRI*) were used for the connection of the different fragments of the construct. The LigAF1 vector was used as a backbone. (B-C) Live images of a transgenic HyPON hp. C1<sub>endo\_ecto</sub> polyp (left) and a control polyp (HyPON hp. C1<sub>control</sub>) (right), recorded with white light (B) or a GFP-filter (C). (D) Cross section of a transgenic HyPON hp. C1<sub>endo\_ecto</sub> polyp, showing an eGFP signal in the endoderm and ectoderm. (E) Confirmation of the HyPON knockdown by qRT-PCR. The HyPON transcript is ca. 50% down regulated in HyPON hp. C1<sub>endo\_ecto</sub> polyps compared to the control. (F) Analysis of the quorum quenching (QQ) activity of HyPON knockdown polyps. The transgenic *Hydra vulgaris* (AEP) polyps were incubated with 10  $\mu$ M 3OC10-HSL for 8 h at 18°C. The remaining AHLs were extracted with ethyl acetate and semi-quantification was performed with bacterial reporter strains. The differences between QQ by control and HyPON knockdown Hydra polyps were calculated with a Two-Way ANOVA with Bonferroni post-test ( $P > 0.05$ ,  $n = 3$ ).

After injection of 33 Hydra embryos only one transgenic polyp (C1) with mosaic eGFP expression in the endoderm and ectoderm hatched. Due to the clonal proliferation and screening for GFP positive cells, out of the mosaic polyp three lines could be established. The line C1\_ecto expresses the hairpin construct in all cells of the ectodermal epithelial stem cells, while line C1\_endo possesses a transgenic endoderm, which can be detected by the eGFP expression. Furthermore, a control line C1\_control, which lost all transgenic cells, was generated (Figure 17B-C). All three lines inherit the same genetic background. To generate a polyp with a transgenic endo- and ectoderm, the transgenic tissues of line C1\_endo and C1\_ecto were transplanted. Therefore the tissue layers of line C1\_endo and C1\_ecto, each 10 polyps, were separated by procaine treatment (chapter 0). Only one transgenic endoderm could be successfully transplanted into the transgenic ectoderm (chapter 5.3). After a week a complete Hydra with transgenic endo- and ectodermal stem cells was arisen out of the transplant. The polyp was used to raise a clonal line C1\_endo\_ecto (Figure 17B-D). In this transgenic line 75% of the HyPON transcripts can be targeted by the hairpin construct, the rest are present in the unaffected interstitial stem cell line (distribution of the HyPON transcript in the 454 transcriptome of the Hydra stem cell lineages: ectodermal: 31, endodermal: 6, interstitial: 12).

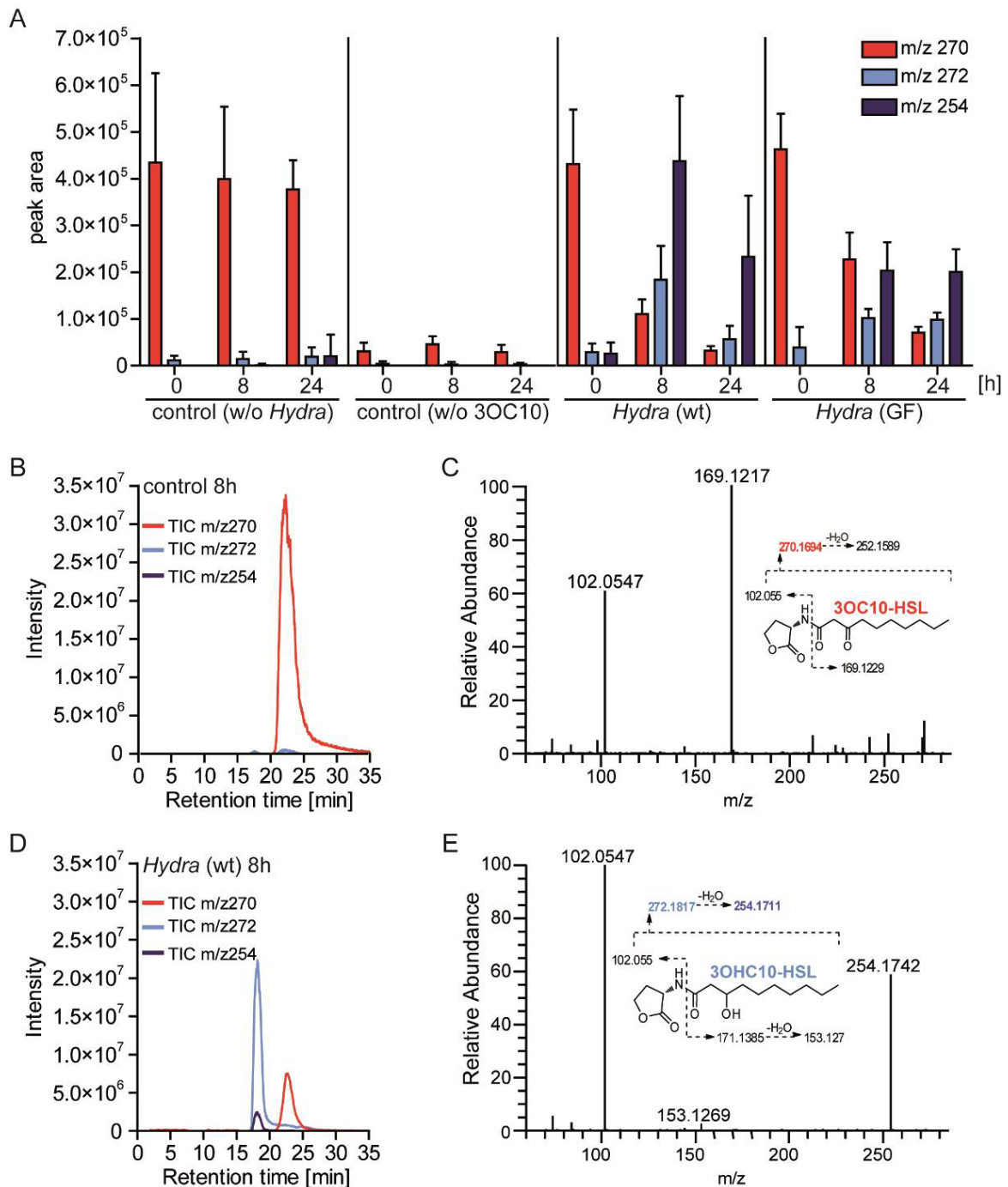
The down regulation of the HyPON transcript, in the transgenic animals by RNA interference (Gao and Zhang, 2007), in comparison to the control polyps, was checked by qRT-PCR (chapter 5.6.4). Therefore mRNA was isolated out of 15 transgenic and control Hydra and transcribed into cDNA (chapter 5.4.1). As demonstrated in Figure 17E, by the introduction of the HyPON hairpin construct into the ecto- and endoderm, an overall knockdown of the HyPON about 50% was achieved.

Because of the reduced HyPON transcript level in the transgenic Hydra, it was analyzed if the QQ activity of transgenic animals, compared to control polyps, was extenuated. Therefore transgenic and control polyps were incubated for 8 hours in 100  $\mu$ l S-medium with 10  $\mu$ M 3OC10-HSL. This AHL was chosen based on its high degradation rate by Hydra polyps (chapter 2.1.1). As a control for the natural AHL degradation process, 3OC10-HSL incubated in S-medium without Hydra. At three time points (after 0 h, 4 h, and 8 h) the remaining AHLs were extracted with ethyl acetate out of three replicates. The reporter strain pSB1075, which recognizes long-chain AHLs, was used for the AHL detection (chapter 5.30.1). As shown in Figure 17F *Hydra vulgaris* (AEP) is inactivating 3OC10-homoserine lactones, but there is no significant difference (Two-Way ANOVA with Bonferroni post-test ( $P > 0.05$ ,  $n = 3$ )) in the quenching activity between control and knockdown animals.

### 2.1.3 Hydra is using an oxidoreductase activity to modify bacterial signaling molecules.

Beside the human paraoxonases (Chun et al., 2004; Draganov et al., 2005; Simanski et al., 2012) no other enzymes are known to quench *N*-acyl-homoserine lactones (AHLs) in a eukaryotic system. Hydra possesses a homologue to the human paraoxonases (HyPON), but the knock-down of the paraoxonase in Hydra by transgenesis did not lead to reduced QQ activity (chapter 2.1.2). Therefore the modification of one AHL should be analysed to conclude the reaction mechanism, used by Hydra polyps for their quorum quenching (QQ). *Hydra vulgaris* (AEP) polyps were incubated with 100  $\mu$ M of the long-chain AHL 3OC10-HSL for 24 hours, remaining AHLs were extracted with ethyl acetate. This AHL species was chosen, as it gets inactivated by different Hydra species (chapter 2.1.1). As a control, the experiment was conducted in parallel with the same AHL concentration in S-medium and Hydra polyps without supplement (chapter 5.30.2). Two different mass spectrometry analyses were used to identify the quorum quenching product (in collaboration with Christian Treitz and Prof. Dr. Andreas Tholey).

As a first step, MALDI-TOF mass spectrometry was used for high-throughput measurements (chapter 5.26.1). Furthermore, the QQ products were fractionized by high-performance liquid chromatography (HPLC) coupled to electrospray ionization (ESI) using the orbitrap velos mass analyzer to identify the quorum quenching product (chapter 5.26.2). Figure 18A illustrates, that the substrate 3OC10-HSL with an  $[M+H]^+$  ion at an  $m/z$  of 270 stayed stable over 24 hours in control, while in the presence of Hydra polyps it was strongly decreased over time. In parallel, two  $[M+H]^+$  ions at an  $m/z$  of 272 and 254 occurred. A similar pattern could be observed in the presence of germfree polyps. Furthermore, the data show that the metaorganism Hydra did not produce 3OC10-HSLs on its own (Figure 18A, control (w/o 3OC10-HSL)).



**Figure 18: Hydra's QQ mechanism**

(A) MALDI-TOF analysis of Hydra's QQ activity. The substrate 3OC10-HSL (m/z 270) and the substances with an  $[M+H]^+$  ion at a m/z of 272 and 254 were measured after 0, 8 and 24 h in different samples: control (w/o Hydra), medium with 10  $\mu$ M 3OC10-HSL; control (w/o 3OC10-HSL), *Hydra vulgaris* (AEP) without 3OC10-HSL addition; wt, wild type *Hydra vulgaris* (AEP); GF, germfree *Hydra vulgaris* (AEP). (B-E) Liquid chromatography coupled to electrospray ionization mass spectrometry (LC-ESI-MS) were used to analyse AHLs and their derivatives in samples of *Hydra vulgaris* (AEP) wild type (wt) or medium (control) incubated with 100  $\mu$ M 3OC10-HSL for 8 h. Only the changed  $[M+H]^+$  ions with an m/z of 270 (3OC10-HSL), 272 and 254 are depicted (B) Time ion chromatograms (TIC) for the respective ions of interest in the control sample after 8 h. (C) MS2 spectrum of the  $[M+H]^+$  ion at an m/z of 270, corresponding to the substrate 3OC10-HSL, originating from the MS1 in B. (D) Shows the respective TIC in the Hydra sample after 8 h. (E) MS2 spectrum of the  $[M+H]^+$  ion at an m/z of 272, origination from D. 3OHC10-HSL was identified by comparison with reference spectra of commercial molecule. Loss of a water from 3OHC10-HSL in source fragmentation explains the  $[M+H]^+$  ion at an m/z of 254, which was found in the MALDI-TOF-MS and in the LC-ESI-MS.

To clarify the identity of the QQ products, an LC-ESI-MS was used. The MS1 data confirmed the results of the MALDI-TOF MS, as the same  $[M+H]^+$  ions at an  $m/z$  of 270, 272 and 254 could be detected (Figure 18B, D). The fragments of the substrate 3OC10-HSL, corresponding to the homoserine lactone ( $m/z$  102.05) and the acyl-side chain ( $m/z$  169.12), were detected as the main fragments in the MS2 (Figure 18C). The quorum quenching product with an  $m/z$  272 was fragmented in the MS2 into two main fragments with  $m/z$  102.05 and 153.12 (Figure 18E). Through comparison with fragment spectra of AHL standards the fragments could be assigned as a homoserine lactone and a 3-hydroxy-decanoyl-side-chain, after the loss of water. By this the  $[M+H]^+$  ion at an  $m/z$  of 272 could be identified as a 3-hydroxy-decanoyl-homoserine lactone (3OHC10-HSL) (Figure 18E). The  $[M+H]^+$  ion at a  $m/z$  of 254 correspond to an unsaturated C10-HSL, which originate from 3OHC10-HSL after water elimination, probably due to in source fragmentation (Figure 18D-E) (Hsu et al., 2006).

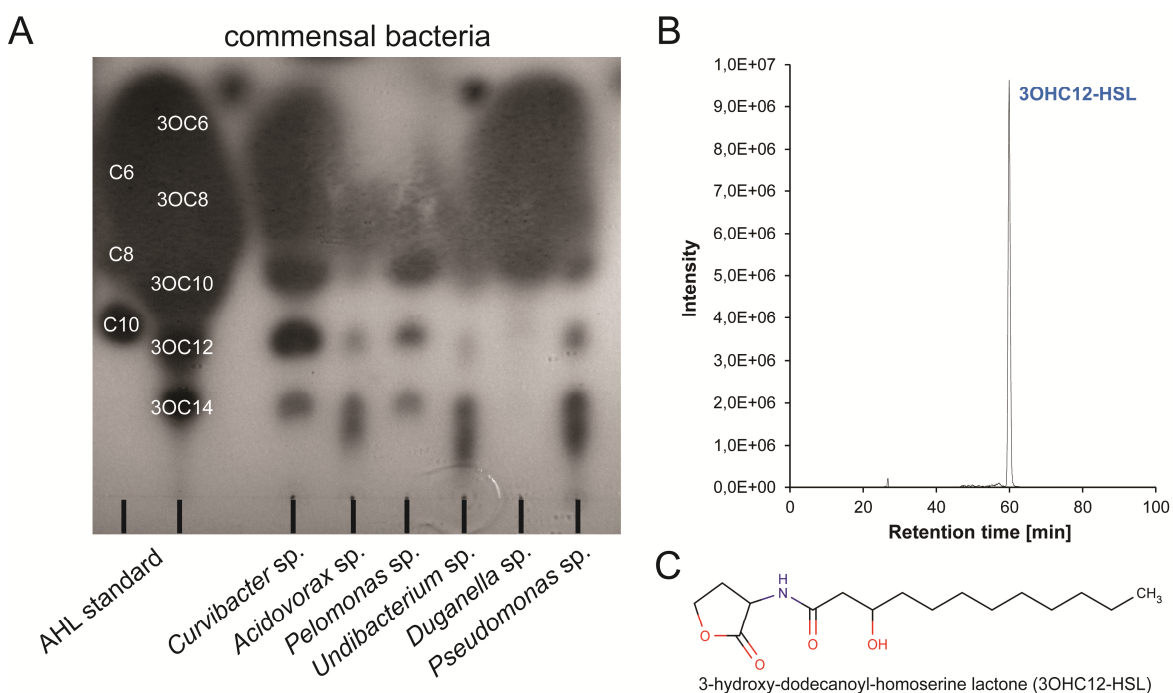
The two different mass spectrometry analyses revealed that Hydra modify the long-chain AHL 3OC10-HSL ( $m/z$  270) into its 3-hydroxy-counterpart 3OHC12-HSL ( $m/z$  272). The reduction of an oxo-group to a hydroxy group can be performed by an enzyme comprising an oxidoreductase activity (Figure 5C). This is to the author's knowledge the first report for a eukaryote, using an oxidoreductase activity to modify the quorum sensing signaling molecules of Gram-negative bacteria (AHLs).

## **2.2 Quorum sensing in the special neighborhood of *Hydra vulgaris* (AEP)**

In previous studies, it was shown that the different Hydra species possess species specific bacterial communities, which are dominated by Gram-negative bacteria (Franzenburg et al., 2013a, 2013b; Fraune and Bosch, 2007). For many eukaryotes it was shown that their commensal bacteria are producing several quorum sensing (QS) molecules (Cha et al., 1998a; Golberg et al., 2011; Ransome et al., 2014) and furthermore that these molecules are influencing the host physiology (Hartmann and Schikora, 2012; Hartmann et al., 2014; Mathesius et al., 2003; Shiner et al., 2005). Therefore it was investigated if Hydra's Gram-negative bacteria are also using QS.

## 2.2.1 Commensal and environmental bacteria of *Hydra vulgaris* (AEP) are producing *N*-acyl-homoserine lactones

To estimate the relevance of quorum sensing (QS) in the metaorganism Hydra, thin-layer chromatography (TLC) combined with the *Agrobacterium tumefaciens* NTL4 reporter was used to detect *N*-acyl-homoserine lactones (AHLs), the signaling molecules of Gram-negative bacteria (Ng and Bassler, 2009). Bacterial cultures of six commensal bacteria of *Hydra vulgaris* (AEP) and six environmental bacteria were screened for the production of AHLs (chapter 5.26). The selected commensal bacteria represent 90% of the bacterial community of *H. vulgaris* (AEP) (Fraune et al., 2014), while the environmental bacteria are present in the Hydra culture dishes, but are not able to colonize Hydra polyps (chapter 5.1.4). All analysed bacteria produced different AHLs, which varies in their chain lengths (from six to sixteen carbons) and in concentration, as shown in Figure 19A and Figure 20A.



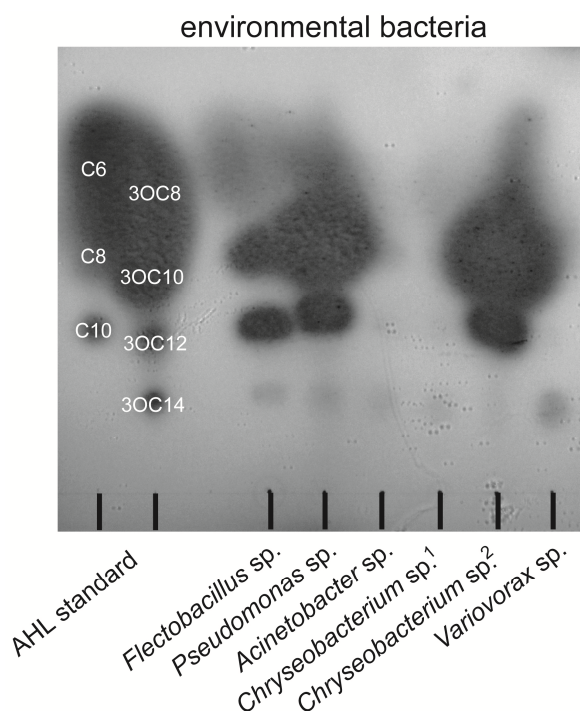
**Figure 19: AHL production by commensal bacteria of *Hydra vulgaris* (AEP)**

(A) AHLs, produced after 48 h in a 50 ml liquid culture of the six commensal bacteria, which represent 90% of Hydra's bacterial community, analysed by a thin-layer chromatography (TLC) combined with the AHL-reporter *Agrobacterium tumefaciens* NTL4. As an AHL standard the following concentration of commercially available AHLs were used: 100 pmol C6-, 10 fmol C8-, 100 pmol C10-, 10 fmol 3OC6-, 0.1 fmol 3OC8-, 1 pmol 3OC10-, 1 pmol 3OC12- and 100 pmol 3OC14-HSL. (B) Mass spectrometry analysis of AHLs, produced by *Curvibacter* sp. after 96 h. Only one AHL, 3OHC12-HSL, could be identified. (C) Molecule structure of 3OHC12-HSL, which could be identified by MS in a liquid culture of *Curvibacter* sp.

The main colonizer of *Hydra vulgaris* (AEP), *Curvibacter* sp. produces five different AHLs; by comparison to the migration patterns of the AHL standards, one can assume that the acyl-side chain of *Curvibacter*'s AHLs varies between six to fourteen carbons. Compared to the other commensal bacteria *Curvibacter* sp. produced, under the analysed conditions

(48 h culture inoculated with  $1 \times 10^5$  CFU/ml), the most AHL species and the highest AHL concentrations (Figure 19A). For further characterization of the produced *N*-acyl-homoserine lactones they were analysed by a LC-ESI-mass spectrometry (chapter 5.26.2). In the culture of *Curvibacter* sp. only the AHL 3OHC12-HSL could be clearly identified (Figure 19B-C).

The *Pelomonas* sp. has as similar TLC pattern to *Curvibacter* sp., but C6- and C8-AHLs are missing and the intensity of the signal is less (Figure 19A). The AHLs of the three commensals *Acidovorax* sp., *Undibacterium* sp. and *Pseudomonas* sp. show similar migration patterns, with a stretched spot at the position of AHLs with 14 and more carbons. Their AHLs run similar as those of *Curvibacter* sp., but they exhibit another spot-pattern, indicating that they have another side-modification (Figure 19A) (Shaw et al., 1997). *Duganella* sp. is the only strain which is producing exclusively short-chain AHLs under these conditions (48 h culture inoculated with  $1 \times 10^5$  CFU/ml) (Figure 19A).



**Figure 20: AHL production in the neighborhood of *Hydra vulgaris* (AEP)**

AHLs, produced after 48 h in a 50 ml liquid culture of six environmental bacteria of *Hydra* analysed by a thin-layer chromatography (TLC) combined with the AHL-reporter *Agrobacterium tumefaciens* NTL4 (<sup>1</sup> = *Chryseobacterium* sp. strain 5, <sup>2</sup> = *Chryseobacterium* sp. strain 8). As an AHL standard the following concentration of commercially available AHLs were used: 100 pmol C6-, 10 fmol C8-, 100 pmol C10-, 10 fmol 3OC6-, 0.1 fmol 3OC8-, 1 pmol 3OC10-, 1 pmol 3OC12- and 100 pmol 3OC14-HSL.

Environmental bacteria are also producing AHLs, but their migration pattern in the TLC differs from that of the commensal bacteria (Figure 20A). *Pseudomonas* sp. and *Chryseobacterium* sp. strain 8 are producing high amounts of AHLs showing similar migration pattern as AHLs with an acyl-side chain length of eight and ten C-atoms.



Furthermore, small amounts of AHLs, which are running at the position of 3OC6- and 3OC14-HSL, could be detected (Figure 20A). The AHLs produced by *Flectobacillus* sp. show a very similar pattern, but because of slightly different position in the chromatogram and the shape of the spots, they possess most probably different side-modifications (Figure 20A). For *Acinetobacter* sp. and *Chryseobacterium* sp. strain 5 small amounts of four AHLs in the same range, as those of *Flectobacillus* sp., could be identified (Figure 20A). *Variovorax* sp. exhibits the highest concentration for AHLs with a chain length of 14 carbons (Figure 20A).

These data show that the Hydra polyp is surrounded and even colonized by bacteria, which are using a broad spectrum of AHLs for quorum sensing. But based on the produced AHL species, one cannot distinguish commensal and environmental bacteria. Furthermore, it seems that different bacteria are using the same AHL species for their communication; consequently, it would be interesting to see if similar genes or rather similar bacterial behaviors are regulated by these AHLs.

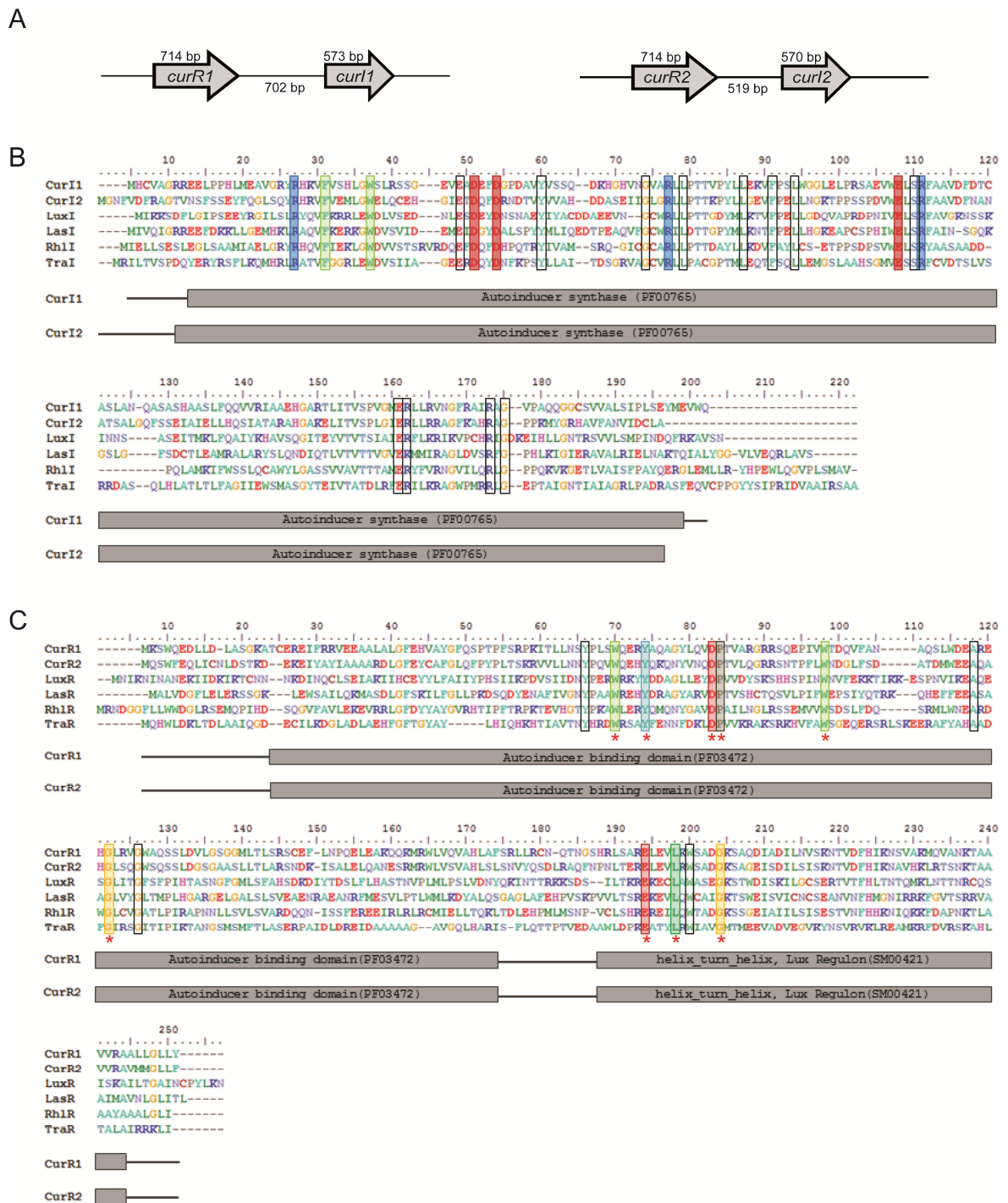
## **2.3 Characterization of the quorum sensing system of *Curvibacter* sp. strain AEP1.3**

To get a deeper understanding of the role of quorum sensing (QS) within the metaorganism Hydra, the QS system of *Curvibacter* sp. (strain AEP1.3), representing up to 80% of the bacterial community of *Hydra vulgaris* (AEP), was investigated.

### **2.3.1 *In silico* analysis of *Curvibacter*'s quorum sensing system**

So far it could be shown that the main colonizer *Curvibacter* sp. is able to produce *N*-acyl-homoserine lactones (AHLs), but the genetic background of this bacterium has not yet been investigated. In the genome of *Curvibacter* sp. (unpublished data) two operons could be identified, each encoding for an AHL-synthase (*curl1*, *curl2*) and an AHL-receptor (*curR1*, *curR2*) (Figure 21A); the sequences of the corresponding operons can be found in the appendices 8.3.1 and 8.3.4.

The AHL-synthases and -receptors of *Curvibacter* sp. were compared by an alignment with three well studied quorum sensing (QS) systems, LuxI/LuxR of *Vibrio fischeri* (YP\_206882.1/YP\_206883.1), LasI/LasR and RhII/RhIR of *Pseudomonas aeruginosa* (P33883/NP\_250121.1, P54291/NP\_252167.1) and TraI/TraR of *Agrobacterium tumefaciens* (P33907/NP\_396657.1) (Figure 21B).



**Figure 21: AHL-synthases CurI1/CurI2 and AHL-receptors CurR1/CurR2 of *Curvibacter* sp.**

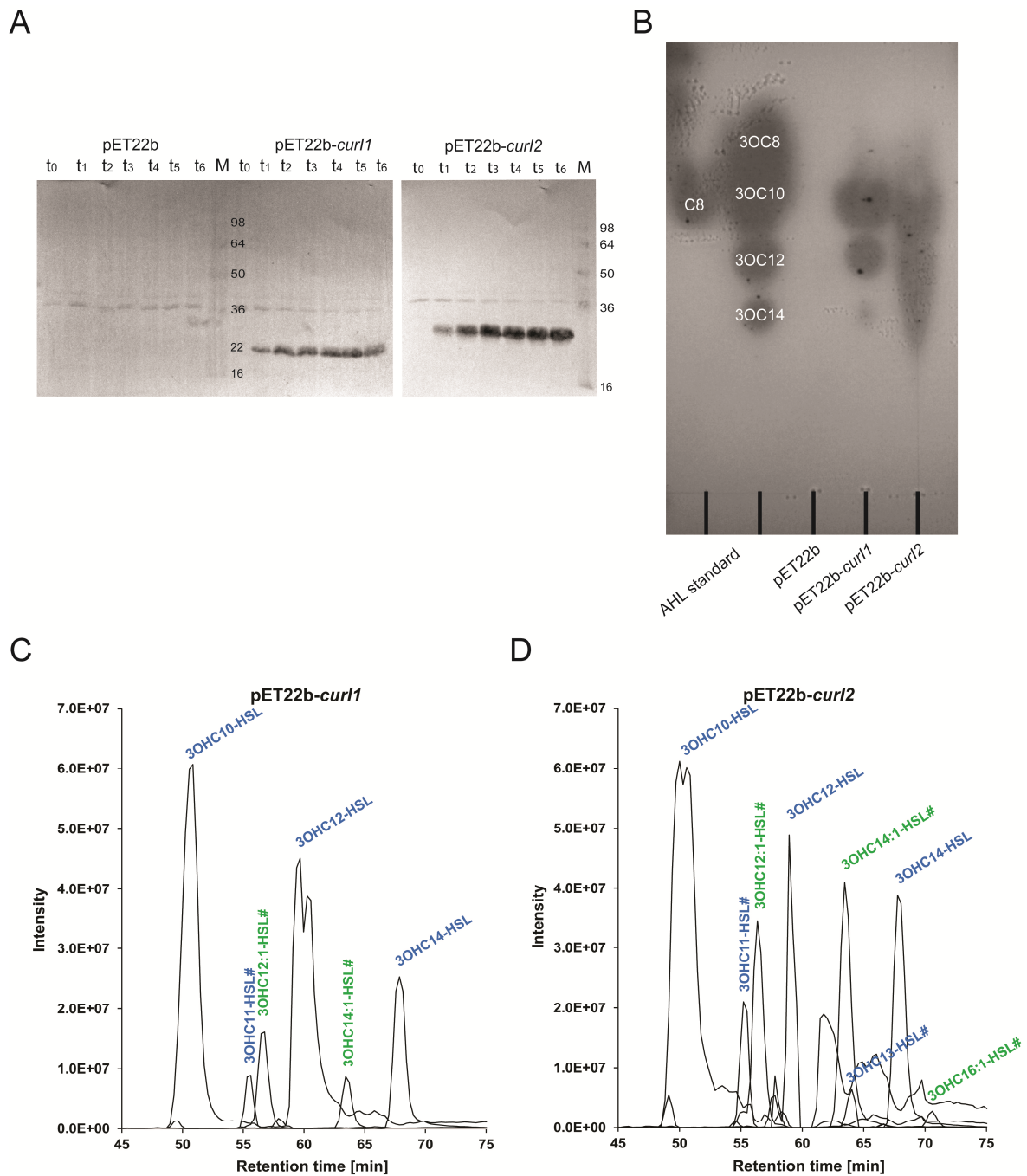
(A) Operon of the AHL-synthases *curI1* and *curI2* and the AHL-receptors *curR1* and *curR2* of *Curvibacter* sp. (B) Alignment of *Curvibacter*'s AHL-synthases CurI1 and CurI2 with the AHL-synthases of *Vibrio fischeri* (LuxI, YP\_206882.1), *Pseudomonas aeruginosa* (LasI, P33883 and RhII P54291) and *Agrobacterium tumefaciens* (TraI, P33907). The eight conserved residues of the AHL-synthase family are highlighted in solid colors, and those that are additionally conserved among these AHL-synthases are boxed. Protein domains of CurI1 and CurI2, predicted by SMART, are indicated below the alignment of the primary structures. (C) Alignment of *Curvibacter*'s AHL-receptors CurR1 and CurR2 against the AHL-receptors of *V. fischeri* (LuxR, YP\_206883.1), *P. aeruginosa* (LasR, NP\_250121.1 and RhIR NP\_252167.1) and *A. tumefaciens* (TraR, NP\_396657.1). The conserved WYDPWG-motif of the AHL binding domain and the conserved residues (E, L, G) in the DNA-binding domain of the AHL-receptors are highlighted in solid colors and red asterisks, and those that are additionally conserved among these AHL-receptors are boxed. Protein domains of CurR1 and CurR2, predicted by SMART, are indicated below the alignment of the primary structures.

*Curvibacter* sp. AHL-synthases can be assigned to the LuxI-type AHL-synthase family because they inherit the eight conserved amino acid residues of this protein family (Churchill and Chen, 2011; Hanzelka et al., 1997; Parsek et al., 1997), as shown in Figure 21B. The protein domain prediction was performed by the program SMART and is indicated below the alignment of the primary structure in Figure 21B. Thereby, the AHL-synthases were classified as an “Autoinducer synthase” (PF00765), while for the AHL-receptors their essential domains “Autoinducer-binding domain” (PF03472) and a “helix-turn-helix, Lux-Regulon” (SM00421) were identified (Koch et al., 2005). The two AHL-receptors CurR1 and CurR2 possess the conserved WYDPWG-motif in the AHL binding domain and also the DNA-binding domain contains the conserved amino acids (E, L, G) (Brameyer and Heermann, 2015; Patankar and González, 2009). These structural features of the coding sequences of *Curvibacter*’s AHL-synthases and AHL-receptors suggest that they could be functional.

### **2.3.2 The AHL-synthases of *Curvibacter* sp. are producing a set of common and distinct *N*-acyl-homoserine lactones**

To test the functionality of the two *Curvibacter* AHL-synthases, both genes (*curl1* and *curl2*) were cloned and heterologously expressed in *E. coli* cells. Expression was determined by SDS-PAGE and Western blot (Figure 22A) (chapter 5.28). To test the functionality of the synthases, the cell free fraction after expression was extracted, separated by thin-layer chromatography (TLC) and potentially produced *N*-acyl-homoserine lactones (AHLs) were detected with the *A. tumefaciens* NTL4 reporter (Figure 22B) (chapter 5.28). Through this it could be proven that the heterologous expression led to functional AHL-synthases, which catalyze the synthesis of AHLs. The TLC predicts for both synthases production of three different AHLs with chain-lengths between eight and sixteen carbons, but the different migration patterns indicate that the produced AHLs possess different modifications at their acyl-side chain.

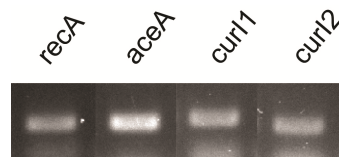
To confirm these results and to obtain a better resolution for the AHL species, the expression extract was additionally analysed by mass spectrometry (Christian Treitz and Prof. Dr. Andreas Tholey from the Institute for Experimental Medicine of the Christian Albrechts University of Kiel), as described elsewhere (Gould et al., 2006) (chapter 5.26.2). Both AHL-synthases are producing saturated, mainly 3OHC10-, 3OHC12- and 3OHC14-HSLs, fitting to the three observed AHL spots in the TLC (Figure 22B). Furthermore, unsaturated long-chain 3-hydroxy-AHLs could be identified in both samples, but *Curl2* is producing much more of the unsaturated AHLs 3OHC12:1- and 3OHC14:1-HSL than *Curl1* (Figure 22C-D), which could be an explanation for the differences in their AHL pattern in the TLC (Figure 22B).



**Figure 22: AHL-syntheses of *Curvibacter* sp.**

(A) The AHL-synthases curl1 and curl2 of *Curvibacter* sp., introduced into the pET22b vector (pET22b-curl1, pET22b-curl2), were heterologously expressed in *E. coli* Rosetta 2 (DE3) pLysS cells for six hours at 25°C. Every hour (t<sub>0</sub>-t<sub>6</sub>) an aliquot of 10 µg protein was taken for Western blot analysis. Numbers indicate the size of the proteins, belonging to the protein ladder, in kDa. (B) After heterologous expression of *Curvibacter*'s AHL-synthases Curl1 and Curl2, the cell free fraction was extracted with ethyl acetate, concentrated and a volume equivalent to 1 ml culture was analysed with a thin-layer chromatography (TLC) combined with the AHL-reporter *A. tumefaciens* NTL4. As an AHL standard the following concentration of commercially available AHLs were used: 10 pmol C8-, 1 pmol 3OC8-, 10 pmol 3OC10-, 1 nmol 3OC12- and 1 nmol 3OC14-HSL. (C-D) After heterologous expression of *Curvibacter*'s AHL-synthases Curl1 and Curl2, AHLs were extracted and characterized by LC-ESI-MS. (C) MS of AHLs produced by Curl1 heterologously expressed in *E. coli*. (D) MS of AHLs produced by Curl2 heterologously expressed in *E. coli*. # Retention time and m/z value of the MS analysis predicted these molecules, but no AHL standard was measured for these substances.

To estimate if both AHL-synthases in *Curvibacter* sp. are expressed in the context of host colonization, mRNA expression of the AHL-synthases of *Curvibacter* sp. was analysed by RT-PCR. To do so, the outer layer of the glycocalyx with colonizing bacteria was detached from Hydra polyps with PBS-treatment. Total RNA was isolated from the colonizing glycocalyx bacteria and cDNA was synthesized (chapter 5.4.3). Expression of the two AHL-synthases of *Curvibacter* sp. *curl1* and *curl2* were compared to expression of two housekeeping genes recombinase A (*recA*) and isocitrate lyase (*aceA*) by a RT-PCR (Figure 23) (chapter 0).



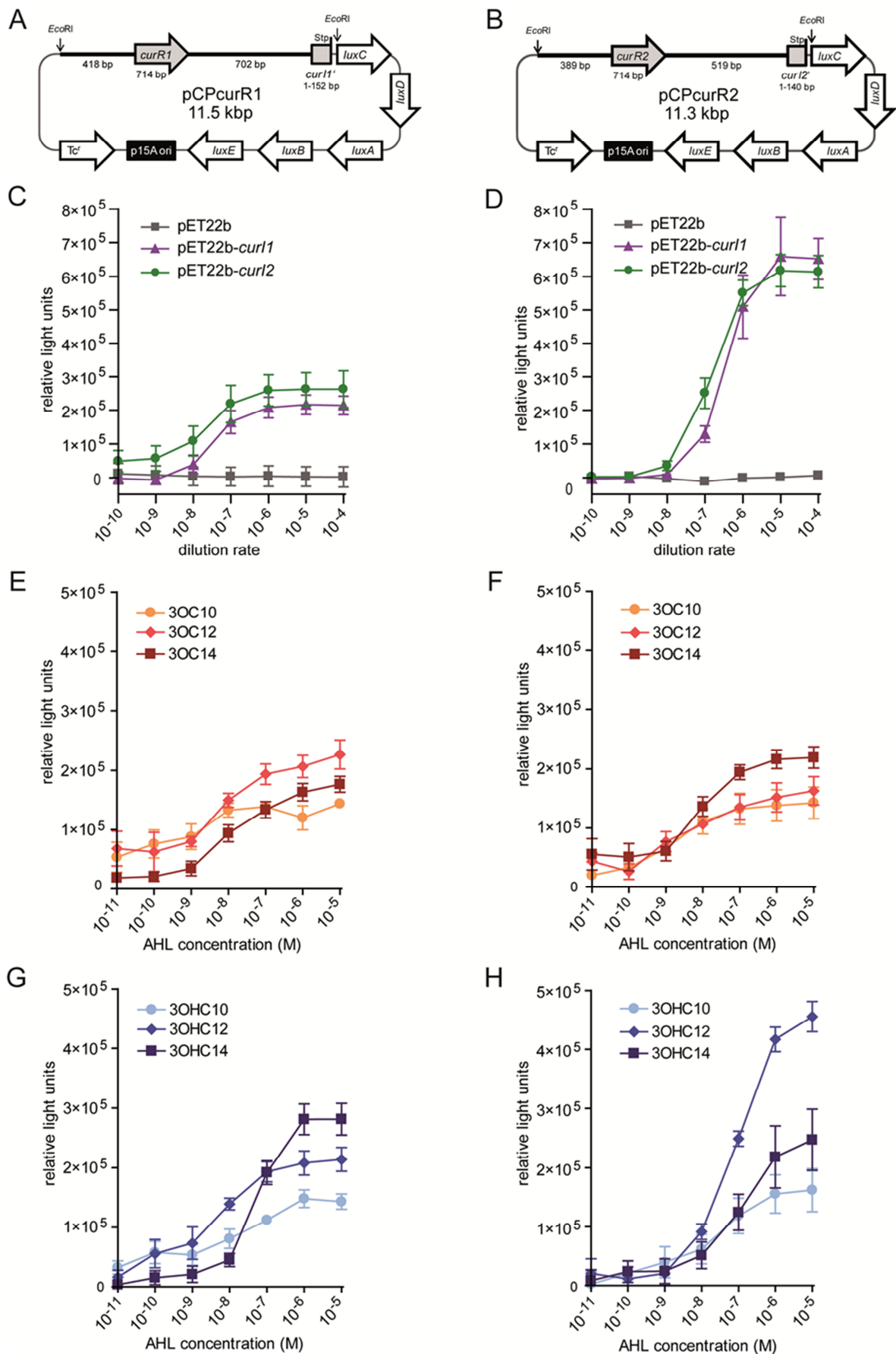
**Figure 23: Expression of *Curvibacter*'s AHL-synthases *Curl1* and *Curl2* while host colonization.**

Through PBS-treatment, colonizing bacteria were detached from the Hydra polyps and were used to isolate RNA. After cDNA synthesis, gene expression of the two AHL-synthases (*curl1*, *curl2*) was compared to that of the two house-keeping genes recombinase A (*recA*) and isocitrate lyase (*aceA*) by PCR.

These results show that *Curvibacter* sp. is expressing both AHL-synthases, while colonizing the Hydra polyp and have the potential to produce AHLs. Therefore, it could be proven that both AHL-synthases are functional and are potentially producing saturated and unsaturated long-chain 3-hydroxy-AHLs in the context of host colonization.

### 2.3.3 *Curvibacter*'s AHL-receptors *CurR1* and *CurR2* recognize both 3-oxo- and 3-hydroxy-HSLs.

Some bacteria not only recognize their own produced *N*-acyl-homoserine lactones (AHLs), but are also able to respond to the presence of AHLs, synthesized by bacteria in their neighborhood (Schauder and Bassler, 2001). Consequently, the affinity of the AHL-receptors doesn't have to coincide with the AHL spectrum of the AHL-synthases. Therefore, the autoinducer responses of the two AHL-receptors *CurR1* and *CurR2* of *Curvibacter* sp. were analysed in a reporter strain assay. A cassette of AHL-receptor and promoter of the corresponding AHL-synthase was cloned in front of the *lux* operon of the pSB401 plasmid (Winson et al., 1998) to create the reporter constructs pCP*curR1* and pCP*curR2* (Figure 24A-B). Through this construct, the efficiency of AHL binding to the receptor and activation of transcription of the AHL-synthase can be read out by emission of light (chapter 5.19). The functionality of the receptors was verified by measuring dilution series of the heterologous expression extracts of the AHL-synthases *Curl1* and *Curl2* (chapter 5.29) (Figure 24C-D). Both AHL-extracts induced similar bioreporter responses, but the *CurR2* leads to a higher light emission than the *CurR1*.



**Figure 24: AHL-receptor reporter constructs pCPcurR1 and pCPcurR2**

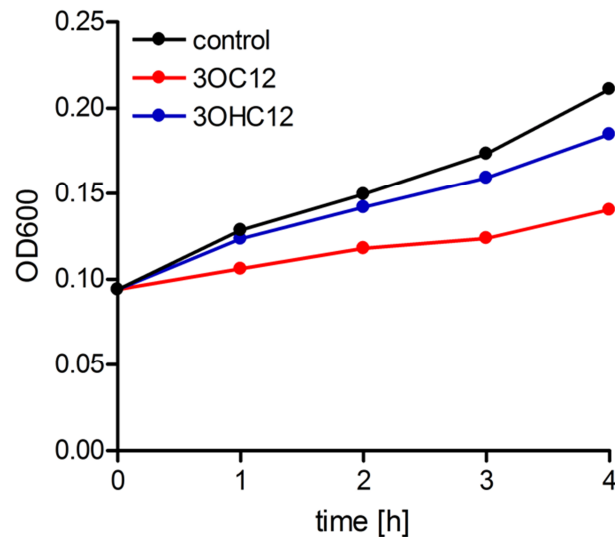
(A) A 1986 bp section, including the open reading frame (ORF) of *curR1* and 152 bp of *curl1*, was cloned by *EcoRI* restriction into the pSB401 plasmid (Winson et al., 1998), harboring the *lux* operon

(luxCDABE), to create the reporter construct pCPcurR1. (B) For the pCPcurR2 construct, a 1762 bp fragment of the *curR2/curI2* operon was introduced into the pSB401 plasmid, like in the pCPcurR1 construct. (C-D) To test the functionality of the two AHL-receptor constructs pCPcurR1 and pCPcurR2 the two AHL-synthases CurI1 and CurI2 were heterologously expressed (pET22b, pET22b-*curI1*, pET22b-*curI2*). The produced AHLs were extracted and a dilution series ( $10^{-4}$ - $10^{-10}$ ) were measured by pCPcurR1 (C) and pCPcurR2 (D) in a bioreporter plate assay. (E-H) For further characterization of the AHL specificity of the CurR-receptors, dilution series ( $10^{-5}$ - $10^{-11}$  M) of commercially available 3-oxo- and 3-hydroxy-HSLs (3OC6-C14, 3OHC8-C14) were measured with pCPcurR1 (E, G) and pCPcurR2 (F, H) in a plate assay. Only AHLs which led to a specific activation of the reporter constructs are indicated, the complete set can be found in appendix 8.4.

To decode the recognition of AHLs by the two AHL-receptors (CurR1/CurR2) in more detail, dilution series of commercially available AHLs with oxo- and hydroxy-modification at the third carbon of the acyl chain were measured with the CurR-reporter constructs pCPcurR1 and pCPcurR2. Figure 24E-H shows that only AHLs with an acyl-side chain of ten or more carbons lead to a specific activation of the transcription of the CurI-synthases (data for all measured AHL-species can be found in appendix 8.4, Figure 36) (chapter 5.29). Similarly, long-chain 3-oxo- and 3-hydroxy-HSLs lead to an activation level as the extract of the heterologously expressed CurI1 and CurI2, indicating that the used AHLs are the native ligands of the receptors. Both receptors have a similar affinity, but it seems that the sensitivity is slightly changed. CurR1 exhibits the highest activation with 3OC12- and 3OHC14-HSL, while CurR2 shows the strongest response to 3OHC12-HSL and 3OC14-HSL. Interestingly the sensitivity for AHLs with twelve and fourteen carbons is inverted for both receptors. These different sensitivities could indicate that the AHL-receptors regulate different sets of genes.

## **2.4 The main colonizer of *Hydra vulgaris* (AEP) *Curvibacter* sp. responds differentially to 3-oxo- and 3-hydroxy-HSLs**

Because of the observed differences in the sensitivities of the AHL-receptors CurR1 and CurR2 toward AHLs with an oxo- or a hydroxy-modification and based on the fact that *Curvibacter*'s AHL-synthases CurI1 and CurI2 are only producing long-chain AHLs, the response of *Curvibacter* sp. to 3-oxo- and 3-hydroxy-long chain-HSLs was investigated. A culture of *Curvibacter* sp. strain AEP1.3 in an early exponential growth phase ( $OD_{600} = 0.1$ ) was washed to remove already produced AHLs and split 15 times into 50 ml cultures. Five cultures each were treated with 10  $\mu$ M 3OC12-HSL, 10  $\mu$ M 3OHC12-HSL or with the AHL solvent ethyl acetate (control). The cultures were incubated for 4 hours (18°C, 225 rpm) and the optical density ( $OD_{600}$ ) were monitored every hour (Figure 25) (chapter 5.31).



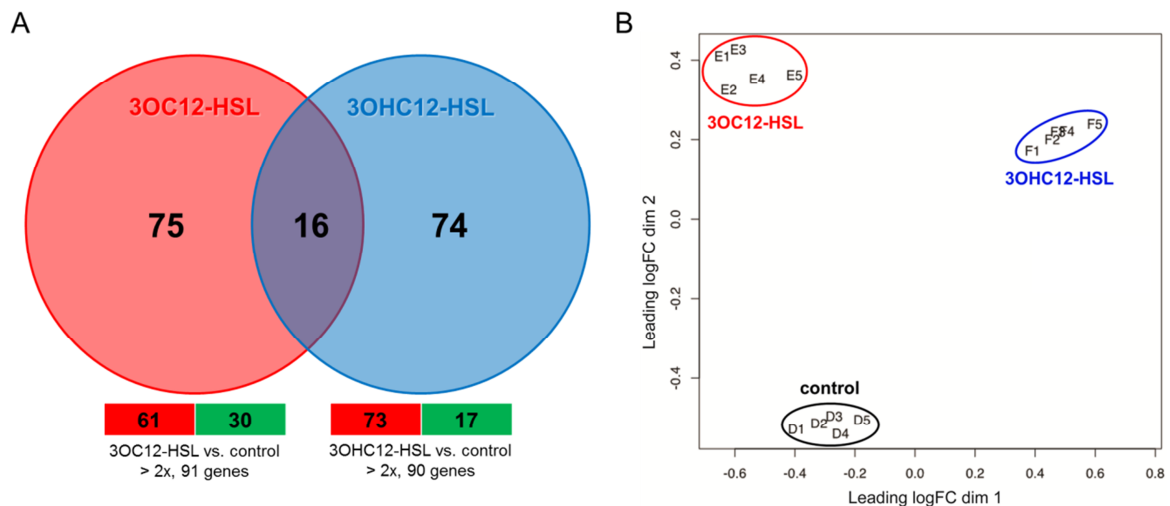
**Figure 25: Effects of AHLs on growth of *Curvibacter* sp.**

*Curvibacter* sp. in an early exponential growth was incubated with 10  $\mu$ M 3OC12- or 3OHC12-HSL for four hours at 18°C and 225 rpm. As a control *Curvibacter* sp. incubated with 1:2000 ethyl acetate, the AHL solvent. The optical density (OD<sub>600</sub>) was monitored every hour. Already after one hour all samples of one time point differ significantly (Two-Way-ANOVA with Bonferroni post-test;  $P \leq 0.001$ , only control vs. 3OHC12 after one hour has a  $P \leq 0.01$ ).

Statistical analysis by a Two-Way-ANOVA with a Bonferroni post-test showed that the addition of 10  $\mu$ M 3OC12-HSL significantly reduced the growth rate of *Curvibacter* sp. within one hour ( $P < 0.001$ ,  $n = 5$ ). After 4 h incubation total RNA was isolated from  $1 \times 10^8$  CFU and used for transcriptomic sequencing in cooperation with the Max Planck Institute for Evolutionary Biology (Dr. Sven Künzel) (chapter 5.16.2). The cDNA libraries for sequencing were constructed with the TrueSeq Stranded mRNA LT – RiboZero Kit (Illumina) and were sequenced using a NextSeq 500 machine (Illumina). The primary bioinformatics analysis was done by PhD Sylvaine Forêt (chapter 5.16.2).

The sequencing yield for most samples was between 10 and 20 million reads, which were mapped to the genome of *Curvibacter* sp. strain AEP1.3 (unpublished). Around 80% of the reads mapped uniquely to the reference genome and around 90% of the reads were mapped (appendix 8.5). Only reads which mapped uniquely were used for further analysis. All 4115 genes of *Curvibacter* sp. could be identified at least with five reads in five conditions. Even if one only considers the genes with at least 10 counts in all 15 conditions, 4085 of the 4115 genes of *Curvibacter* sp. are detected, so an overwhelming majority of the genes could be identified. Because of a low biological variance, a very large number of statistical differentially expressed genes can be found in the comparisons of the three treatments, but most of these changes are associated with a small fold change. Therefore, a fold change of at least two ( $\log_{2}FC = 1$ ) was used as cut-off. Differentially regulated genes in 3OC12-HSL and 3OHC12-HSL compared to control are graphically illustrated in Figure 26A.





**Figure 26: 3-oxo- and 3-hydroxy-HSLs have strong effects on *Curvibacter*'s transcriptome**

(A) Graphic representation of differentially regulated ( $\geq 2$ -fold change) genes in 3OC12-HSL and 3OHC12-HSL compared to control *Curvibacter* sp. Down-regulated genes are highlighted in red, up-regulated genes in green. (B) *Curvibacter* sp. was incubated for 4 h with 1:2000 ethyl acetate (D), 10  $\mu$ M 3OC12-HSL (E) or 10  $\mu$ M 3OHC12-HSL (F). The experiment was conducted in five replicates. The transcriptome of these samples were sequenced and a multidimensional scaling (MDS) plot was used to visualize the relationships between all samples.

In both treatments around 90 genes are differentially regulated compared to control and around 75 genes are specifically regulated for one of the two AHL species. The dissimilarity of the three treatments is visualized by a multidimensional scaling (MDS) plot (Figure 26B). The MDS plot illustrates that the two AHL species lead to two distinct responses in *Curvibacter* sp.

For further characterization of the specific responses, a pathway analysis with the GoSeq package (Young et al., 2010) was used to identify KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways enriched in the differentially expressed genes. As shown in Table 1 ten classes of KEGG pathways were differentially regulated under the two conditions compared to control.

Addition of 3OC12-HSL led to the up-regulation of genes involved in the pathways "Flagellar assembly", "Ribosome" and "Citrate cycle (TCA cycle)". In contrast, genes in "Glycan biosynthesis and metabolism", especially "Lipopolysaccharide biosynthesis" and "Peptidoglycan biosynthesis", were down-regulated. Indicating that 3OC12-HSL induced the formation of flagella, which entails an elevated metabolism (TCA cycle) and translation rate (Ribosome) and for integration of the flagella into the bacterial cell wall peptidoglycans and lipopolysaccharides have to be reduced (Johnson et al., 2013; Scheurwater et al., 2008). In contrast, 3OHC12-HSL causes the down-regulation of "Ribosome" related genes and the up-regulation of the following KEGG classes: "Cell motility", "Signal transduction", "Carbohydrate metabolism", "Lipid metabolism", "Amino acid metabolism", "Carbon metabolism" and "Fatty acid metabolism".

The transcriptional changes, induced by 3OHC12-HSL, does not lead to such a clear phenotype as those induced by 3OC12-HSL. Interestingly, both *Curvibacter* sp. phenotypes invest in “Cell motility”. While the 3OC12-HSL phenotype is characterized by its flagella motility, 3OHC12-HSL induces “Bacterial chemotaxis”.

**Table 1: KEGG pathways effected by 3OC12- and 3OHC12-HSLs**

KEGG pathway			3OC12		3OHC12	
Entry	Name	Class	up	down	up	down
ko00020	Citrate cycle (TCA cycle)	Metabolism; Carbohydrate metabolism	+	-	+	-
ko00071	Fatty acid degradation	Metabolism; Lipid metabolism	-	-	+	-
ko00072	Synthesis and degradation of ketone bodies	Metabolism; Lipid metabolism	-	-	+	-
ko00280	Valine, leucine and isoleucine degradation	Metabolism; Amino acid metabolism	-	-	+	-
ko00540	Lipopolysaccharide biosynthesis	Metabolism; Glycan biosynthesis and metabolism	-	+	-	-
ko00550	Peptidoglycan biosynthesis	Metabolism; Glycan biosynthesis and metabolism	-	+	-	-
ko00620	Pyruvate metabolism	Metabolism; Carbohydrate metabolism	-	-	+	-
ko00630	Glyoxylate and dicarboxylate metabolism	Metabolism; Carbohydrate metabolism	-	-	+	-
ko00640	Propanoate metabolism	Metabolism; Carbohydrate metabolism	-	-	+	-
ko00650	Butanoate metabolism	Metabolism; Carbohydrate metabolism	-	-	+	-
ko01200	Carbon metabolism	Metabolism; Overview	-	-	+	-
ko01212	Fatty acid metabolism	Metabolism; Overview	-	-	+	-
ko02020	Two-component system	Environmental Information Processing; Signal transduction	-	-	+	-
ko02030	Bacterial chemotaxis	Cellular Processes; Cell motility	-	-	+	-
ko02040	Flagellar assembly	Cellular Processes; Cell motility	+	-	-	-
ko03010	Ribosome	Genetic Information Processing; Translation	+	-	-	+

The pathway analysis tool GoSeq identified KEGG pathways enriched by significantly differentially expressed genes in both conditions. Regulation is indicated as: +, up-regulation; +, down-regulation; -, unaltered expression.

By looking manually through the list of differentially regulated genes and to further characterize both phenotypes, one operon attracted attention, as it was reversely regulated by 3OC12- and 3OHC12-HSL. In the presence of 3OC12-HSL, the genes were down-regulated, while 3OHC12-HSL led to an induced expression of these genes (Table 2).

In this operon several polyketide synthases were identified. To prove this, secondary metabolite analysis using the AntiSMASH program version 3.0.1 (Weber et al., 2015), of the *Curvibacter* sp. strain AEP1.3 genome (unpublished) was performed. This analysis confirmed that the differentially regulated operon was lying in a trans-AT polyketide synthases (trans atpks) cluster. Polyketides are structurally complex organic compounds, which could possess a wide range of biological activities, including bactericidal activity (Piel, 2010).

Taking these findings together, the 3OC12-HSL phenotype of *Curvibacter* sp. is characterized by its flagella production, while in the presence of 3OHC12-HSL, genes involved in bacterial chemotaxis and polyketide production are up-regulated in *Curvibacter* sp.

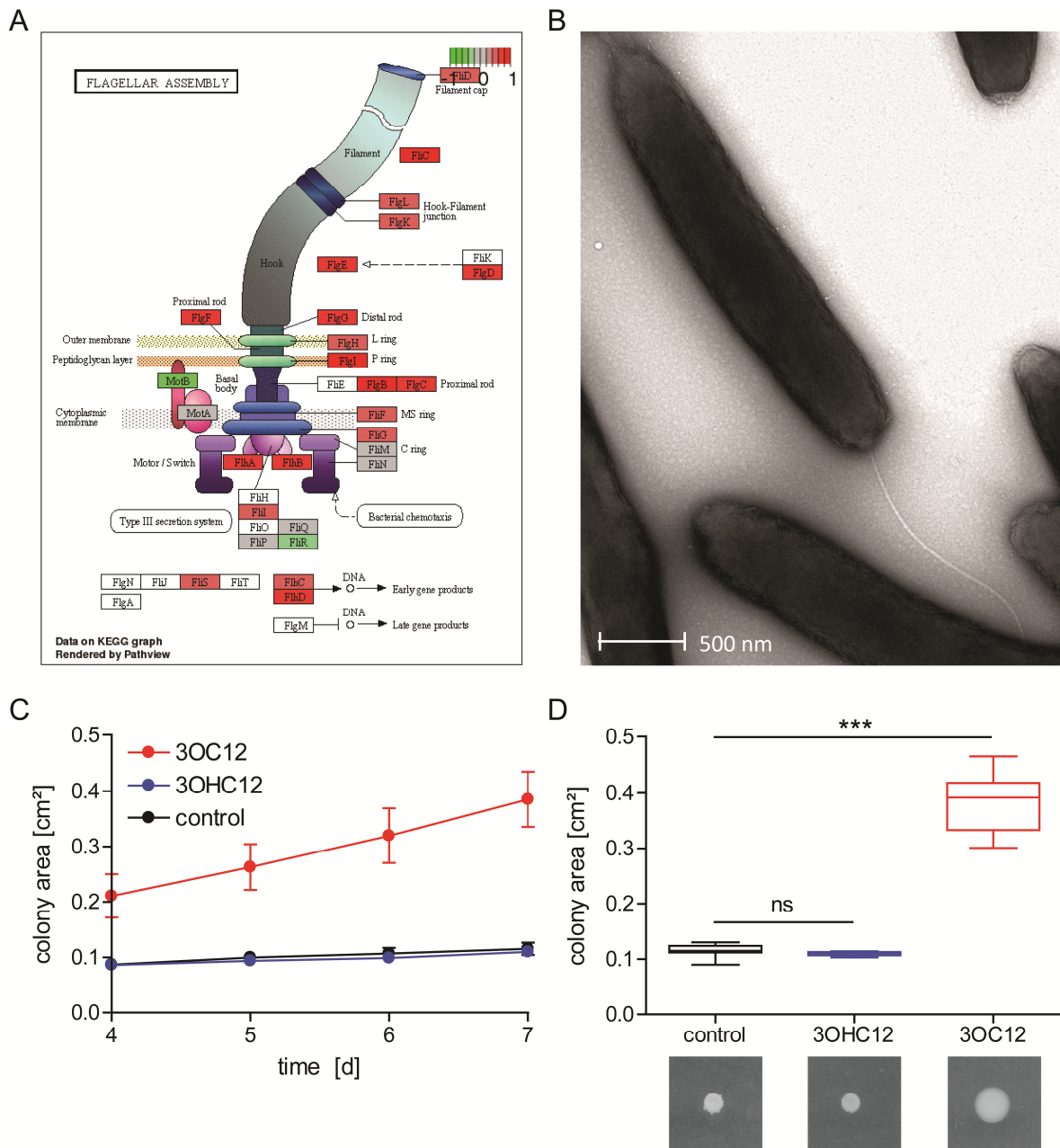
**Table 2: Differentially regulation of the polyketide operon by AHLs**

Locus tag	3OHC12-HSL		3OC12-HSL		antiSMASH - antibiotics & Secondary Metabolite Analysis Shell
	FC	FDR	FC	FDR	Predicted function
AEP_03681	1,14	0,0206	0,63	0,0000	2-amino-4, 5-dihydroxy-6-oxo-7-(phosphonoxy)heptanoate synthase
AEP_03682	1,29	0,0003	0,62	0,0000	Long-chain-fatty-acid-CoA ligase
AEP_03683	1,35	0,0000	0,65	0,0000	Peroxiredoxin OsmC
AEP_03684	1,45	0,0000	0,58	0,0000	<b>Polyketide synthase PksN</b>
AEP_03685	1,39	0,0001	0,67	0,0000	<b>Polyketide synthase PksN</b>
AEP_03686	1,33	0,0000	0,75	0,0000	<b>Polyketide synthase PksL</b>
AEP_03687	1,29	0,0023	0,78	0,0020	Anthranilate 3-monooxygenase oxygenase component
AEP_03688	1,31	0,0000	0,80	0,0001	hypothetical protein
AEP_03689	1,18	0,0023	0,90	0,0549	hypothetical protein
AEP_03690	1,13	0,0204	0,88	0,0091	hypothetical protein
AEP_03691	1,22	0,0281	0,78	0,0045	4'-phosphopantetheinyl transferase sfp
AEP_03692	1,14	0,1265	0,79	0,0018	Linear gramicidin dehydrogenase LgrE
AEP_03693	1,32	0,0007	0,78	0,0021	<b>Polyketide biosynthesis malonyl CoA-acyl carrier protein transacylase PksC</b>
AEP_03694	1,28	0,0000	0,88	0,0451	PqqC-like protein
AEP_03695	1,21	0,0031	0,84	0,0049	hypothetical protein

The program antiSMASH - antibiotics & Secondary Metabolite Analysis SHell Version 3.0.1 was used to analyse the genome of *Curvibacter* sp. strain AEP1.3 for production of secondary metabolites. By this a trans-AT polyketide synthases (trans atpks) operon could be identified. The fold change (FC) of these genes in 3OHC12- or 3OC12-HSL compared to control are indicated and the false discovery rate (FDR). A significantly down-regulation is marked in green, while an up-regulation is indicated by a red mark. The significance is indicated in different shade of grey (dark grey, FDR  $\leq$  0.001; middle grey, FDR  $\leq$  0.01; light grey, FDR  $\leq$  0.05).

#### 2.4.1 *Curvibacter's* swarming is dependent on 3-oxo-HSLs *in vivo*

The analysis of the *Curvibacter* sp. (strain AEP1.3) transcriptomes revealed that *Curvibacter* sp. possess all genes to produce flagella and furthermore that they are regulated by AHLs. A host has to suppress the flagella motility of its commensals, as epithelial contact with the MAMP (microbe-associated molecular pattern) flagellin, a principal component of bacterial flagella, induces the host immune system and causes inflammation (Cullender et al., 2013; Hayashi et al., 2001; Letran et al., 2011; Lodes et al., 2004). As *Curvibacter's* flagella production is AHL dependent, quorum quenching (QQ) could be an instrument to regulate the bacterial motility. Therefore, *Curvibacter's* motility was further investigated. On closer examination of the KEGG pathway "Flagellar Assembly" it became apparent that almost all genes necessary for the assembly of the flagellum are up-regulated by 3OC10-HSL, confirming a specific regulation of the flagella production (Figure 27A). Furthermore, the monotrichous flagellum of *Curvibacter* sp. could be visualized by transmission electron microscopy (TEM) (Figure 27B) (chapter 5.31).



**Figure 27: 3OC12-HSL dependent motility of *Curvibacter* sp.**

(A) Differential regulation of the KEGG pathway “Flagellar assembly” in *Curvibacter* sp. in the presence of 3OC12-HSL. Relative differential expression of the involved genes is indicated in shades of red (up-regulation) and green (down-regulation) (the figure is an output of the Gage analysis (chapter 5.16.2)). (B) Transmission electron microscopy (TEM) image of *Curvibacter* sp. was taken by Dr. Friederike Anton-Erxleben. A single flagellum attached to one cell pole can be seen. Cells were fixed with glutaraldehyde and stained with uranyl acetate. (C-D) *Curvibacter* sp. (1  $\mu$ l) was spotted on swarming plates supplemented with 10  $\mu$ M 3OC12-HSL, 10  $\mu$ M 3OHC12-HSL or 1:2000 ethyl acetate (control). Between four and seven days the colony area was measured. (C) Colony expansion of *Curvibacter* sp. between four and seven days. (D) After seven days the colony area of 3OC12-HSL treated *Curvibacter* sp. is significantly different to the 3OHC12-HSL treatment and control. A One-Way-ANOVA with Bonferroni post-test was used for statistical analysis (\*\*\*,  $P \leq 0.001$ ; ns, not significant,  $n = 10$ ). Images of the corresponding *Curvibacter* sp. colonies are depicted under the graph.

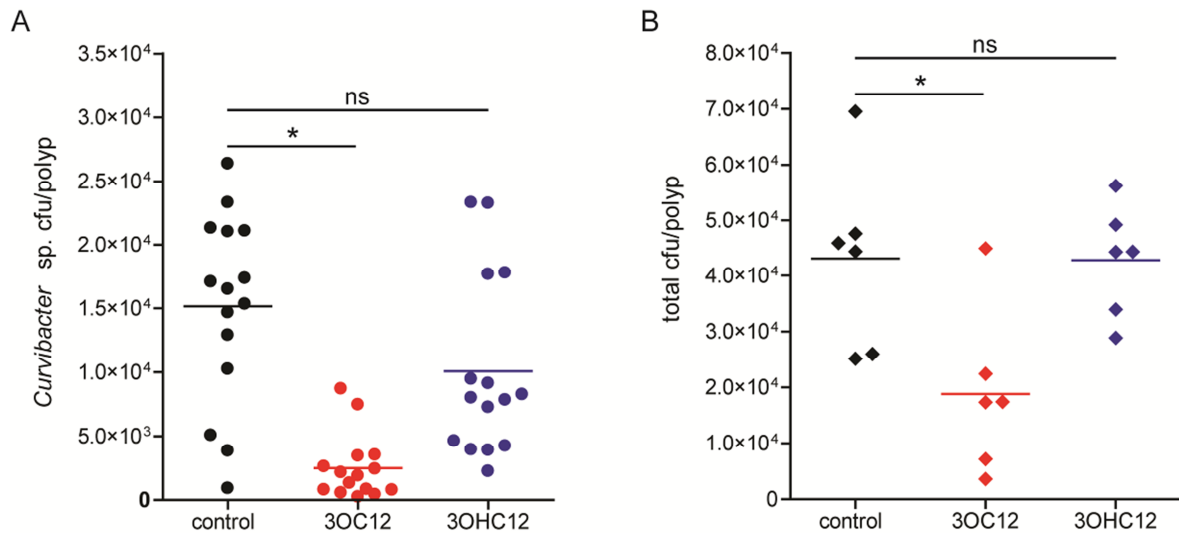
To analyse if the induced flagellar gene expression by 3OC12-HSL has consequences on the *in vivo* motility, it was investigated if the addition of 3OC12-HSL leads to swarming of *Curvibacter* sp. (strain AEP1.3). Therefore 1  $\mu$ l of a liquid culture of *Curvibacter* sp. was

spotted on swarming plates, supplemented with 10  $\mu$ M 3OC12-HSL, 10  $\mu$ M 3OHC12-HSL or 1:2000 ethyl acetate. Between four and seven days after spotting, the colony area was measured (chapter 5.32). Only in the presence of 3OC12-HSL was a continuous expansion of *Curvibacter*'s colony observed (Figure 27C & D). A One-Way-ANOVA with Bonferroni post-test was used for statistical analysis ( $P \leq 0.001$ ;  $n = 10$ ). Through these experiments, the 3-oxo-HSL-dependent induction of the flagella production and motion was confirmed *in vivo*.

## 2.5 Response of the metaorganism Hydra to 3-oxo- and 3-hydroxy-HSLs

On one hand it had been shown that the AHLs 3OC12- and 3OHC12-HSLs have drastically different effects on *Curvibacter*'s physiology in suspension (chapter 2.4), on the other hand Hydra's quorum quenching (QQ) mechanism converts long-chain 3-oxo-HSLs to 3-hydroxy-HSLs (chapter 2.1.3). These findings indicate, that while colonizing Hydra, certain *Curvibacter* genes, regulated by 3OC12-HSL, are suppressed, while other genes controlled by 3OHC12-HSL get supported. To investigate this hypothesis, the effects of 3OC12- and 3OHC12-HSLs addition on the metaorganism Hydra were investigated.

First, recolonization of germfree *Hydra vulgaris* (AEP) by the main colonizer *Curvibacter* sp. after four days were compared in the presence and absence of these two *N*-acyl homoserine lactones (AHLs) (Figure 28A). Thereby, one had to ensure that a saturated concentration of 3OC12-HSL was used, because Hydra is modifying the 3OC12-HSL into 3OHC12-HSL. Based on the calculated reaction rate for *Hydra vulgaris* (AEP) (18 pmol 3OC12-HSL/hour/polyp) (chapter 2.1.1) it was determined that after 16 h incubation 9 nmol 3OC12-HSL will be transformed into 3OHC12-HSL by the Hydra polyps. Consequently, 10  $\mu$ M (100 nmol/10 ml) 3OC12-HSL concentration was used to compensate Hydra's QQ activity. Recolonization efficiency was determined by counting colony forming units (CFUs) per Hydra polyp after four days of colonization (chapter 5.33). In the presence of 3OC12-HSL, only one sixth of *Curvibacter* sp. was able to recolonize the germfree polyps compared to control. The addition of 3OHC12-HSL had no significant effect on the colonization compared to control (Figure 28A). Statistical analysis was performed with a One-Way-ANOVA including a Bonferroni's Multiple Comparison Test (\*,  $P \leq 0.05$ ;  $n = 15$ ). These data shows that 3OC12-HSL induced changes in *Hydra* sp. or *Curvibacter* sp. reduce *Curvibacter*'s ability to colonize the Hydra polyp. As the addition of 3OHC12 HSL had no effect compared to control, it seems that the 3OHC12-HSL induced phenotype is the normal state for colonization.



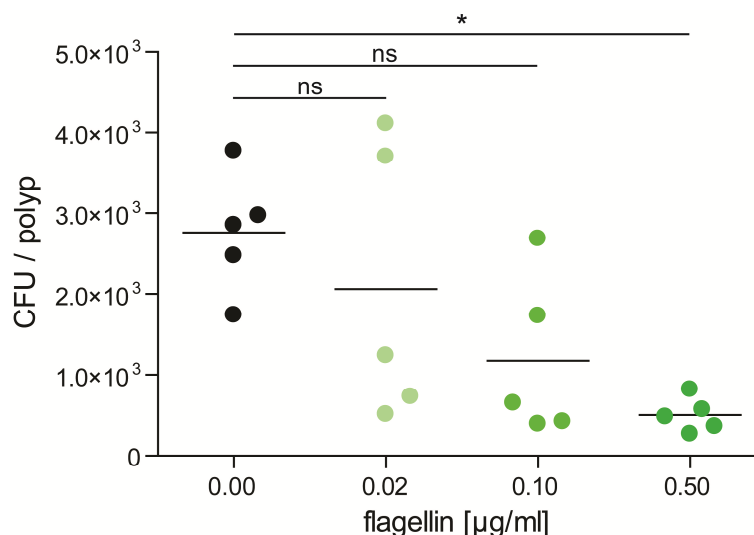
**Figure 28: Effects of AHLs on the metaorganism Hydra**

(A) Germfree *Hydra vulgaris* (AEP) polyps were colonized with *Curvibacter* sp. in the presence of  $10 \mu\text{M}$  3OC12-HSLs,  $10 \mu\text{M}$  3OHC12-HSLs or 1:2000 acidified ethyl acetate (control). After four days the recolonized polyps were plated and colony forming units (CFUs) were counted after three days. (B) Effects of AHLs on the bacterial load of the metaorganism Hydra. *Hydra vulgaris* (AEP) polyps were incubated for five days with  $5 \mu\text{M}$  3OC12-HSLs,  $5 \mu\text{M}$  3OHC12-HSLs or 1:4000 acidified ethyl acetate (control), afterwards polyps were plated on R2A agar and CFUs were counted after three days. (A-B) Statistical analysis was performed with a One-Way-ANOVA including a Bonferroni's Multiple Comparison Test (\*,  $P \leq 0.05$ ; ns, not significant; (A)  $n = 15$ , (B)  $n = 6$ ).

Furthermore, it was investigated whether 3OC12-HSL has a similar effect on the complete bacterial community of *Hydra vulgaris* (AEP) compared to *Curvibacter*'s recolonization. Therefore, *Hydra vulgaris* (AEP) polyps were treated with the two AHL species for 5 days, followed by determination of CFUs/polyp (Figure 28B) (chapter 5.34). Statistical analysis was performed with a One-Way-ANOVA including a Bonferroni's Multiple Comparison Test (\*,  $P \leq 0.05$ ;  $n = 6$ ). The treatment with 3OC12-HSL reduced the bacterial community within five days to about two thirds, while 3OHC12-HSL displayed no effect in this assay (Figure 28B).

An explanation for the observed phenomena could be that the 3OC12-HSL induced flagella, including the MAMP flagellin, of *Curvibacter* sp. leads to an immune response of Hydra, which results in a reduced bacterial load of the metaorganism Hydra.

To test the hypothesis that an immune reaction triggered by *Curvibacter*'s 3OC12-HSL induced flagella is responsible for decreased bacterial load, *Hydra vulgaris* (AEP) polyps were treated for 24 h with different concentrations of flagellin ( $0.02$ ,  $0.1$  and  $0.5 \mu\text{g/ml}$ ). The flagellin effect was determined by counting the CFUs/polyp (chapter 5.35). Flagellin of *Salmonella typhimurium* led to a concentration dependent decrease of the bacterial load of *Hydra vulgaris* (AEP) polyps, while only  $0.5 \mu\text{g/ml}$  caused a significant reduction ( $P \leq 0.05$ ,  $n = 5$ ) (Figure 29).



**Figure 29: Flagellin induced reduction of bacterial load of *Hydra vulgaris* (AEP)**

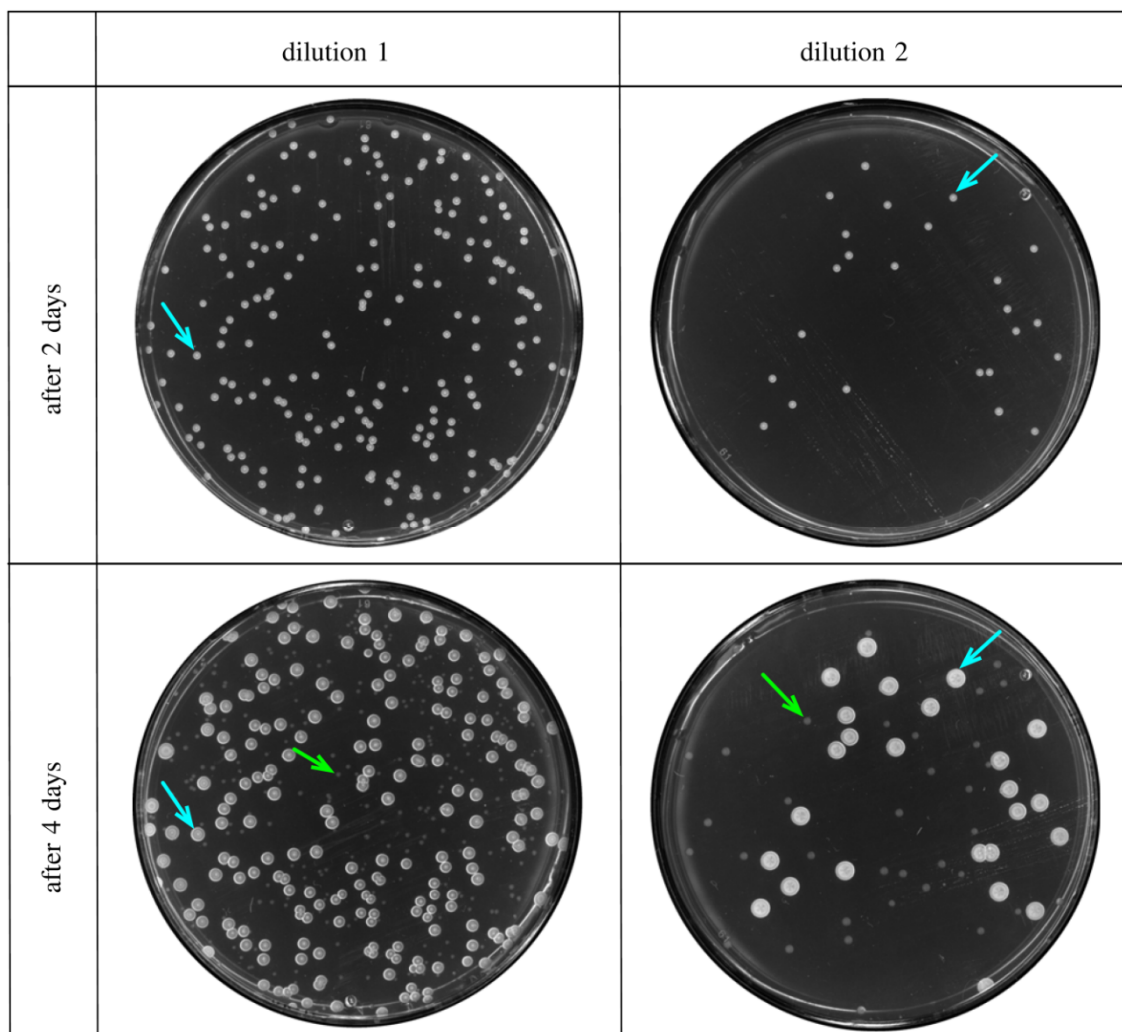
*Hydra vulgaris* (AEP) polyps were incubated for 24 h with three concentration of flagellin of *Salmonella typhimurium* resuspended in S-medium (0.02, 0.1 and 0.5 µg/ml). As a control *Hydra vulgaris* polyps incubated in S-medium with 0.2% PBS, as the flagellin was lyophilized in PBS. Afterwards 1/10 of a polyp was plated on R2A plates and CFUs were counted. Statistical analysis was performed with a One-Way-ANOVA including a Dunnett's Multiple Comparison Test (\*,  $P \leq 0.05$ ; ns, not significant,  $n = 5$ ).

These results indicate that 3OC12-HSL induces the production of flagella in *Curvibacter* sp. strain AEP1.3 to trigger a so far undetermined immune response in *Hydra vulgaris* (AEP), which causes the reduction of the bacterial load of the metaorganism Hydra. In contrast, 3OHC12-HSL showed no differences to control, indicating that the 3OHC12-HSL *Curvibacter* sp. phenotype is adapted to host colonization. Consequently, Hydra is promoting by its quorum quenching activity the colonizing phenotype of *Curvibacter* sp.

## 2.6 Combination of a modeling and experimental approach will help to understand the complex interactions in a metaorganism

Due to its simplicity, the model organism Hydra is well-suited, for the investigation of the basal regulatory network underlying the metaorganism, however for studying the network in its full complexity one needs cooperation with computer scientists and mathematicians to establish fundamental models, which can be applied on other metaorganisms. The problem is that assumptions taken by theoreticians and by biologists differ substantially. For example, classic evolutionary game theory models typically assume constant population size, which often does not hold for microbial populations. Consequently, theoreticians and biologists have to work together, to develop models for the fundamental interactions within the different partners of a metaorganism, before one can start to build models for the system in its full complexity.

In cooperation with Xiang-Yi Li and Prof. Dr. Arne Traulsen (Department of Evolutionary Theory, Max Planck Institute for Evolutionary Biology, Plön), the interactions between the two species of Betaproteobacteria *Curvibacter* sp. AEP1.3 (C) and *Duganella* sp. C1.2 (D) were studied by a theoretical (Xiang-Yi Li) and a biological (Cleo Pietschke) view (the data were published in Li et al., 2015). Both bacteria belong to the order of Burkholderiales, while C represents a Comamonadaceae and D an Oxalobacteraceae (Fraune et al., 2014). These two bacteria were chosen because (i) they are the two most abundant species in the bacterial community of the freshwater polyp *Hydra vulgaris* (AEP) (C: 75.6% and D: 11.1%) (Fraune et al., 2014); (ii) the synergistic interaction between the two bacteria species effectively provides anti-fungal protection for the host (Fraune et al., 2014); and (iii) the morphology of their colonies can be distinguished from each other on agar plates (Figure 30).

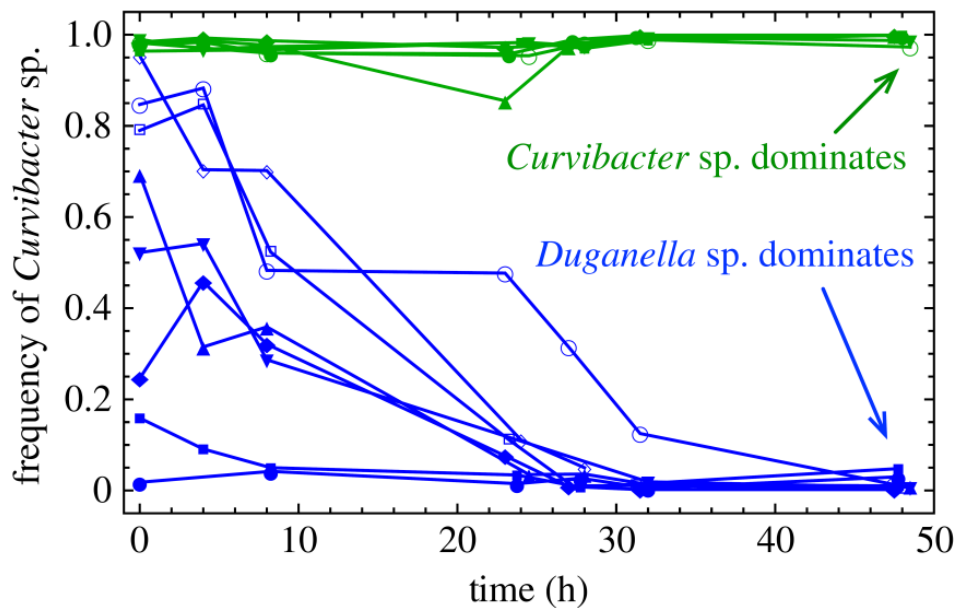


**Figure 30: Determination of colony forming units (CFUs) of *Duganella* sp. and *Curvibacter* sp. in di-association**

At each time point, a sample of the bacterial culture was taken and two dilution steps were plated on R2A plates. After 2 days, already clear and distinct colonies were formed by the fast-growing *Duganella* sp. (indicated by cyan arrows), which were counted to determine the colony forming units per sample. Colonies formed by the slow-growing *Curvibacter* sp. (indicated by green arrows) were counted after 4 days (taken from Li et al., 2015).

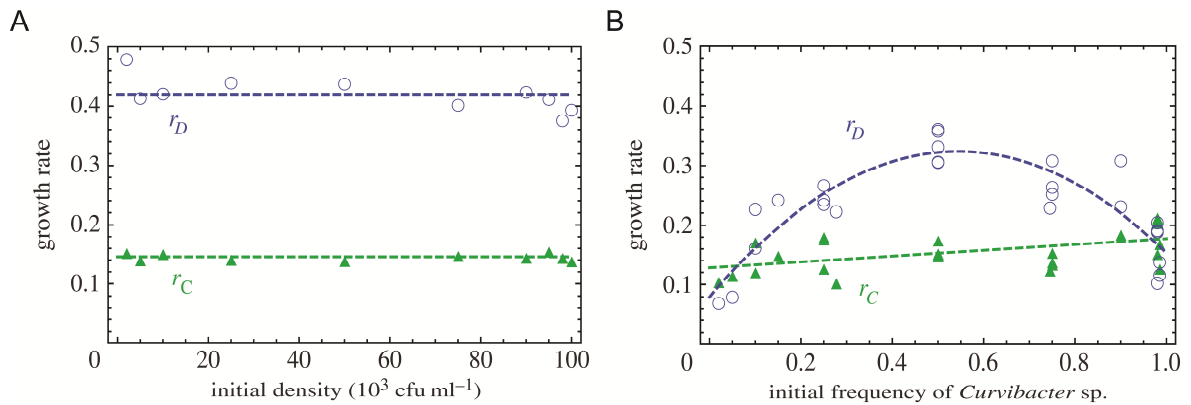


To investigate the interactions between *Curvibacter* sp. (C) and *Duganella* sp. (D) double culture experiments were performed, where a total initial concentration of  $1.0 \times 10^5$  CFU/ml was kept and a gradient of different initial frequencies of species C and D were applied. Over the course of 3 days, three times a day, the optical density ( $OD_{600}$ ) of the cultures was measured and two dilutions were plated on R2A agar plates. After 2 days, the number of colonies of D and after 4 days those of C were counted. This difference in counting times was due to different growth rates of the two bacteria on agar plates (Figure 30). The results showed that only if *Curvibacter* sp. possessed a high initial frequency it was able to dominate the culture (Figure 31).



**Figure 31: Change in the frequency of *Curvibacter* sp. in double cultures with *Duganella* sp.** The frequency of *Curvibacter* sp. approaches the 0 or 1 boundaries over time, depending on the initial frequency of both types. If the culture was inoculated with a high frequency of *Curvibacter* sp., the frequency of it remains high (green trajectories), otherwise *Duganella* sp. quickly outgrows and eventually pushes the frequency of *Curvibacter* sp. towards 0 (blue trajectories). This resembles the dynamics in a coordination game, where the two homogeneous populations are stable (taken from Li et al., 2015).

This was confirmed experimentally by determining that the maximum growth rates of the two bacteria did not change with the initial density in monoculture experiments (Figure 32A). The maximum growth rates of *Curvibacter* sp. and *Duganella* sp. in monoculture inoculated with a gradient of initial densities fit to constant functions  $r_C = 0.146$  ( $R^2 = 0.998$ ) and  $r_D = 0.420$  ( $R^2 = 0.996$ ). The frequency-dependent growth rate of *Curvibacter* sp. in double culture fit to a linear function  $r_C = 0.049x + 0.128$  (adjusted  $R^2 = 0.970$ ), and the growth rate of *Duganella* sp. in double culture fit to a quadratic function  $r_D = -0.825x + 0.898x + 0.080$  (adjusted  $R^2 = 0.972$ ), shown in Figure 32B. This suggests that differences in the growth rate in double cultures depend on the frequency combinations of the two species, rather than their absolute densities.

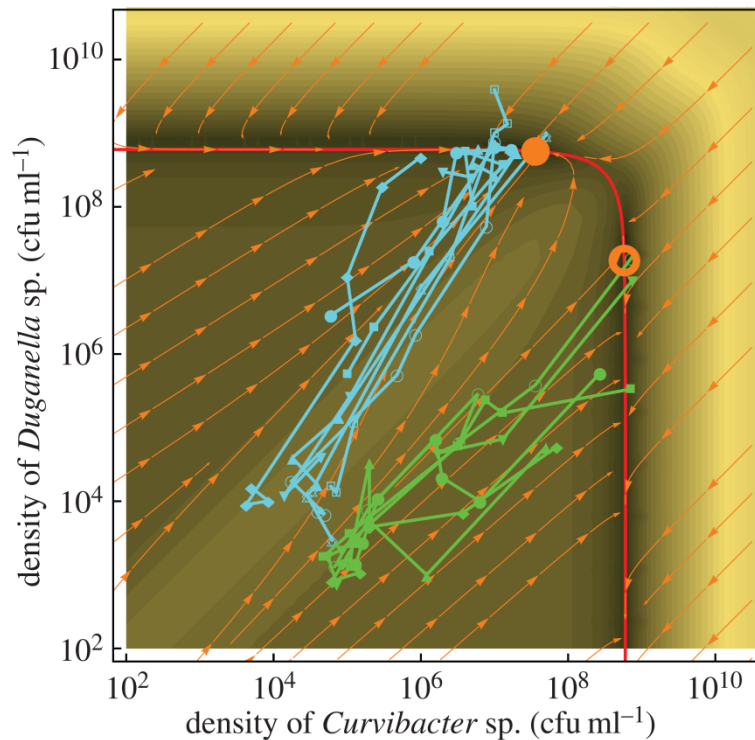


**Figure 32: Growth rates of *Curvibacter sp.* and *Duganella sp.* in mono- and di-association**

(A) The growth rates of *Curvibacter sp.* and *Duganella sp.* do not depend on the initial density in monoculture experiments.  $r_C = 0.146$  ( $R^2 = 0.998$ ),  $r_D = 0.420$  ( $R^2 = 0.996$ ). (B) The growth rate during the exponential growth phase changes with initial frequencies in double cultures. This depends on the frequencies of both types, not necessarily in a linear fashion. The frequency-dependent growth rates of *Curvibacter sp.* were fitted to a linear function  $r_C = 0.049x + 0.128$  (adjusted  $R^2 = 0.970$ ), and those of *Duganella sp.* were fitted to a quadratic function  $r_D = -0.825x^2 + 0.898x + 0.080$  (adjusted  $R^2 = 0.972$ ). The presence of *Duganella sp.* affects the growth rate of *Curvibacter sp.*, but not substantially. By contrast, *Curvibacter sp.* has strong inhibiting effects on the growth rate of *Duganella sp.*, across a wide range, even at very low frequency (taken from Li et al., 2015).

From comparison between growth rates in monoculture and double culture experiments (Figure 32), one can observe the presence of *Duganella sp.* does not profoundly influence the growth rates of *Curvibacter sp.*, across a wide range of initial frequencies. Interestingly, the presence of *Curvibacter sp.* greatly influences the growth rate of *Duganella sp.*, even at very low frequency (2%). When the initial frequency of *Curvibacter sp.* approaches 0 in double culture experiments, the growth rates of *Duganella sp.* do not converge to its growth rate when cultured alone. This large effect of *Curvibacter sp.* on *Duganella sp.* could be an explanation, why *Curvibacter sp.* is the main colonizer of *Hydra vulgaris* (AEP) even though *Duganella sp.* possess a 3 fold higher growth rate in mono-culture than *Curvibacter sp.*

These experimental findings were used by Xiang-Yi Li to develop a model for this bacteria-bacteria interaction (Li et al., 2015). Parameters measured in the experiments were inserted into her equations and the obtained phase plane dynamics are illustrated in Figure 33. Thereby she was able to recapitulate the formation of two fix endpoints, as it was observed in the double culture; *Curvibacter sp.* or *Duganella sp.* will dominate the culture (Figure 31).



**Figure 33: Growth trajectories plotted on the phase plane predicted by the model.**

The green trajectories lead to the unstable interior fixed point where *Curvibacter* sp. dominates in frequency. The cyan trajectories lead to the stable interior fixed point where *Duganella* sp. dominates. Filled and empty circles are stable and unstable fixed points, respectively. The red line is the invariant manifold  $C + D = K$ . The darkness of the contour plot represents the speed of change. The fast region along the diagonal represented by light colors separates the trajectories and pushes them to the two distinct fixed points in the interior. Near the fixed points the speed of change is very low; therefore, the two bacteria can coexist for a long time even near the internal unstable fixed point (taken from Li et al., 2015).

Beside the modeling of the experimental findings, she furthermore predicted that the observed pattern is only explainable if one assumes at least the interactions of three partners. As an interaction of two bacterial species was analysed, most probably no biologist would have assumed that the studied interaction takes place between more than two partners. The only explanation could be that at least one of the phages located in the genomes of *Curvibacter* sp. and *Duganella* sp. (unpublished data) are involved in this interaction. These results illustrate the advantages of the collaboration of theoreticians and biologists to understand biological systems.

### 3 Discussion

Every multicellular organism possesses an associated bacterial community, influencing each other in evolution, leading to the formation of a finely regulated homeostasis between the two partners (McFall-Ngai et al., 2013; Zilber-Rosenberg and Rosenberg, 2008). The host is dependent on its bacteria, as they are crucial for maturation of the host immune system (Clarke et al., 2010), organ morphogenesis (Wagner et al., 2008) and metabolism (Tremaroli and Bäckhed, 2012). Consequently, an imbalance of the system is associated with many diseases; in humans a dysregulated microbiota is involved in inflammatory bowel diseases (Frank et al., 2007; Hoffmann et al., 2015; Sartor and Mazmanian, 2012), obesity (Backhed et al., 2004; Ley et al., 2006), neurological disorders (Collins et al., 2012; Gonzalez et al., 2011) and cancer (Garrett, 2015; Lupton, 2004; De Martel et al., 2012) (reviewed in Sommer and Bäckhed, 2013). Therefore it is crucial to understand the regulatory network responsible for host-bacteria homeostasis and dysbiosis. Furthermore, in the last decades bacterial associated diseases were attempted to be cured with antibiotics, which led to multi-resistant bacterial strains (WHO, 2014) and often did not result in the desired recovery. Therefore, it is fundamental to understand not only the regulatory network within the host, but also the involved bacterial processes. Bacterial behavior, including virulence and biofilm formation, are mostly regulated by their communication system, called quorum sensing (QS). Influencing QS could be a mechanism to repress bacterial pathogenicity and to restore homeostasis in a bacterial community. However, the complexity of the human microbiota makes it difficult to study the fundamental regulatory circuits of the host-microbe homeostasis. The freshwater polyp *Hydra vulgaris* (AEP) has been established in previous years as a model organism for host-microbe interactions. It is a convincing model because of its simple body plan and its low bacterial diversity, consisting of six bacteria, which represent 90% of colonizing bacteria.

In the present thesis, it could be shown that *Hydra* possesses a quorum quenching (QQ) mechanism to modify the QS molecules of Gram-negative bacteria. Thereby, *Hydra* is able to manipulate bacterial behavior. The QQ activity of *Hydra vulgaris* (AEP) suppresses the flagellar motility but promotes chemotaxis and production of polyketides of its main colonizer *Curvibacter* sp. Therefore, *Hydra* allows its colonization by *Curvibacter* sp. and contributes to the homeostasis within the metaorganism *Hydra*.

In discussion, five different aspects of the thesis will be elucidated in more detail: (i) Hydra's QQ activity compared to already known QQ mechanisms, (ii) the role of QS in metaorganisms, (iii) the function of the 3-oxo- and 3-hydroxy-HSL phenotype of *Curvibacter* sp. (iv) the importance of QQ mechanisms for the host-bacteria homeostasis within a metaorganism and (v) importance of the interactions between *Curvibacter* sp. and *Duganella* sp. for the metaorganism Hydra.

### **3.1 Hydra sp. is using a novel eukaryotic quorum quenching mechanism**

In this thesis it has been shown that the three distantly related Hydra species *Hydra vulgaris* (AEP), *Hydra oligactis* (strain St. Petersburg) and *Hydra viridis* (strain A99) possess a general quorum quenching (QQ) activity against long-chain-3-oxo-HSL (3OC10- to 3OC14-HSL). Additionally, *H. oligactis* and *H. viridis* are able to degrade unmodified AHLs with a mid-chain length, as C6- and C8-HSL. An explanation for these additional QQ activities can most probably be found within their species-specific bacterial communities. For the antimicrobial peptide Arminin it is known that different Hydra species possess a species-specific set of Arminins to regulate their bacterial community (Franzenburg et al., 2013a), thus the species specific QQ activities could be explained by an adaptation of the Hydra species to their distinct bacterial community. Furthermore, as many bacteria are known to possess a QQ mechanism (Dong et al., 2001; Fetzner, 2015), the observed QQ activity could also be caused by Hydra's microbes. To investigate these hypotheses, the QQ activities of wild type and germfree *H. oligactis* and *H. viridis* have to be compared, as it has been done for *H. vulgaris* (AEP). Here, the detectable QQ activity originates clearly from the host epithelium.

The only eukaryotic enzymatic QQ mechanism so far described was reported for the human paraoxonases (PON) (Simanski et al., 2012). They use lactonase activity to degrade *N*-acyl-homoserine lactones (AHLs), the signaling molecules of Gram-negative bacteria. First, it was discovered that the human airway epithelia cells are able to inactivate the quorum sensing (QS) molecule of *Pseudomonas aeruginosa* 3OC12-HSL (Chun et al., 2004). Recently it was shown *in vitro*, that the PON2 of human keratinocytes can also degrade 3OC12-HSL (Simanski et al., 2012) and that PON2 deficient human airway epithelia cells possess impaired AHL degradation, which led to enhanced QS of *P. aeruginosa* (Stoltz et al., 2007). Through loss-of function experiments using *Hydra vulgaris* (AEP) polyps with an endodermal and ectodermal knockdown of Hydra's homologue to the human paraoxonases (HyPON), it was analysed if the QQ activity is an ancient function of PONs and responsible for the QQ of Hydra. In Hydra, no QQ activity

could be assigned to the HyPON. Although the paraoxonases are conserved enzymes, as they can be found along the phylogenetic tree of metazoans, their function seem to be diverged. Most probably the QQ activity, as identified in humans, is restricted to mammals, where the ancestral PON gene has twice been duplicated to give three mammalian paralogues, PON1, 2, and 3. The human PONs possess similar but distinct activities, but it has yet to be elucidated if other mammals also possess QQ activity (Bar-Rogovsky et al., 2013; Costa et al., 2014; Draganov et al., 2005; Mackness and Mackness, 2015; Reddy et al., 2001; Simanski et al., 2012). By identifying oxidoreductase activity as a QQ mechanism of Hydra, it could be confirmed that HyPON is not responsible for Hydra's QQ activity, as it is a lactonase.

It was interesting to identify an oxidoreductase activity responsible for the modification of 3-oxo-HSLs to 3-hydroxy-HSLs in *Hydra* sp., as this is the first report of a eukaryote using an oxidoreductase activity to modify *N*-acyl-homoserine lactones (AHLs). Furthermore, little is known about QQ by an oxidoreductase in general. The bacteria *Rhodococcus erythropolis* and Burkholderia strain GG4 show a similar oxidoreductase activity as *Hydra* sp., as they also target the oxo-group at the C3-carbon of the acyl-side chain, but have different substrate specificities. *R. erythropolis* modify similar AHLs (3OC6- to 3OC14-HSLs) as *Hydra* sp., but beside AHLs also alters molecules without the lactone ring or an additional phenyl group (Uroz et al., 2005). It has to be investigated if *Hydra* sp. is also able to modify a broader substrate spectrum than AHLs. The QQ activity of Burkholderia strain GG4 differs from Hydra's activity in that way, it is able to modify the short-chain 3-oxo-HSL, 3OC4-, 3OC6- and 3OC8-HSLs (Chan et al., 2011). Furthermore, in a soil-metagenome an oxidoreductase called BpiB09 was identified, which converts 3-oxo-C12-HSL to 3-hydroxy-C12-HSL (Bijtenhoorn et al., 2011). BpiB09 was characterized as short-chain dehydrogenase/reductase (SDR), which probably uses NADPH as co-substrate. Heterologous expression in *P. aeruginosa* influenced expression of QS-regulated genes and attenuated virulence on *Caenorhabditis elegans*. However, because of the low turnover of 3OC12-HSL by BpiB09, the authors assumed that 3OC12-HSL is probably not the native substrate (Bijtenhoorn et al., 2011). The monooxygenase CYP102A1 from *Bacillus megaterium* oxidize the acyl-side chain of the AHLs, which led to the introduction of a hydroxy group at the  $\omega$ -1,  $\omega$ -2 or  $\omega$ -3 carbon (Chowdhary et al., 2007). As the CYP102A1 oxidize the AHLs and do not act on the oxo-group at the third carbon, it clearly differs from the other AHL-oxidoreductases and Hydra's oxidoreductase activity. None of the so far described AHL-oxidoreductase activities reflect completely the QQ mechanism observed in Hydra. The identification of the corresponding gene is difficult, as oxidoreductases are quite abundant and no good comparable example is known. The

development of methods for the identification of *in vivo* relevant QQ enzymes is important to get a deeper understanding for the evolution and relevance of QQ in metaorganisms.

### 3.2 Bacterial quorum sensing – a common language in metaorganisms

To characterize the distribution of *N*-acyl-homoserine lactones (AHLs) and the relevance of quorum sensing (QS) in the metaorganism Hydra, the production of AHLs by environmental and commensal bacteria of *Hydra vulgaris* (AEP) were investigated. Surprisingly for all twelve analysed bacterial strains the production of AHLs could be demonstrated.

In general, relatively few information could be found in the literature about bacterial species related to Hydra's commensal bacteria. To my knowledge this is the first report of an AHL production for the species *Curvibacter* sp., *Pelomonas* sp., *Undibacterium* sp. and *Duganella* sp. By mass spectrometry analysis, one AHL, produced by the main colonizer *Curvibacter* sp., could be identified as 3OHC12-HSL. Even though little is known about these bacteria, it is striking that for all four bacterial species, closely related bacteria were reported to colonize eukaryotic host organisms, while reports about pathogenicity are missing. Related species to *Curvibacter* sp., the main colonizer of *Hydra vulgaris* (AEP), can also be found in the bacterial community of the coral *Montastraea annularis* (Barott et al., 2011), *Daphnia magna* (Gorokhova et al., 2015), leech (Ott et al., 2015), different amphibian species (McKenzie et al., 2012) and benthic algae (Barott et al., 2011). *Pelomonas* sp. belongs to the core microbiota of *Arabidopsis thaliana* (Schlaeppli et al., 2014), while *Undibacterium* sp. was identified in the gut intestine of tiger shrimps (Rungrassamee et al., 2014) and *Duganella* sp. can be found in the rhizosphere of sugar cane (Madhaiyan et al., 2013).

The production of 3OHC8- and 3OHC10-HSL was described for strains of *Acidovorax* sp., colonizing rhizospheres of wheat (Fekete et al., 2007) and tobacco (d'Angelo-Picard et al., 2005). Through use of thin-layer-chromatography (TLC), similar AHL molecules were identified in this thesis, but additionally further molecules with a longer acyl-side chain were detected. A comparable AHL spectrum was identified in *Acidovorax citrulli*, which is a cucurbits pathogen, including growth rate, swimming motility and virulence were shown to be controlled by QS (Fan et al., 2010). In contrast to the other commensal bacteria, much is known about *Pseudomonas* sp., as it includes the human pathogen *P. aeruginosa*. It is known to produce the *N*-acyl-homoserine lactones C4- and 3OC12-HSL and possess a huge, but well-characterized virulence machinery (reviewed in Jimenez et al., 2012). Besides pathogenic *Pseudomonas* sp., many commensal *Pseudomonas* sp.

have also been identified; for example, *P. fluorescens*, which inhabits rhizospheres, suppresses plant pathogens by secretion of bioactive secondary metabolites and furthermore promotes plant growth (Paulsen et al., 2005; Rainey, 1999). In Pseudomonads, a broad spectrum of AHLs were identified, ranging from unmodified to 3-oxo- and 3-hydroxy-HSLs, with chain lengths between C4 and C14 (Rehm, 2008). Concerning the chain-length, similar AHLs were identified in Hydra's commensal *Pseudomonas* sp.

The described results are in line with findings in sponges (Mohamed et al., 2008; Taylor et al., 2004), corals (Golberg et al., 2011; Ransome et al., 2014), squid (Verma and Miyashiro, 2013) and plants (Cha et al., 1998b), showing that many eukaryotes are colonized by bacteria using quorum sensing for their communication and demonstrating the high relevance of QS in a metaorganism.

The environmental bacteria of *Hydra vulgaris* (AEP) produced at least low amounts of four AHL species with 6 to 14 carbons at their acyl-side chain. Only the bacteria *Flectobacillus* sp., *Pseudomonas* sp. and *Chryseobacterium* strain 8 showed a high production of AHL species with an acyl-side chain between eight and twelve carbons.

To my knowledge this is the first report of an AHL production for the species *Flectobacillus* sp. and *Chryseobacterium* sp. The AHL production by the two *Chryseobacteria* are intriguing, as both strains are identical on 16S-rDNA, but differ drastically in their AHL spectrum. This fact illustrates how different the produced AHLs from two different strains of one species could be. For Pseudomonads and *Acinetobacter* sp., production of a broad spectrum of AHLs were reported, while Pseudomonads synthesize AHL ranging from short to long chain AHLs, with or without modification of the third carbon (see above) (Rehm, 2008), *Acinetobacter* sp. mainly produces 3-hydroxy-C12-HSLs, but also small amounts of unmodified AHLs (C10-, to C16-HSLs) and 3-oxo-C13-HSL (How et al., 2015; Kalia, 2015; Niu et al., 2008). *Acinetobacter* sp. of Hydra's environment exhibit only a low AHL production, but the produced AHLs were within a similar range as reported for other *Acinetobacter* sp. The AHL spectrum of the environmental *Pseudomonas* sp. is interesting, as it significantly differs to its commensal counterpart. Its AHL production is characterized by higher amount of produced AHLs and is dominated by two mid-chain AHLs, while the commensal *Pseudomonas* sp. produces more long-chain AHLs and possesses an overall lower AHL production. These differences could indicate a possible adaptation to the two different live stages, planktonic versus symbiotic.

For *Variovorax paradoxus*, production of 3-hydroxy-C8-HSLs was reported before (d'Angelo-Picard et al., 2005), however for the environmental *Variovorax* sp. only long-chain AHL was detected. Furthermore, *Variovorax paradoxus* and *Chryseobacterium* sp.



strain StRB126 are known to possess quorum quenching activity (Leadbetter and Greenberg, 2000; Wang et al., 2012), it would be interesting to see if bacterial QQ could also be found in the neighborhood of Hydra.

In contrast to Hydra's commensal bacteria, five of six environmental bacteria are related to bacterial species which are associated with pathogenicity. *Flectobacillus roseus* is a pathogen for Rohu Carps (Adikesavalu et al., 2015), *Pseudomonas aeruginosa* (de Bentzmann and Plésiat, 2011; Fujii et al., 2014), *Acinetobacter baumannii* (Antunes et al., 2014; Falagas et al., 2008) and *Chryseobacterium gleum* (Lo and Chang, 2014) are human pathogens, which are responsible for severe infections in hospitals. But of course not all species of the genera are pathogens, for example *Flectobacillus* sp. belongs to the microbial community of the coral *Porites astreoides* (Rohwer et al., 2002) but is furthermore known to be a freshwater bacterium (Sheu et al., 2009), fitting to its isolation source, Hydra's culture dish. *Chryseobacterium* sp. is part of the cucumber spermosphere and rhizosphere (Green et al., 2007), while *Variovorax paradoxus* is a soil bacterium (Pehl et al., 2012).

Taken together, Hydra is colonized by bacterial species mostly known to be in symbiosis with eukaryotic hosts, while the characterized environmental bacteria tend to live in a non-symbiotic stage, colonizing water and soil or becoming pathogenic. But based on the AHL pattern, one cannot distinguish between the commensal and environmental bacteria of *Hydra vulgaris* (AEP). This indicates that bacteria are regulating different set of genes with the same AHL species, resulting in different bacterial phenotypes. Consequently, eukaryotes would only allow colonization by specific bacterial phenotypes, which have to be characterized.

### **3.3 3-oxo- and 3-hydroxy-HSLs regulate distinct *Curvibacter* sp. phenotypes**

The general principal of a quorum sensing (QS) system is common to all bacteria, but which AHL species are produced and which genes are regulated by AHLs, at least slightly differ between the different bacterial species. In this thesis two LuxI/LuxR type QS systems of *Curvibacter* sp., the main colonizer of *Hydra vulgaris* (AEP), were identified and characterized. Through mass spectrometry analysis of a *Curvibacter* sp. culture, the 3-hydroxy-HSL, 3OHC12-HSL, could be identified. QS is a very dynamic process and is regulated by many factors, such as cell density (Choi et al., 2011; Darch et al., 2012), nutrient disposability (Kalia, 2015; Lazazzera, 2000) and oxygen concentration (Rutherford and Bassler, 2012; Wagner et al., 2003). As AHL production is dependent on fatty acid biosynthesis (Hoang et al., 2002), which is influenced by the aforementioned

conditions, the amount and species of AHLs can vary during bacterial growth. Therefore, heterologous expression of *Curvibacter*'s AHL-synthases Curl1 and Curl2 was performed to determine a broader view on *Curvibacter*'s AHL spectrum. It was expected that heterologous expression of AHL-synthases in *E. coli* may lead to the production of additional AHLs with unusual chain length (Gould et al., 2006), which is most probably caused by a different metabolism in *E. coli* compared to *Curvibacter* sp., resulting in a different mixture of available fatty acids for AHL production (Hoang et al., 2002). Nevertheless, the AHLs identified in the heterologous *E. coli* system are comparable to AHLs found *in vivo*, in liquid culture of *Curvibacter* sp., indicating that the specificity of the Curls is quite high. Both AHL-synthases are producing long-chain 3-hydroxy-HSLs, while Curl2 synthesizes higher amounts of unsaturated long-chain 3-hydroxy-HSLs than Curl1. Similar AHL patterns of the two AHL-synthases suggest that the 3-hydroxy-HSLs fulfill an important function for *Curvibacter* sp. On the other hand, the production of the AHLs in different amounts by the two AHL-synthases lets one suppose that both synthases perform different functions.

Through use of reporter constructs for the two AHL-receptor of *Curvibacter* sp., CurR1 and CurR2, it was analysed if the different AHL spectra have an effect on the expression level of Curl1 and Curl2. Both extracts of heterologously expressed Curl led to similar activation, suggesting that 3-hydroxy-HSLs are necessary for the activation of AHL-synthase transcription. The activation of CurR1 was only half as strong as for the CurR2, indicating that CurR2 is the key regulator of the AHL-synthases. Whether CurR1 has higher activation potential for other genes remains to be elucidated. By measuring single AHL species, it could be shown that the activation pattern of CurR1 can be recapitulated by addition of long-chain 3-oxo- and 3-hydroxy-AHLs, whereas 3OC12- and 3OHC14-HSL showed the highest response. The same AHL species led to a similar activation for CurR2, but 3OHC12-HSL, which could be identified in the liquid culture of *Curvibacter* sp, was responsible for the doubled activation compared to CurR1. In contrast to CurR1, CurR2 preferentially recognizes 3OC14- and 3OHC12-HSL, showing that the AHL-receptors also exhibit, as with the AHL-synthases, a slightly different activity. As already the analysed AHL species could recapitulate the observed activation pattern by the expressed Curls, it would be interesting to analyse if unsaturated AHLs exhibit the same expression pattern like the saturated AHLs or are responsible for different gene regulation. It is known that the binding of AHLs by the AHL-receptor is dependent on the modifications of the acyl-side chain of the AHLs, which also have different impacts on the strength of the gene expression (Schaefer et al., 1996b). These *in vitro* results indicate that the two Curl/CurR sets possess different activities and combined with different AHLs also cause different regulation of *Curvibacter*'s genes.

An *in vivo* study, where the expression profile of *Curvibacter* sp. in the presence of a 3-oxo-HSL or a 3-hydroxy-HSL was analysed, confirmed the *in vitro* results. Thereby, it could be shown that the 3-oxo-HSL 3OC12-HSL induces the development of flagella, resulting in a higher motility, while 3OHC12-HSL led to the up-regulation of genes, related to chemotaxis and production of polyketides, which are known for their bioactivity (Piel, 2010; Teta et al., 2010; Trindade-Silva et al., 2013). Taking Hydra's quorum quenching mechanism into account, which modify 3-oxo-HSLs into 3-hydroxy-HSLs, and the production of 3-hydroxy-HSLs by *Curvibacter* sp. itself, *Curvibacter*'s 3OHC12-HSL phenotype would represent its symbiotic lifestyle, while the 3OC12-HSL transcript profile reflects the non-symbiotic *Curvibacter* sp.

Flagella motility and chemotaxis are essential behavior for bacteria-host interactions. Possession of a flagella increases motility and thereby is beneficial for acquisition of nutrition, avoidance of toxic substances, ability to change hosts and find the proper niche within a host (Fenchel, 2002; Ottemann and Miller, 1997). Otherwise it is connected with a high metabolic cost for the synthesis of the flagella machinery (Macnab, 2003; Martínez-García et al., 2014) and furthermore it possesses antigens, recognized by the immune system (Lodes et al., 2004; Wang et al., 2003). Chemotaxis is necessary to sense changes in the environment, which could cause change in the direction of the bacterial motility (Wadhams and Armitage, 2004). Both mechanisms play important roles in the establishment of a host-bacteria interaction, but also in the development of bacterial pathogenicity.

On one hand for many pathogens it is known that they need motility and chemotaxis to colonize their host and establish their virulence. For example free-living *Vibrio anguillarum*, known to be a fish pathogen, can sense components of the fish intestine mucus (O'Toole et al., 1999), the plant pathogen *Agrobacterium tumefaciens* gets directed to wounded plants by chemotaxis (Kim and Farrand, 1998) and *Clostridium difficile*, the main cause of hospital-acquired infections (Lofgren et al., 2014), needs its flagella for adherence and gut colonization (Tasteyre et al., 2001). On the other hand, chemotaxis and flagella motility are also known to be essential for symbiotic interactions. For example, free-living rhizobia find legume root-hairs, where nodules will be formed and the bacteria fix nitrogen for its host, also by chemotaxis (Pandya et al., 1999) such as with *Vibrio fischeri* will find its way to the prospective light organ of the squid *Euprymna scolopes* also only by use of chemotaxis and its flagella. Interestingly, after establishment of symbiosis in the squid light organ, the *Vibrio* will become non-motile (Millikan and Ruby, 2003; Ruby and Asato, 1993). Recently it was shown that coral reef bacteria show a high chemotaxis towards compounds released by corals and its symbionts, indicating that chemotaxis play an important role for the establishment of the coral-bacteria association (Tout et al., 2015).

It would be interesting to see if components of Hydra act as chemoattractant for *Curvibacter* sp. and the other commensal bacteria.

If one assumes that the 3OHC12-HSL phenotype of *Curvibacter* sp. corresponds to its symbiotic life stage, than one could conclude that *Curvibacter* sp. colonizing Hydra is non-motile but has a high chemotaxis activity. Interestingly, the phenomenon that potentially motile bacteria stay non-motile in association with host, can also be found in human gut. In metagenomes of the human gut many flagella motility related genes can be found (Gill et al., 2006; Kurokawa et al., 2007; Turnbaugh et al., 2006). But by analyzing the metatranscriptome data, it becomes apparent that cell motility related genes are almost not expressed in the gut (Verberkmoes et al., 2009), indicating a suppression machinery by the host or an adaptation of the bacteria to the host colonization. In a healthy gut, flagella become recognized by Toll-like receptor 5 (TLR5) (Hayashi et al., 2001), which leads to the expression of flagellin specific immunoglobulins (Cullender et al., 2013; van der Waaij et al., 1996), causing the repression of bacterial motility (Cullender et al., 2013). A knockout of TLR5 in mice leads to an up-regulation of flagella motility genes in commensal bacteria, which enabled them to penetrate the crypts and villi in the small intestine, bringing them in direct contact with epithelial cells and activating the immune system (Cullender et al., 2013). These findings could explain the phenomenon, that a high level of flagellin is associated with mucosal barrier breakdown and inflammation in the gut (McCole and Barrett, 2003; Sanders et al., 2006). Cullender et al. showed that innate and adaptive immune system interact to modulate the gut microbial gene expression and control bacterial behavior and localization (Cullender et al., 2013). Applying these results on the Hydra-*Curvibacter* system, one can conclude that Hydra suppresses flagellar production of *Curvibacter* by quenching 3-oxo-HSLs and thereby tolerates colonization and maintains homeostasis.

### **3.4 Hydra's quorum quenching activity promotes the symbiotic functions of its main colonizer *Curvibacter* sp.**

Based on the two different *Curvibacter* sp. phenotypes induced by the different AHLs, it was hypothesized that Hydra's quorum quenching (QQ) activity promotes *Curvibacter*'s symbiotic functions, by suppression of flagella production and induction of polyketide synthesis. Therefore, Hydra allows its colonization by *Curvibacter* sp. and keeps the metaorganism in homeostasis. To test this hypothesis it was first investigated if the 3OC12-HSL-phenotype of *Curvibacter* sp., representing the non-symbiotic lifestyle, can recolonize Hydra as well as the symbiotic phenotype, induced by 3OHC12-HSL. Interestingly, the 3OC12-HSL phenotype of *Curvibacter* sp. has only poor ability to

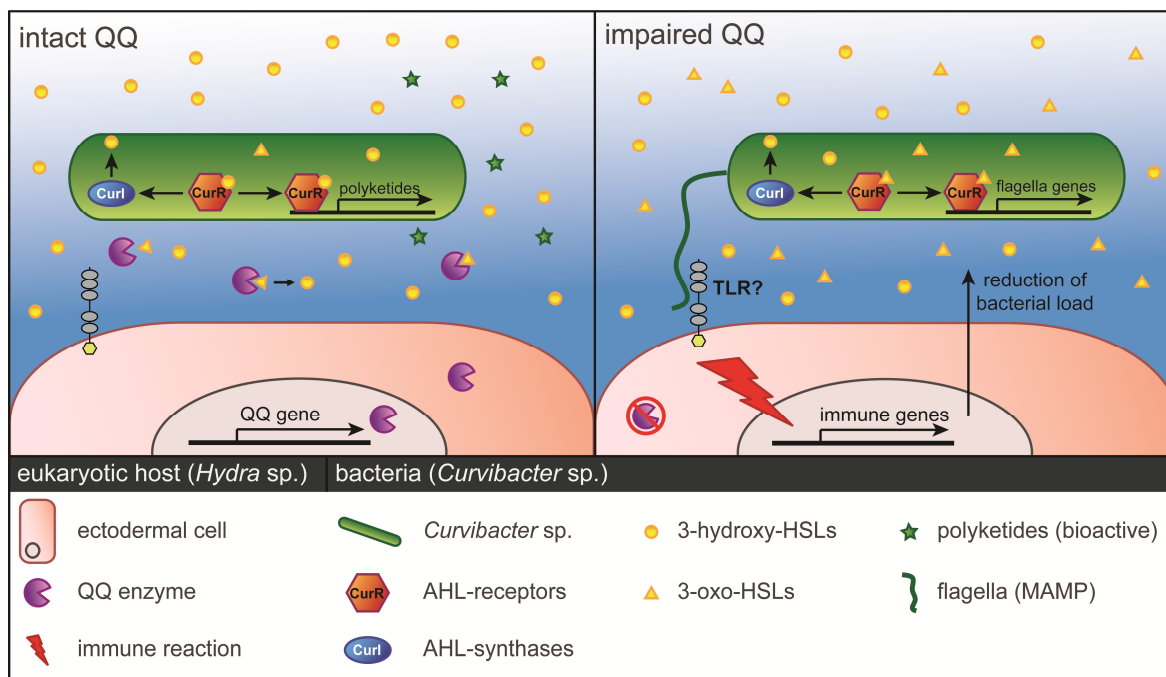
recolonize Hydra compared to wild type *Curvibacter* sp. and the 3OHC12-HSL phenotype. To investigate the role of QQ in keeping the metaorganism in homeostasis, Hydra was incubated in a saturated concentration of 3OC12-HSL. Through this it could be shown that 3OC12-HSLs also reduced the total bacterial colonization of Hydra, indicating a dysbiosis of the metaorganism Hydra. The dysbiosis can be induced by a phenotypical change of the bacteria or by a host response to 3OC12-HSL. It was shown that 3OC12-HSL induced the flagella production of *Curvibacter* sp. and it was hypothesized that a flagellin induced immune response in Hydra caused the reduction of its bacterial community. To support this hypothesis, Hydra was incubated with different concentrations of flagellin and the bacterial colonization was evaluated. The addition of 100 ng/ml flagellin led to a 50% reduction of Hydra's bacterial load, similar to the effect seen when Hydra was incubated with 3OC12-HSL. These findings indicate that indeed *Curvibacter*'s induced flagella are responsible for triggering a reaction in Hydra that leads to the elimination of half of the symbiotic bacteria. Although the underlying mechanisms have to be elucidated, it is likely that Hydra's innate immune system is involved in the reaction. One possibility, how Hydra can sense the phenotypical change of its main colonizer *Curvibacter* sp., is by its Toll-like receptor (TLR), which is known to possess the ability to bind flagellin (Bosch et al., 2009). This is supported in mice and in humans, where flagellin get recognized by the TLR5 and is known to induce inflammation (Cullender et al., 2013; Hayashi et al., 2001).

The missing link in this system is the as yet undetermined production of 3-oxo-HSLs in the neighborhood of Hydra. The fact that *Curvibacter* sp. is recognizing both AHLs (3OC12- and 3OHC12-HSL) and thereby induces two different phenotypes makes it unlikely that in the direct environment of *Curvibacter* sp. no 3-oxo-HSLs can be found. There are two possibilities, as to why 3-oxo-HSL production by *Curvibacter* sp. could not be shown. First, *Curvibacter* sp. is producing 3-oxo-HSLs, but the right conditions have not yet been tested; this is possible as it is known that the produced AHL species can vary with different growth conditions (Wang et al., 2006) and the heterologous expression in *E. coli* can possess a bias (Gould et al., 2006). The second explanation could be that *Curvibacter* sp. lost the ability to produce 3-oxo-HSLs during coevolution with its host. Because of permanently quenching of 3-oxo-HSLs by Hydra, 3-oxo-HSLs cannot fulfill their function in *Curvibacter* sp. colonizing the host; consequently *Curvibacter* sp. had only high costs for the AHL production, but no benefits, resulting in the loss of the ability to produce 3-oxo-HSLs. In this case *Curvibacter* sp. would be dependent on other bacteria, producing 3-oxo-HSL, for production of flagella in high amounts. The specific transcriptional response of *Curvibacter* sp. towards 3OC12-HSL makes it unlikely that there are no 3-oxo-HSLs in Hydra's environment, but the source has to be elucidated.

The fact that flagellated *Curvibacter* sp. can be detected in liquid mono-culture without the addition of 3-oxo-HSLs indicates that *Curvibacter* sp. even possesses flagella in the absence of 3-oxo-HSL. Therefore, one has to assume that the flagella concentration of *Curvibacter* sp., while colonizing *Hydra* sp., is under a certain threshold to induce a reaction in Hydra.

These results are supported by recent findings about the role of quorum sensing (QS) in white band disease of the coral *Acropora cervicornis*. Certner and Vollmer could show that the *Cytophaga-Flavobacterium* spp., which are present in small numbers in a healthy coral, are able to grow unlimitedly on a diseased coral. Interestingly, both the addition of cell free extract of a diseased coral and addition of AHLs to a healthy coral cause the onset of the white band disease, resulting in tissue loss (Certner and Vollmer, 2015). These results support the hypothesis that environmental changes cause a compromised host, resulting in an inactivation of the host QQ, which entails a phenotypical change of the bacteria and results in a dysbiosis. Addition of AHLs can simulate the development of disease. Furthermore, black band disease could be associated with active QS and a bacterial dysbiosis (Zimmer et al., 2014).

Taking these results together, one can conclude that in a healthy metaorganism, Hydra suppresses, by use of its QQ mechanism, flagella production of its main colonizer *Curvibacter* sp. to increase immune tolerance and thereby promotes bacterial colonization and their symbiotic functions. If environmental factors lead to an inactivation of the host QQ, 3-oxo-HSLs can occur and induce the production of bacterial flagella, resulting in inflammation and dysbiosis of the metaorganism Hydra (Figure 34). As QS can be found in many metaorganisms, it is likely that also the regulation of the bacterial behavior by QQ is a general mechanism in host-microbe interactions, but this has to be further investigated.



**Figure 34: Role of quorum quenching in the metaorganism Hydra**

In a healthy metaorganism, Hydra suppresses, by use of its quorum quenching enzyme, flagella production of its main colonizer *Curvibacter* sp. to increase immune tolerance and thereby promotes bacterial colonization and their symbiotic functions. If environmental factors lead to an inactivation of the host QQ, 3-oxo-HSLs can occur and induce the production of bacterial flagella, resulting in inflammation and dysbiosis of the metaorganism Hydra.

### 3.5 Consequences of the interactions between *Curvibacter* sp. and *Duganella* sp. for the metaorganism Hydra

By analyzing the bacterial colonization during ontogeny of *Hydra vulgaris* (AEP) by a modeling approach it was predicted that a host factor and a frequency dependent fitness of the colonizing bacteria are necessary for the modeling of the colonization process (Franzenburg et al., 2013b). Before the complex network within the metaorganism Hydra can be deciphered, the basal interactions of this network was needed to be understood by analyzing the interaction of two bacterial species. The two Burkholderiales *Curvibacter* sp. and *Duganella* sp. were chosen, as they are the main colonizers of *Hydra vulgaris* (AEP) (Franzenburg et al., 2013a; Fraune et al., 2014). Furthermore, it could be shown that the interaction of these two species provides anti-fungal protection for their host Hydra (Fraune et al., 2014) and consequently influence the homeostasis within the metaorganism Hydra. To understand the relationship of these two bacterial species, their frequency dependent fitness in mono-culture and in double culture were analysed.

Interestingly, *Duganella* sp. has three times higher growth rate than *Curvibacter* sp. in mono-culture, although *Curvibacter* sp. is dominating with almost 80% the bacterial community of *Hydra vulgaris* (AEP) and *Duganella* sp. represents only around 10%. An explanation for the dominance of *Curvibacter* sp. in Hydra's bacterial community can be

found in the double culture experiment, where the growth of *Duganella* sp. is strongly inhibited by *Curvibacter* sp. The developed mathematical model of Xiang-Yi Li (Department of Evolutionary Theory, Max Planck Institute for Evolutionary Biology, Plön) (Li et al., 2015) predicted that the studied interaction in double culture takes place with at least three partners, with phages perhaps being involved in the interaction between *Curvibacter* sp. and *Duganella* sp. The role of phages in the *Curvibacter* sp. - *Duganella* sp. interaction has to be elucidated. Furthermore, it could be shown that *Curvibacter* sp. is producing polyketides, which are known for their bioactivity (chapter 2.4). These polyketides could be responsible for *Curvibacter*'s antifungal activity (Fraune et al., 2014) or perhaps they are also used to suppress other bacteria, while colonizing Hydra.

Further analyses will reveal the role of phages in the bacteria-bacteria interaction and the function of the polyketides. The most interesting approach will be the analysis of bacteria-bacteria interactions in a host context. Thereby it will be interesting to identify factors, which are responsible for *Curvibacter*'s dominance and relatively low abundance of *Duganella* sp. in the metaorganism Hydra, in contrast to their growth in culture medium. These studies will help to understand the complex network of interaction within a metaorganism.



## 4 Material

### 4.1 Organisms

#### 4.1.1 General organisms

Analysed organisms	<i>Hydra vulgaris</i> (AEP), <i>Hydra oligactis</i> (strain St. Petersburg), <i>Hydra viridis</i> (strain A99)
Feed	<i>Artemia salina</i>
Recipient	<i>Escherichia coli</i> DH5 $\alpha$ <i>Escherichia coli</i> Rosetta™ 2 (DE3) pLysS

#### 4.1.2 AHL-reporter strains

For the detection of *N*-acyl-homoserine lactones (AHLs) bacterial reporter strains were used. Such a reporter is a host, in this case *Escherichia coli* JM109 or *Agrobacterium tumefaciens* NTL4, possessing a plasmid, on which a gene of interest is fused to a reporter system.

The *E. coli* JM109 strains harbor a plasmid, in which a quorum sensing (QS) system, consisting of the AHL-receptor and the promoter of the AHL-synthase, is fused to the luciferase operon (*luxCDABE*). In the presence of the proper AHLs, the AHL-receptor binds to the molecules and activates the transcription of the luciferase genes. The resulting light emission is proportional to the AHL concentration. The AHL-reporters pSB401, pSB534 and pSB1075 were provided by Dr. Karen Tait from the Plymouth Marine Laboratory.

The AHL detection by the *A. tumefaciens* bioreporter is performed by the products of two plasmids; the pCF218-plasmid harbors the gene of the AHL-receptor *traR*, while the fusion of the *traI*-promoter with the gene for the  $\beta$ -galactosidase *lacZ* is located on the pCF372 plasmid. The reporter fusion allows a detection of AHLs by a blue-white screening; in the presence of compatible AHLs the  $\beta$ -galactosidase gets transcribed and converts X-gal (5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside) into a blue dye. The *A. tumefaciens* NTL4 bioreporter (Fuqua and Winans, 1996, 1994; Luo et al., 2001) was kindly provided by Prof. Dr. Wolfgang Streit. The used AHL-reporter strains are described in Table 3.

**Table 3: AHL-reporter strains**

strain	QS system based on	response to	reference
<i>E. coli</i> JM109 pSB536	Ahyl/R ( <i>A. hydrophyla</i> )	C4-HSL	Swift et al. 1997
<i>E. coli</i> JM109 pSB401	LuxI/R ( <i>V. fischeri</i> )	C6-HSL 3OC6-HSL C8-HSL 3OC8-HSL	Winson et al. 1998
<i>E. coli</i> JM109 pSB1075	LasI/R ( <i>P. aeruginosa</i> )	3OC10-HSL 3OC12-HSL	Winson et al. 1998
<i>A. tumefaciens</i> NTL4 (pCF218)(pCF372)	Tral/R ( <i>A. tumefaciens</i> )	C6-C12-HSL 3OC6-3OC14-HSL 3OHC8-3OHC14-HSL	Fuqua & Winans 1994, 1996; Luo et al. 2001

## 4.2 Chemicals

### 4.2.1 Antibiotics

**Table 4: Antibiotics**

abbreviation	antibiotic	stock solution	resolvent	provider
Amp	ampicillin	100 mg/ml	water	Sigma-Aldrich GmbH
Cam	chloramphenicol	34 mg/ml	ethanol	Sigma-Aldrich GmbH
Kan	kanamycin sulfate	50 mg/ml	water	Sigma-Aldrich GmbH
Neo	neomycin sulfate	50 mg/ml	water	Roth
Tet	tetracycline hydrochloride	10 mg/ml, 5 mg/ml	water	Boehringer Mannheim GmbH
Rif	rifampicin	50 mg/ml	DMSO	Sigma-Aldrich GmbH
Sp	spectinomycin dihydrochloride pentahydrate	60 mg/ml	water	AppliChem
Strep	streptomycin sulfate	50 mg/ml	water	Roth

#### 4.2.2 *N*-acyl-homoserine lactones (AHLs)

**Table 5: *N*-acyl-homoserine lactones (AHLs)**

abbreviation	name	empirical formula	molecular weight
C4-HSL	<i>N</i> -butyryl-DL-homoserine lactone	C <sub>8</sub> H <sub>13</sub> NO <sub>3</sub>	171.19
C6-HSL	<i>N</i> -hexanoyl-DL-homoserine lactone	C <sub>10</sub> H <sub>17</sub> NO <sub>3</sub>	199.25
3OC6-HSL	<i>N</i> -(3-oxohexanoyl)-L-homoserine lactone	C <sub>10</sub> H <sub>15</sub> NO <sub>4</sub>	213.23
C8-HSL	<i>N</i> -octanoyl-DL-homoserine lactone	C <sub>12</sub> H <sub>21</sub> NO <sub>3</sub>	227.30
3OC8-HSL	<i>N</i> -(3-oxooctanoyl)-L-homoserine lactone	C <sub>12</sub> H <sub>19</sub> NO <sub>4</sub>	241.28
3OHC8-HSL	<i>N</i> -(3-hydroxyoctanoyl)-L-homoserine lactone	C <sub>12</sub> H <sub>21</sub> NO <sub>4</sub>	243.30
C10-HSL	<i>N</i> -decanoyl-DL-homoserine lactone	C <sub>14</sub> H <sub>25</sub> NO <sub>3</sub>	255.35
3OC10-HSL	<i>N</i> -(3-oxodecanoyl)-L-homoserine lactone	C <sub>14</sub> H <sub>23</sub> NO <sub>4</sub>	269.34
3OHC10-HSL	<i>N</i> -(3-hydroxydecanoyl)-L-homoserine lactone	C <sub>14</sub> H <sub>25</sub> NO <sub>4</sub>	271.35
C12-HSL	<i>N</i> -dodecanoyl-DL-homoserine lactone	C <sub>16</sub> H <sub>29</sub> NO <sub>3</sub>	283.41
3OC12-HSL	<i>N</i> -(3-oxododecanoyl)-L-homoserine lactone	C <sub>16</sub> H <sub>27</sub> NO <sub>4</sub>	297.39
3OHC12-HSL	<i>N</i> -(3-hydroxydodecanoyl)-DL-homoserine lactone	C <sub>16</sub> H <sub>29</sub> NO <sub>4</sub>	299.41
3OC14-HSL	<i>N</i> -(3-oxotetradecanoyl)-L-homoserine lactone	C <sub>18</sub> H <sub>31</sub> NO <sub>4</sub>	325.44
3OHC14-HSL	<i>N</i> -(3-hydroxytetradecanoyl)-L-homoserine lactone	C <sub>18</sub> H <sub>33</sub> NO <sub>4</sub>	327.46

All *N*-acyl-homoserine lactones were acquired from Sigma-Aldrich.

#### 4.2.3 General chemicals

Acetic acid	Roth
Acetonitrile, LC-MS Grade	Roth
Agar-Agar	Roth
Agarose Low Melt	Roth
Agarose NEEO Ultra	Roth
Ammonium persulfate (APS)	Roth
Ammonium sulfate	Roth
β-Mercaptoethanol	Merck
Boric acid	Roth
Bromophenol blue	Fluka
BSA FraktionV	Roth
Calcium chloride dihydrate	Roth
dNTPs (100 mM)	Thermo Scientific
DMSO (Dimethylsulfoxid)	Roth
EDTA	Sigma
Eiken agar	Eiken Chemicals
Ethanol	Roth

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Glucose	Roth
Glutaraldehyde (50%)	Sigma
Glycerol	Roth
Glycine	Roth
IPTG	Roth
Iron(II) sulfate heptahydrate	Sigma
Isopropanol	Roth
LB Broth Base	Roth
Magnesium chloride	Roth
Magnesium sulfate heptahydrate	Roth
Manganese(II) sulfate heptahydrate	Sigma
Methanol	Roth
NBT / BCIP	Roth
Pepstatin A	Roth
peqGREEN	Peqlab
Potassium carbonate	Roth
Potassium chloride	Roth
Potassium dihydrogen orthophosphate	Merck
Potassium hydroxide	Roth
Procaine hydrochloride	Sigma
R2A-Agar	Roth
R2A Broth	Lab M
RapidGel-XL-40 % Concentrate	Roth
RNAprotect <sup>®</sup> Bacteria Reagent	Qiagen
Roti <sup>®</sup> -Blue	Roth
Rotiphorese <sup>®</sup> Gel 30	Roth
Sodium chloride	Roth
Sodium citrate	Roth
Sodium dodecyl sulfate (SDS)	Merck
Sodium hydrogen carbonate	Roth
Sodium pyruvate	Sigma
TEMED	Merck
TES	Sigma
Tris acetate	Roth
TRIzol <sup>®</sup>	Invitrogen
Tryptone / Peptone from casein	Roth
Tween <sup>®</sup> 20	Roth

Uranyl acetate	Sigma
X-gal	Sigma
Xylene cyanol	Fluka
Yeast extract	Roth

## 4.3 Media

### 4.3.1 Media for the rearing of Hydra polyps

Artemia medium	31.8 g sea salt / 1 l Millipore H <sub>2</sub> O
S-medium	0.29 mM CaCl <sub>2</sub> x 2 H <sub>2</sub> O, 0.33 mM MgSO <sub>4</sub> x 7 H <sub>2</sub> O, 0.5 mM NaHCO <sub>3</sub> , 0.08 mM K <sub>2</sub> CO <sub>3</sub>
Sterile S-medium	
Stock solution 1 (1000x):	42.18 g CaCl <sub>2</sub> x 2 H <sub>2</sub> O / 1 l Millipore H <sub>2</sub> O
Stock solution 2 (100x):	8.116 g MgSO <sub>4</sub> x 7 H <sub>2</sub> O, 4.238 g NaHCO <sub>3</sub> , 1.0958 g K <sub>2</sub> CO <sub>3</sub> / 1 l Millipore H <sub>2</sub> O

The sterile S-medium was always freshly made; a 1fold solution 1 was autoclaved and a sterile filtered solution 2 was 1fold added.

### 4.3.2 Media for the cultivation of bacteria

According to requirements, antibiotics were added to the plates and media for bacteria.

AT buffer (20x)	214 g KH <sub>2</sub> PO <sub>4</sub> , ad 1 l Millipore H <sub>2</sub> O, KOH pH 7.0
AT medium	1x AT salt solution, 1x AT buffer, 0.5% glucose in Millipore H <sub>2</sub> O
AT salt solution (20x)	40 g (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , 3.2 g MgSO <sub>4</sub> x 7 H <sub>2</sub> O, 0.2 g CaCl <sub>2</sub> x 2 H <sub>2</sub> O, 0.1 g FeSO <sub>4</sub> x 7 H <sub>2</sub> O, 0.04 g MnSO <sub>4</sub> x 7 H <sub>2</sub> O, ad 1 l Millipore H <sub>2</sub> O
AT soft agar	1 g Eiken agar in 89 ml Millipore H <sub>2</sub> O (autoclaved, 42°C), 5 ml AT buffer (20x), 5 ml AT salt solution (20x), 1 ml glucose solution (50%)
LB medium	10 g NaCl, 10 g tryptone, 5 g yeast extract, 1 l Millipore H <sub>2</sub> O

LB plate	1 l LB medium, 15 g agar-agar
R2A medium	3 g R2A Broth / 1 l Millipore H <sub>2</sub> O
R2A plate	18 g R2A-Agar / 1 l Millipore H <sub>2</sub> O
SOB medium	20 g tryptone, 5 g yeast extract, 0.58 g NaCl, 0.19 g KCl, 1 l Millipore H <sub>2</sub> O, 100 µl MgCl <sub>2</sub> , 10 mM MgSO <sub>4</sub>
SOC medium	10 ml SOB medium, 100 µl 2 M glucose solution (sterile filtered)
Swarming plate	1 l R2A medium, 5 g Agarose Low Melt

## 4.4 Buffer and solutions

### 4.4.1 General solutions

APS stock solution	10% (w/v) in Millipore H <sub>2</sub> O
DNA loading dye	10 mM EDTA pH 8.0, 0.1% SDS, 0.025% bromophenol blue, 0.025% xylene cyanol
IPTG stock solution	1 M in Millipore H <sub>2</sub> O, store at -20°C
PBS	150 mM NaCl, 80 mM Na <sub>2</sub> HPO <sub>4</sub> , 21 mM NaH <sub>2</sub> PO <sub>4</sub> , pH 7.2 in Millipore H <sub>2</sub> O
PBT	PBS + 0.1% Tween 20
TAE buffer (50 x)	2 M Tris acetate, 1 mM EDTA, pH 8
TBE buffer (10 x)	162 g Tris Base, 27.5 g boracic acid, 50 ml 0.5 M EDTA, add 1 l Millipore H <sub>2</sub> O
X-Gal stock solution	20 mg/ml in DMSO

### 4.4.2 Buffer and solutions for SDS-PAGE and Western blot

blocking solution	PBT, 3% BSA
loading dye (2x)	135 mM Tris-HCl (pH 6.8), 20% glycerol, 4% SDS, 2% β-mercaptoethanol, 0.001% bromophenol blue
running buffer (5x)	125 mM Tris, 0.96 M glycine, 0.5% SDS

NTM buffer	100 mM NaCl, 100 mM Tris-HCl, 5 mM MgCl <sub>2</sub> , pH 9.5
NTMT buffer	NTM, 0.1% Tween20
stacking gel (4%)	0.88 ml 30% acrylamide/0.8% bisacrylamide, 1.66 ml stacking gel buffer, 66.6 µl 10% SDS, 13.2 µl TEMED, 66.8 µl 10% APS, 4.08 ml Millipore H <sub>2</sub> O
stacking gel buffer	0.5 M Tris-HCl (pH 6.8)
SDS stock solution	10% (w/v) in Millipore H <sub>2</sub> O
transfer buffer	25 mM Tris, 192 mM glycine, 10% methanol
separation gel (10%)	4.44 ml 30% acrylamide/0.8% bisacrylamide, 3.34 ml separation gel buffer, 133.4 µl 10% SDS, 13.2 µl TEMED, 133.2 µl 10% APS, 5.28 ml Millipore H <sub>2</sub> O
separation gel buffer	1.5 M Tris-HCl (pH 8.8)

#### 4.4.3 Buffer and solutions for Procaine treatment

dissociation medium	3.6 mM KCl, 6.0 mM CaCl <sub>2</sub> , 1.2 mM MgSO <sub>4</sub> , 6.0 mM sodium citrate, 6.0 mM sodium pyruvate, 12.5 mM TES, pH 6.9, sterile filtered
solution A	1:1:1 dilution of 1% procaine-HCl in Millipore H <sub>2</sub> O, dissociation medium, S-medium, pH 4.5
solution B	1:1:1 dilution of 1% procaine-HCl in Millipore H <sub>2</sub> O, dissociation medium, S-medium, pH 2.5

#### 4.5 Kits

DNeasy <sup>®</sup> Blood & Tissue Kit	Qiagen
First Strand cDNA Synthesis Kit	Thermo Scientific
NucleoSpin <sup>®</sup> Gel and PCR clean-up-Kit	Macherey-Nagel
NucleoSpin <sup>®</sup> Plasmid QuickPure-Kit	Macherey-Nagel
pGEM <sup>®</sup> -T Vector System	Promega

PureLink® RNA Mini Kit	Invitrogen
QuantiTect® Reverse Transcription Kit	Qiagen
SYBR® Green PCR Kit	Qiagen
QIAfilter Plasmid Midi Kit (25)	Qiagen
RNeasy® Mini Kit	Qiagen
Roti®-Store Cryoröhrchen	Roth

## 4.6 Enzymes

### 4.6.1 General enzymes

DNase I	Roche
GoTaq® DNA polymerase	Promega
Platinum® Taq DNA Polymerase	invitrogen
Platinum® Taq DNA Polymerase High Fidelity	invitrogen
Proteinase K	Qiagen
PureLink® DNase	Invitrogen
RNase H	invitrogen
T4 DNA ligase	New England Biolabs

### 4.6.2 Restriction enzymes

<i>Asi</i> SI	New England Biolabs
<i>Bs</i> WI	New England Biolabs
<i>Eco</i> RI	Thermo Scientific
<i>Eco</i> RI-High Fidelity (HF)	New England Biolabs
<i>Nco</i> I	Thermo Scientific
<i>Xho</i> I	Thermo Scientific



## 4.7 Vectors

LigAF-1	modified HoTG vector, by K. Khalturin
pET 22b	Novagen
pET 28a(+)	Novagen
pGEM <sup>®</sup> -T	Promega

## 4.8 Antibodies

mouse anti-His-tagged-protein	Calbiochem
sheep anti-mouse-Ig-AP	Millipore

## 4.9 DNA and protein ladders

GeneRuler™ DNA Ladder Mix (100-10.000 bp)	Thermo Scientific
SeeBlue <sup>®</sup> Plus2 Pre-Stained Standard (4-250 kDa)	Invitrogen

## 4.10 Oligonucleotids (Primer)

**Table 6: Oligonucleotids (Primer)**

description	identifier	sequence (5'-3')	T <sub>m</sub> [°C]
<b>standard primers</b>			
Eubacteria	Eub_27F	AGRGTTTGATCMTGGCTCAG	57,3
	Eub_1492R	GGHTACCTTGTTACGACTT	53,1
eGFP	GFP_F(625)	CGAAAGATCCCAACGAAAAGA	55,9
Hydra actin terminator	HAT_R(2342)	GGACGTCTTTTATATTACAGC	54,0
pGEM <sup>®</sup> -T	Sp6	CTATTTAGGTGACACTATAG	51,2
pGEM <sup>®</sup> -T	T7	TAATACGACTCACTATAGGG	53,2

**primers for the generation of expression constructs**

paraoxonase hyPON	SF_paroax_F	GTGGGAGTTCAGTTTTATAATGTC	57,6
	SF_paroax_R	TTATCTGTGCGGCAGTAAACC	57,9
	SF_paroax_F_Nco	ATATACCATGGctGTGGGAGTTCAG	63,0
	SF_paroax_R_Xho	TAATGCTCGAGGATATTATCTGTGCGG	63,4
	SF_PON_F_s_Asi	ACAAAGCGATCGCGTAATGGGAGTTCA GTTTTATAATG	68,4
	SF_PON_R_s_S_E	TGTGAATTCATTCGTACGGTAAACTATA TTAGATGTGGGCA	68,4
	SF_PON_F_as_Spl	TTTACCGTACGGACTAAAACACTGGG TAATATACTGTTACT	68,5
	SF_PON_R_as_Eco	ATTGTGAATTCTGGGAGTTCAGTTTTAT AATGTCTATCCTGG	68,5
AHL-synthase curl1	Ahl_syn1_NdeI_F	ATATACATATGCATTGCGTTGCTGG	59,7
	Ahl_syn1_Xho_R	TATTACTCGAGTTGCCACACCTC	60,6
AHL-synthase curl2	Ahl_syn2_NdeI_F	ATATACATATGGGGAATTTTCGTGGA	58,1
	Ahl_syn2_Xho_R	TAATACTCGAGTGCCAAGCAGTC	60,6
AHL-receptor curR1	AEP1.3_luxR1_F	CTGATTTCTTTGTAACGCACG	55,9
	AEP1.3_luxR1_R	GCGTCAGGCCCATCG	56
	AEP1.3_luxR1_sR	TGTAATCCAGAGATAGCGG	55,3
	CPluxR1/401_F	ATGAATTCCTGATTTCTTTGTAACGC	58,5
	CPluxR1/401_stpR	TAGAATTCATGCGTCAGGCC	57,3
AHL-receptor curR2	AEP1.3_luxR2_F	CCGCACTTCTTGAGACTCG	58,8
	AEP1.3_luxR2_R	GTTTCAATGCCGTGCTCGC	58,8

			Material
	AEP1.3_luxR2_sF	TGACCAGCAAACGGGTGG	58,2
	CPluxR2/401_F	ATGAATTCCCGCACTTCTTGAG	58,4
	CPluxR2/401_stpR	TAGAATTCATGTTTCAATGCCGTG	57,6
<b>primers for qRT-PCR</b>			
Hydra actin	SF_actin_RT_F	GAATCAGCTGGTATCCATGAAAC	58,9
	SF_actin_RT_R	AACATTGTCGTACCACCTGATAG	58,9
Hydra elongation factor	EF1a_F_qRT	GCAGTACTGGTGAGTTTGAAG	57,9
	EF1a_R_qRT	CTTCGCTGTATGGTGGTTCAG	59,8
paraoxonase hyPON	SF_paraox_RT_F2	GGCAGTAAGAACTTAACTCTCA	57,1
	SF_paraox_RT_R2	GCGGCAGTAAACCAATGTGTTA	58,4
Curvibacter recombinase A (recA)	CP_recA_qRT_F	TTCGGCAAGGGCACCATC	58,2
	CP_recA_qRT_R	ACGACTCCGGGCCATAGA	58,2
Curvibacter isocitrate lyase (aceA)	CP_aceA_qRT_F	CGCATTCCGGTGCATCTC	58,2
	CP_aceA_qRT_R	ACCGAGTCGTAGGCGTAC	58,2
AHL-synthase curl1	Ahl_syn1_qRT_F	TTGTTAGAGAAAGTATTTCCATCGC	58,1
	Ahl_syn1_qRT_R	GCTGCAATCCGAACCACC	58,2
AHL-synthase curl2	Ahl_syn2_qRT_F	GCATCTGAAATCATTGGATTAGG	57,1
	Ahl_syn2_qRT_R	TTGGCATTGAAGTCCACTGC	57,3

All primers were ordered from the company Eurofins MWG Operon.

## 4.11 Devices

### 4.11.1 Centrifuges

Centrifuge 5424	Eppendorf
Centrifuge 5415 D	Eppendorf
Centrifuge 5417 R (refrigerated centrifuge)	Eppendorf
Mini Spin	Eppendorf
Multifuge 3 S-R	Heraeus Instruments
Multi-Spin MSC-6000	Kisker-Biotech
Sorvall RC 5B	Du Pont Instruments
Speed Vac® Plus	Savant
Universal Vacuum System Plus	Savant

### 4.11.2 Devices for gel electrophoresis

PerfectBlue™ Twin L	Peqlab
Electrophoresis Power Supply Consort EV 231	peqLab
Electrophoresis Power Supply EPS 500/400	Pharmacia Fine Chemicals
SE250 PAGE gel chamber	Hoefer Scientific Instr.
Separation system B1A	Owl Separation Systems
Separation system B2	Owl Separation Systems
Separation system D3	peqLab

### 4.11.3 Devices for microinjection

CellTram air pump	Eppendorf
CellTram vario pump	Eppendorf
Micromanipulator	Leitz
Micromanipulator 5171	Eppendorf
Vertical Pipette Puller 700 C	Kopf Instruments

#### 4.11.4 Electroporation devices

Gene Pulser II	Biorad
Pulse Controller Plus	Biorad
Capacitance Extender Plus	Biorad

#### 4.11.5 Incubators / shakers

Certomat <sup>®</sup> R (shaker)	B. Braun
Certomat <sup>®</sup> H (incubator)	B. Braun
KS 4000 ic control (incubator)	IKA
KS10 (shaker)	Edmund Buhler
RC 2 basic (cooler)	IKA
REAX 2000 (vortex)	Heidolph
RM 5 (rolling mixer)	Karl Hecht
Thermo-Inkubator	Heraeus Instruments
Thermomixer compact	Eppendorf
VF2 (vortex)	Jankel and Kunkel
Vortex Genie 2	Scientific Industries

#### 4.11.6 PCR thermal cyclers

Primus 25	MWG-Biotech
Primus 96 <i>advanced</i>	peqLab
Primus 96 <i>plus</i>	MWG-Biotech
Real-Time Cyclers 7300	Applied Biosystems
Robocycler Gradient 96	Statagene

#### 4.11.7 Photometers

BioPhotometer	Eppendorf
NanoDrop ND-1000	Eppendorf
Tecan GENios Plus	Tecan

#### 4.11.8 UV-devices

Molecular Imager® Gel Doc™ XR+	Bio-Rad
UV-bulb Chroma 43	Vetter GmbH
UV-Stratalinker 1800	Stratagene

#### 4.11.9 Miscellaneous devices

1205 MP balance	Sartorius
Freezer -20°C	Liebherr
Freezer -80°C	Forma Scientific
HI9321 (pH-Meter)	Hanna Instruments
Ice machine Scotsman AF 10	Mathner Kalte-Klima
IKA-Combimag RCT (magnetic stirrer)	Jankel and Kunkel
IKA-Combimag RET (magnetic stirrer)	Jankel and Kunkel
Julabo 5B (water bath)	Eydam
Julabo MB (water bath)	Eydam
Kern 770 (balance)	Kern
LaminAir® HB 2448 (cleanbench)	Heraeus Instruments
Milli-Q Academic System	Millipore
pH 211 (pH-Meter)	Hanna Instruments
Refrigerator	Liebherr
Rotavapor R-114 (rotating evaporator)	Büchi
SZX 16 (Binocular)	Olympus
Transmission electron microscope EM 208 S	Philips
Vakuum-Controller CVC 24	vacuubrand
VARIOKLAV Typ 400 (steam-sterilizer)	EV H+P Labortechnik GmbH

## 4.12 Webpages

AntiSMASH	<a href="http://antismash.secondarymetabolites.org/">http://antismash.secondarymetabolites.org/</a>
BLAST	<a href="http://blast.ncbi.nlm.nih.gov/Blast.cgi">http://blast.ncbi.nlm.nih.gov/Blast.cgi</a>
Compagen	<a href="http://compagen.zoologie.uni-kiel.de/">http://compagen.zoologie.uni-kiel.de/</a>
ExPASy	<a href="http://au.expasy.org/">http://au.expasy.org/</a>
MWG	<a href="http://www.eurofindna.com/de/home.html">http://www.eurofindna.com/de/home.html</a>
NCBI	<a href="http://www.ncbi.nlm.nih.gov">http://www.ncbi.nlm.nih.gov</a>
Ribosomal Database Project (RDP)	<a href="http://rdp.cme.msu.edu/">http://rdp.cme.msu.edu/</a>
SignalP	<a href="http://www.cbs.dtu.dk/services/SignalP/">http://www.cbs.dtu.dk/services/SignalP/</a>
SMART	<a href="http://smart.embl-heidelberg.de/">http://smart.embl-heidelberg.de/</a>
Translatorx	<a href="http://translatorx.co.uk/">http://translatorx.co.uk/</a>
Unifrac	<a href="http://bmf2.colorado.edu/unifrac/index.psp">http://bmf2.colorado.edu/unifrac/index.psp</a>
UniProt	<a href="http://www.uniprot.org/">http://www.uniprot.org/</a>

## 4.13 Computer programs

Image editing	Adobe Photoshop CS4 ImageJ
Illustration	Adobe Illustrator CS4 Microsoft Powerpoint 2010
Microscopy	CELL*
Sequence analysis	BioEdit DNAMAN 4.15 MEGA 5

## 5 Methods

### 5.1 Cultivation of organisms

#### 5.1.1 Rearing of Hydra polyps

Experiments were carried out using *Hydra vulgaris* (AEP) (Hemrich et al., 2007), *Hydra viridis* (strain A99) and *Hydra oligactis* (strain St. Petersburg). All laboratory-cultured strains are available at the University of Kiel. The animals were cultured under constant environmental conditions, including culture medium (S-medium: 0.29 mM CaCl<sub>2</sub> x 2 H<sub>2</sub>O, 0.33 mM MgSO<sub>4</sub> x 7 H<sub>2</sub>O, 0.5 mM NaHCO<sub>3</sub>, 0.08 mM K<sub>2</sub>CO<sub>3</sub>), temperature (18°C), light (12 h/12 h light/dark rhythm) and food (naupli of *Artemia salina*, fed three times a week), after standard protocol (Lenhoff and Brown, 1970).

#### 5.1.2 Cultivation of *Artemia salina*

Freshly hatched nauplii of the aquatic crustacean *Artemia salina* were used to feed Hydra polyps. For the breeding two teaspoons of *Artemia salina* cysts were incubated for 24 hours in 500 ml Artemia medium at 30°C. After hatching, the larvae were kept at room-temperature with permanent aeration. Before the Artemia could be used up as prey for Hydra polyps, they were washed with water to remove salts and transferred into S-medium.

#### 5.1.3 Generation of germfree Hydra polyps

For the generation of germfree Hydra polyps, up to 50 polyps were transferred in a 50 ml sterile plastic tube, 40 ml of sterile S-medium with 50 µg/ml of five different antibiotics (Amp, Neo, Rif, Sp, Strep) were added and air tight sealed. The polyps incubated for ten days at 18°C under light protected conditions. After three, five and seven days the vessel and the sterile S-medium with antibiotics were exchanged. After 10 days the polyps were transferred in a sterile 50 ml tube, washed with sterile S-medium and incubated for 24 h in 50 ml sterile S-medium. As a control, from every 50 ml batch one polyp were homogenized with a pestle in 100 µl sterile S-medium and plated on a R2A plate. Absence of colony forming units (CFUs), on the plates after incubation for three days at 18°C, indicated a successful antibiotic treatment. The remaining animals were directly conducted to the experiments.



### 5.1.4 Cultivation of Hydra-associated bacteria

Commensal bacteria of *Hydra vulgaris* (AEP) were cultivated and identified previously (Table 7) (Fraune et al., 2014). For isolation of environmental bacteria of Hydra, the biofilm of a culture dish of *Hydra vulgaris* (AEP) was plated on R2A plates. A fragment of the 16S-rDNA of the bacterial colonies was amplified by colony-check PCR and used for sequencing to identify the environmental bacteria (Table 7) (chapter 0 and 5.16.1). By comparison with sequencing data for the bacterial colonization during ontogeny and for the different Hydra species (Franzenburg et al., 2013a, 2013b), it could be excluded that these strains are commensals of *Hydra vulgaris* (AEP).

Hydra-associated bacteria were cultivated for three days at 18°C and 225 rpm in 5 ml R2A broth (LabM). They were diluted 1:10 in R2A and after an additional growth for 10 to 16 h,  $1 \times 10^5$  colony forming units (CFUs) per ml of each bacterium (Table 7) was inoculated in 50 ml R2A. The cultures incubated at 18°C and 225 rpm. The incubation time varies between the experiments. The colony-forming units (CFUs) per milliliter at an optical density of  $OD_{600nm} = 0.1$  were determined for the bacteria by plating 100  $\mu$ l of dilution series on R2A plates and counting the bacterial colonies after 3 days incubation at 18°C (Table 7).

**Table 7: Commensal and environmental bacterial strains cultivated from Hydra's neighborhood**

Bacterium	Class	Order	Family	Clone	CFUs/ml <sup>a</sup>
<b>commensal bacteria<sup>b</sup></b>					
<i>Curvibacter</i> sp.	Betaproteobacteria	Burkholderiales	Comamonadaceae	AEP1.3	$1.0 \times 10^8$
<i>Acidovorax</i> sp.	Betaproteobacteria	Burkholderiales	Comamonadaceae	AEP1.4	$9.0 \times 10^7$
<i>Pelomonas</i> sp.	Betaproteobacteria	Burkholderiales	Comamonadaceae	AEP2.2	$9.0 \times 10^7$
<i>Undibacterium</i> sp.	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	C1.1	$2.3 \times 10^7$
<i>Duganella</i> sp.	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	C1.2	$2.0 \times 10^7$
<i>Pseudomonas</i> sp.	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	C2.2	$7.0 \times 10^7$
<b>environmental bacteria</b>					
<i>Flectobacillus</i> sp.	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Biofilm 1	$1.8 \times 10^7$
<i>Pseudomonas</i> sp.	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Biofilm 3	$1.3 \times 10^8$
<i>Acinetobacter</i> sp.	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Biofilm 4	$1.2 \times 10^9$
<i>Chryseobacterium</i> sp.	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Biofilm 5	$7.0 \times 10^8$
<i>Chryseobacterium</i> sp.	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Biofilm 8	$7.4 \times 10^8$
<i>Variovorax</i> sp.	Betaproteobacteria	Burkholderiales	Comamonadaceae	Biofilm 10	$1.0 \times 10^9$

<sup>a</sup>colony-forming units (CFUs) per ml at  $OD_{600nm} = 0.1$

<sup>b</sup>bacterial strains were cultivated previously (Fraune et al., 2014).

### 5.1.5 Monoculture and double culture experiments

To determine the growth rate in monocultures, each bacterium was inoculated in 50 ml R2A medium with 10 concentrations between  $2.0 \times 10^3$  and  $1.0 \times 10^5$  CFU/ml of *Curvibacter* sp. (C) or *Duganella* sp. (D) of an overnight culture. The cell numbers of each bacteria were estimated by counting the colony forming units (CFUs) and were crosschecked with optical density (OD) measurements at  $OD_{600} = 0.1$  ( $1.0 \times 10^8$  CFU/ml for C and  $2.0 \times 10^7$  CFU/ml for D). In double culture experiments, a total initial concentration of  $1.0 \times 10^5$  CFU/ml was kept and applied a gradient of different initial frequencies of species C and D. Over the course of 3 days, three times a day, the  $OD_{600}$  of the cultures was measured and two dilutions, which were adjusted individually to the OD, were plated on R2A agar plates. After 2 days, the number of colonies of D and after 4 days those of C were counted. This difference in counting times was due to different growth rates of the two bacteria on agar plates.

### 5.1.6 Cultivation of standard bacterial strains

*Escherichia coli* was grown at 37°C in Luria-Bertani broth (LB) with the appropriate antibiotic. *E. coli* DH5 $\alpha$  (Invitrogen) were used for standard cloning. Heterologous overexpression were executed with *E. coli* strain Rosetta 2 (DE3) pLysS (Novagen), its plasmid was selected with 34  $\mu$ g/ml chloramphenicol. The plasmid pET22b (Merck Millipore) was used to express curl1 and curl2, by addition of 100  $\mu$ g/ml ampicillin the plasmids were selected. The hyPON, introduced into pET28a(+) vector (Novagen), was selected with 50  $\mu$ g/ml kanamycin.

### 5.1.7 Cultivation of bacterial AHL-reporter strains

The AHL-bioreporters *Escherichia coli* JM109 pSB401, pSB536 and pSB1075 strains were grown under continuously shaking (250 rpm) at 37°C in Luria-Bertani broth (LB) supplemented with the corresponding antibiotic (10  $\mu$ g/ml tetracycline (pSB401 and pSB1075) or 100  $\mu$ g/ml ampicillin (pSB401)). The *Agrobacterium tumefaciens* NTL4 (pCF218)(pCF372) AHL-reporter strain was grown at 29°C and shaking (225 rpm) in different media supplemented with 60  $\mu$ g/ml spectinomycin and 5  $\mu$ g/ml tetracycline. An overnight culture of the reporter strain in LB was used to inoculate AT medium containing 0.5% glucose (Krysciak et al., 2014; Tempé et al., 1977).

## 5.2 Separation of endoderm and ectoderm by procaine treatment

A modified protocol after Epp et al. and Bode et al. for the separation of endoderm and ectoderm of Hydra was used (Bode et al., 1987; Epp et al., 1979). Head and foot of *Hydra vulgaris* (AEP) polyps were removed with a scalpel. The trimmed polyp incubated at 4°C for 5 min in solution A and 1.5 min in solution B. The tissue was transferred into 18°C warm dissociation medium. The ectodermal tissue layer coil up, which led to the formation of a ring (ectoderm) and a rod (endoderm) shaped structure. Both tissue layers could be separated with forceps.

## 5.3 Transplantation of endoderm and ectoderm

For the transplantation of an endoderm and an ectoderm of two different transgenic Hydra lines, first their tissues were separated by procaine treatment (chapter 0). While still laying into 18°C dissociation medium, the rod shaped endoderm was introduced with forceps into the ring shaped ectoderm. Afterwards, the dissociation medium has been gradually decreased in the S-medium. The transplant was kept for 4 h in 75% dissociation medium and then transferred into 50% dissociation medium, where it incubated for 16 h. The now rounded transplant incubated first for 4 h in 25% dissociation medium, then 4 h in 10% dissociation medium and finally the dissociation medium was reduced to 5%. After three days an intact Hydra polyp with a transgenic endo- and ectoderm was grown out of the transplant and transferred into S-medium.

## 5.4 Isolation of RNA and cDNA synthesis

Dependent on the raw material different kits were used for the RNA isolation and the synthesis of cDNA.

### 5.4.1 Isolation of total RNA out of Hydra polyps and cDNA synthesis

Fifteen Hydra polyps, if not indicated differently, were applied to the total RNA isolation after the protocol of the TRIzol<sup>®</sup> Reagent with the PureLink<sup>®</sup> RNA Mini Kit (Invitrogen) combined with the on-column PureLink<sup>®</sup> DNase (Invitrogen) Treatment. The First Strand cDNA Synthesis Kit (Thermo Scientific) was used to synthesize cDNA out of 500 ng total RNA with Oligo (dT)18 Primer according to the manufacturer's protocol. A 1:20 dilution of cDNA was used for the experiments.

### 5.4.2 Isolation of total RNA out of bacterial cells

For the isolation of total RNA out of bacterial cells, first two volumes of RNeasy Protect<sup>®</sup> Bacteria Reagent (Qiagen) was added to  $1 \times 10^8$  colony forming units (CFU) per milliliter bacterial cells, if not indicated differently. The RNA was isolated after the manufacturer's protocol of the RNeasy Mini Kit (Qiagen). The CFU/ml of the cultures was calculated based on the value for an OD<sub>600</sub> of 0.1 (Table 7).

### 5.4.3 Isolation of total RNA out of Hydra colonizing bacteria and cDNA synthesis

For the bacterial isolation, 1200 *Hydra vulgaris* (AEP) polyps, washed three times with sterile S-medium, incubated for 3 min in 3 ml sterile PBS and were inverted every minute. Afterwards the supernatant was taken and an aliquot, equivalent to 1/10 of a polyp, was plated on R2A plates to determine the colony forming units per milliliter (CFU/ml). Additionally, two volumes of RNeasy Protect<sup>®</sup> Bacteria Reagent (Qiagen) was added to an aliquot of the bacterial fraction and after determination of the CFU/ml, total RNA was isolated out of  $5 \times 10^8$  CFU (chapter 5.4.2). The cDNA synthesis was performed after the manufacturer's protocol of the QuantiTect<sup>®</sup> Reverse Transcription Kit (Qiagen). The maximal amount of RNA was applied.

## 5.5 Isolation of bacterial genomic DNA

Genomic DNA was isolated out of a liquid culture of Hydra associated bacteria after the protocol for Gram-negative bacteria of the DNeasy Blood & Tissue Kit (Qiagen).

## 5.6 Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) is used for the exponential amplification of DNA. In general, the PCR is structured into three steps: denaturation, annealing and elongation. In the denaturation step, the PCR reaction gets heated up to 95°C that the two strands of the template-DNA get separated. In the following the oligonucleotides hybridize with the single-stranded template-DNA at a primer specific temperature (annealing). The last step is for the binding of the DNA polymerase and the synthesis of the complementary strand (elongation). One PCR-cycle leads to the duplication of the template-DNA.

### 5.6.1 Standard PCR

A standard PCR is the simplest application of the principle of a polymerase-chain-reaction (PCR) for the amplification of a DNA fragment. It was applied with the conditions indicated under Table 8 and Table 9. The GoTaq<sup>®</sup> DNA polymerase from Promega was used, if not indicated differently.

**Table 8: Reaction for a standard PCR**

component	volume	final concentration
GoTaq <sup>®</sup> Reaction Buffer (5x)	2,00 µl	1x
dNTP-Mix (10 mM)	0,20 µl	0,2 mM
upstream primer (10 µM)	1,00 µl	1,0 µM
downstream primer (10 µM)	1,00 µl	1,0 µM
GoTaq <sup>®</sup> DNA Polymerase (5 U/µl)	0,05 µl	0,25 U
template DNA	X µl	~100 ng
Millipore H <sub>2</sub> O	5,75 µl - X µl	
total volume	10,00 µl	

Dependent on the concentration of the template DNA, the total volume varied between 10 µl and 25 µl. Indicated is an exemplary 10 µl reaction.

**Table 9: Thermal cycling conditions of a standard PCR**

step	temperature	time	number of cycles
initial denaturation	95°C	2 min	1
denaturation	95°C	30 sec	
annealing	T <sub>m</sub> -1	30 sec	30 - 40
elongation	72°C	1 min/kb	
final elongation	72°C	5 min	1
soak	8°C	∞	1

The theoretical melting temperature of the primers is indicated with T<sub>m</sub> (chapter 4.10). The time of elongation was calculated with the assumption, that the DNA polymerase synthesizes 1 kb per minute.

### 5.6.2 Colony-check PCR

Colony-check PCR was mainly used to check if bacterial colonies, resulting from a transformation, comprise the plasmid with the inserted fragment of interest. Therefore the bacterial colony was picked from plate with a tip and streaked on another plate for further usage before the bacterial cells serve as template for the standard PCR instead of DNA (Table 8). The cycling conditions were used from a standard PCR with forty cycles for the amplification (Table 9).

### 5.6.3 Insertion of restriction sides by PCR

Polymerase-chain-reaction (PCR) can also be used to add restriction sides or overhangs to DNA fragments. For the adding of a cutting side, one has to create a primer, which binds partially to the template DNA and the overhang contains the sequence of the restriction side. In the beginning the primer is only able to bind incompletely to the DNA, as a consequence the first ten cycles have a lower annealing temperature ( $T_{m1}$ ), which is calculated based on the hybridizing nucleotides. After the third cycle, the PCR reaction contains DNA fragments, which can be bound completely by the primer, consequently the last 30 cycles have the melting temperature of the complete primer ( $T_{m2}$ ) (Table 11). For DNA synthesis, a DNA polymerase with proof reading, like the Platinum<sup>®</sup> Taq DNA polymerase High Fidelity from Invitrogen, was use (Table 10).

**Table 10: Reaction for a PCR to insert restriction sides**

component	volume	final concentration
High Fidelity PCR Buffer (10x)	5,00 $\mu$ l	1x
dNTP-Mix (10 mM)	1,00 $\mu$ l	0,2 mM
MgSO <sub>4</sub> (50 mM)	2,00 $\mu$ l	2 mM
upstream primer (10 $\mu$ M)	5,00 $\mu$ l	1,0 $\mu$ M
downstream primer (10 $\mu$ M)	5,00 $\mu$ l	1,0 $\mu$ M
Platinum Taq High Fidelity (5 U/ $\mu$ l)	0,25 $\mu$ l	0,25 U
template DNA	5,00 $\mu$ l	~100 ng
Millipore H <sub>2</sub> O	24,50 $\mu$ l	
total volume	50,00 $\mu$ l	

**Table 11: Thermal cycling conditions for the insertion of restriction sites by PCR**

step	temperature	time	number of cycles
initial denaturation	94°C	2 min	1
denaturation	94°C	30 sec	
annealing	$T_{m1}-1$	30 sec	10
elongation	68°C	1 min/kb	
denaturation	94°C	30 sec	
annealing	$T_{m2}-1$	30 sec	30
elongation	68°C	1 min/kb	
final elongation	68°C	5 min	1
soak	8°C	$\infty$	1

The theoretical melting temperature of the primers is indicated with  $T_m$  (chapter 2.10), while  $T_{m1}$  is the temperature for the partially binding primer and  $T_{m2}$  is the temperature for the complete sequence of the primer. The time of elongation was calculated with the assumption, that the DNA polymerase synthesizes 1 kb per minute

#### 5.6.4 quantitative Real-Time PCR

The quantitative Real-Time PCR (qRT-PCR) is a method to amplify nucleic acids on the principle of a polymerase chain reaction (PCR, chapter 5.6) with the additional quantification of DNA. The quantification can be achieved by fluorescence measurement during a PCR cycle. The fluorescence is proportional to the amount of the PCR product, because the fluorescent dye SYBR-Green binds to the newly generated double stranded DNA. Due to the additional measurement of a reference gene the probes can be relatively quantified. The qRT-PCR is used for the determination of the relative expression level of a gene in different samples. Therefore, cDNA is needed as raw material, which was synthesized out of total RNA (chapter 5.4). The qRT-PCR was performed after the manufacturer's protocol of the GoTaq qPCR Master Mix kit (Promega, Madison, USA). The real-time PCR cycler 7300 of Applied Biosystems (ABI, Foster City, USA) was used for measurement. The comparative  $\Delta\Delta C_T$  method was applied for the relative quantification of transcripts (Scheffe et al., 2006) (primer can be found in Table 6).

## 5.7 Agarose gel electrophoresis

The agarose gel electrophoresis is a standard method for the separation of DNA. Its principle is based on the size dependent migration kinetics of charged molecules in an electric field. DNA is negatively charged, consequently it migrates from the cathode to the anode. For the determination of the size the standardized DNA ladder GeneRuler™ DNA Ladder Mix (Thermo Scientific, Rochester, USA) was also separated. Depending on the size of the nucleic acids, different percent agarose gels (0.8 % – 1.2 %) were used. The appropriate amount of agarose was dissolved in 1x TAE buffer by heating and after short cooling 1:20,000 peqGREEN (Peqlab) was added. The gel was poured into horizontal gel chambers. After the polymerization of the gel, the chamber was filled with 1x TAE buffer, which served as running buffer of the gel electrophoresis and electrically connects anode and cathode. The samples, added with 1x DNA loading dye, and the standard DNA ladder were filled into the gel pockets. The electrophoretic separation in the gel was performed at 3-5 V/cm<sup>2</sup> (direct-current voltage). The DNA fragments were documented with the Molecular Imager® Gel Doc™ XR+ (Bio-Rad); the excitation with UV light leads to the emission of fluorescence (530 nm) by the peqGREEN intercalated into the DNA.

## 5.8 Extraction of DNA fragments out of agarose gels

The extraction of DNA fragments was performed according to the manufacturers' instructions of the NucleoSpin® Gel and PCR clean-up kit (Macherey-Nagel, Düren, Germany).

## 5.9 Restriction digest

Endonucleases recognize specific sequences of four to eight base pairs in the DNA double helix. They cut both DNA strands at these specific, palindromic sequences; according to restriction enzyme this leads to blunt or sticky ends. All restriction enzymes were ordered from Thermo Scientific (Rochester, USA) or New England Biolabs (chapter 4.6.2). Incubation and inactivation of the restriction enzymes were performed after manufacturers' instructions. A standard reaction of a restriction digest is indicated in Table 12.



**Table 12: Reaction for a standard restriction**

component	volume		final concentration
	approach 1	approach 2	
vector	X $\mu$ l	/	2 $\mu$ g
DNA fragment for insertion*	/	X $\mu$ l	
reaction buffer (10x)	4,0 $\mu$ l	6,0 $\mu$ l	1x
restriction enzyme 1 (10 u/ $\mu$ l)	1,5 $\mu$ l	1,5 $\mu$ l	15 U
restriction enzyme 2 (10 u/ $\mu$ l)	1,5 $\mu$ l	1,5 $\mu$ l	15 U
Millipore H <sub>2</sub> O	40,0 $\mu$ l - X $\mu$ l	60,0 $\mu$ l - X $\mu$ l	
total volume	40,0 $\mu$ l	60,0 $\mu$ l	

\*: The DNA fragment was previously isolated out of a 50  $\mu$ l PCR reaction.

## 5.10 Ligation

Dependent on the application different ligations methods were used.

### 5.10.1 Vector-insert ligation

A vector and a DNA fragment, which were cut before like described in chapter 5.9, were applied in a molar vector:insert ratio of 1:3 to the ligation. The T4 DNA ligase from New England Biolabs ligated the fragments while incubating over night at 4°C. A standard ligation reaction is shown in Table 13. The reaction was inactivated by incubating for 20 min at 70°C.

**Table 13: reaction of a standard ligation**

component	volume
T4 ligations buffer (10x) (NEB)	1,0 $\mu$ l
T4 DNA ligase (NEB)	1,0 $\mu$ l
vector	X $\mu$ l
insert	Y $\mu$ l
Millipore H <sub>2</sub> O	8,0 $\mu$ l - (X+Y)
total volume	10,0 $\mu$ l

The volume for X and Y varies, dependent on the molar vector:insert ratio of 1:3.

### 5.10.2 Ligation into the pGEM<sup>®</sup>-T vector

The pGEM<sup>®</sup>-T vector belongs to the pGEM<sup>®</sup>-T Vector System from Promega (Promega, Madison, USA). The advantage is, that PCR products can be directly ligated into the vector without a previous restriction. The plasmid is already cut and has a thymidine overhang at his 5' ends. The *Taq* DNA polymerase has an intrinsic polyadenylation activity, which leads to the adding of adenosine nucleotides to the ends of the amplified DNA fragment. The T4 DNA ligase hybridizes the thymidine overhangs of the vector with the adenosine ends of the insert. The used ligation reaction is described in Table 14. The ligation incubated over night at 4°C. The reaction was inactivated by incubation for 20 min at 70°C.

**Table 14: Reaction for the ligation into the pGEM<sup>®</sup>-T vector**

component	volume
rapid ligation buffer (2x)	2,5 µl
pGEM <sup>®</sup> -T vector (50 ng/µl)	0,5 µl
T4 DNA ligase (3 U/µl)	0,5 µl
PCR product	1,5 µl
total volume	5,0 µl

## 5.11 Dialysis

For some method, for example electroporation, it is necessary that the plasmid DNA is embedded only in a low salt concentration. To reduce the salt concentration DNA dialyzed for one hour against Millipore water; the filter for dialysis Type VS 0.025 µm from the company Millipore was used.

## 5.12 Production of competent cells

Only competent cells are able to take up DNA through their membrane, which is necessary for transformation. Some bacterial strains possesses a natural competence, other ones has to get prepared for it. Electro and chemically competent *E. coli* cells were used.

### 5.12.1 Production of electro competent cells

For the production of electro competent cells *E. coli* DH5α cells from Invitrogen were used. The cells were inoculated in 20 ml SOB medium and cultivated over night at 37°C and 220 rpm. From the starter culture, 6 ml was transferred into 450 ml SOB medium and incubated at 37°C and 220 rpm till it reached the logarithmic growth phase with an optical

density ( $OD_{600}$ ) of 0.6 to 0.8. The cells were pelleted by centrifugation at 3,600 g and 4°C for 15 min. The pellet was washed three times with 50 ml ice cold, sterile water, followed by one washing step with an ice cold 15 % (v/v) glycerin solution. By this salts should be removed to avoid a short circuit in the electroporation. After a repeated centrifugation the cell pellet was resuspended with 3.6 ml 15 % (v/v) glycerin solution, spitted into 40  $\mu$ l aliquots, froze into liquid nitrogen and stored at -80°C.

### 5.12.2 Chemically competent cells

The chemically competent *E. coli* Rosetta 2 (DE3) pLysS cells from Novagen were used.

## 5.13 Transformation of *Escherichia coli*

Transformation is the insertion of free, soluble DNA into a bacterial cell. Only competent bacteria are able to take up DNA through their membrane. Electro and chemically competent *E. coli* cells were used (chapter 5.12).

### 5.13.1 Transformation of *Escherichia coli* by electroporation

In the process of an electroporation an electric impulse is used to temporarily make the membrane of electro competent cells permeable, which leads to the diffusion of DNA into the bacterial cell. Therefore 1  $\mu$ l - 4  $\mu$ l of a dialyzed plasmid DNA (chapter 5.11), resulting from a ligation (chapter 5.10), or less than 1 ng/ $\mu$ l of pure plasmid DNA were added to 40  $\mu$ l electro competent *E. coli* DH5 $\alpha$  cells (chapter 5.12.1). This mix was added to a prechilled, sterile cuvette for electroporation with a gap width of 1 mm. The devices *Gene Pulser II* and *Pulse Controller II* from Biorad were used for the electroporation. Thereby an electric impulse with a capacity of 25  $\mu$ F, a parallel resistance of 200  $\Omega$  and a voltage of 1.8 kV were applied for ca. five seconds. Afterwards the cells were resuspended with 1 ml preheated SOC medium and incubated for 1 hour at 37°C on a shaker. For the screening 50  $\mu$ l and up to 500  $\mu$ l of the bacterial suspension were plated on a LB agar plate with appropriate antibiotics and incubated overnight at 37°C.

### 5.13.2 Transformation of *Escherichia coli* by heat shock

1 µl plasmid DNA (1-400 ng) was added to 25 µl competent *E. coli* Rosetta 2 (DE3) pLysS cells (chapter 5.12.2). They incubated for 15 min on ice and were then heat shocked by incubation for 30 s at 42°C in a water bath, followed by an additional incubation on ice for 2 min. The cells were resuspended with 500 µl pre-warmed SOC medium and incubated for 1 hour, while continuously shaking, at 37°C. Afterwards, 50 µl and up to 500 µl of the culture were plated on LB plates with the corresponding antibiotic. They incubated overnight at 37°C.

## 5.14 Isolation of plasmid DNA

The method to isolate plasmid DNA was chosen dependent on the amounts of plasmid DNA, which is needed for a specific application.

### 5.14.1 Mini-plasmid preparation

Plasmid DNA was isolated out of a 5-10 ml bacterial overnight culture according to the manufacturer's protocol of the NucleoSpin® Plasmid Quick Pure kit (Macherey & Nagel, Düren, Germany). The DNA was eluted with 50 µl *NE Buffer*.

### 5.14.2 Midi-plasmid preparation

For the preparation of around 2 µg/µl plasmid DNA the QIAfilter Plasmid Midi kit (Qiagen) was used. A 100 ml bacterial overnight culture was applied to the plasmid DNA isolation after manufacturer's introduction. The DNA was resuspended with 100 µl Millipore water. To increase the purity, the DNA was precipitated by adding 1/10 volume 3 M sodium acetate and 2.5 volumes of 100% ethanol followed by an incubation for one hour at -20°C. After centrifugation for 30 min at 15000 g and 4°C, the pellet was washed with 1.5 ml 70% ethanol, followed by an extra centrifugation step and then air-dried. The DNA was resuspended with 50 µl Millipore water overnight at 4°C.

## 5.15 Measurement of nucleic acid concentration

The nucleic acid concentration was determined with the spectrophotometer NanoDrop ND-1000. For the measurement 2.5 µl of a RNA or DNA probe was applied.

## 5.16 Sequencing technics

### 5.16.1 DNA sequencing

Sanger sequencing of plasmid DNA was performed in cooperation with the Institute of Clinical Molecular Biology of the CAU Kiel.

### 5.16.2 Transcriptomic sequencing with NextSeq (Illumina)

The transcriptomic sequencing was conducted in cooperation with the Max Planck Institute for Evolutionary Biology in Plön (Dr. Sven Künzel). The cDNA libraries for sequencing were constructed after manufacturer's protocol of the TrueSeq Stranded mRNA LT - RiboZero Kit (Illumina). The resulting cDNA libraries were sequenced using a NextSeq 500 machine (Illumina) in paired-ends mode and running 150 cycles for high output. The Illumina basecaller bcl2fastq2-v2.16.0.10 was used to convert the raw data into FASTQ format. The primary bioinformatics analysis was done by PhD Sylvaine Forêt from Research School of Biology at the Australian National University, Canberra. RNA-seq reads were mapped to the genome assembly of *Curvibacter* sp. strain AEP1.3 (unpublished) using Subreads (Liao et al., 2013). The number fragments per gene was calculated using FeatureCounts (Liao et al., 2014). Differential gene expression was assessed using edgeR (Robinson et al., 2009), modelling the treatments (solvent control, 3OC12, 3OHC12) as a single factor with three levels. Gene enrichment analysis was carried out with Gage (Luo et al., 2009) and GoSeq (Young et al., 2010).

## 5.17 Short term response of *Curvibacter* sp. to AHL addition

For the investigation of the short term response of *Curvibacter* sp. to 3-oxo- and 3-hydroxy-AHLs 500 ml R2A broth were inoculated with  $1 \times 10^5$  CFU/ml *Curvibacter* sp. of a fresh culture (chapter 5.1.4, Table 7) and incubated at 18°C and 200 rpm till an optical density ( $OD_{600}$ ) of 0.145 was reached. The culture was centrifuged for 15 min at 3600 g at 18°C and the cell pellet was resuspended with 2 volume of R2A medium to discard already produced AHLs. After an additional centrifugation the cells were resuspended with R2A medium till an  $OD_{600}$  of 0.1 was reached. The culture was split into 15 times 50 ml per 100 ml flask. To each five replicates 10  $\mu$ M 3OC12-HSL, 10  $\mu$ M 3OHC12-HSL or 1:2000 acidified ethyl acetate were added. The AHL solvent acidified ethyl acetate was diluted in all fifteen cultures 1:2000. The 15 cultures incubated for four hours at 18°C and 225 rpm, while every hour the  $OD_{600}$  was measured. After the incubation an aliquot of  $1 \times 10^8$  CFU was taken and used for the isolation of total RNA (chapter 5.4.2). RNA was applied to transcriptomic sequencing (chapter 5.16.2).

## 5.18 Generation of expression constructs

### 5.18.1 Cloning of the AHL-synthases *curl1* and *curl2* of *Curvibacter* sp.

The AHL-synthases from *Curvibacter* sp. (*curl1* and *curl2*) were cloned into the pET22b-vector. The *curl1* and *curl2* genes were amplified out of genomic DNA (chapter 5.5) of *Curvibacter* sp. and restriction sites *NdeI* and *XhoI* were fused to the fragment with primers (primers can be found in Table 6) (chapter 5.6.3). The restriction sites were used to clone the sequences of the AHL-synthases into the pET22b-vector (chapter 5.18.1). The plasmids were introduced into *E. coli* DH5 $\alpha$  cells by electroporation for amplification. The construct was checked by a colony-check PCR and sequencing (chapter 0 and 5.16). For heterologous expression the plasmids were transformed into *E. coli* Rosetta 2 (DE3) pLysS.

### 5.18.2 Cloning of the AHL-receptors *curR1* and *curR2* of *Curvibacter* sp.

The sequences of the AHL-receptors of *Curvibacter* sp. *curR1* and *curR2* were amplified out of genomic DNA of *Curvibacter* sp. (chapter 5.5) and cloned into the pGEM<sup>®</sup>-T-vector (chapter 0). For amplification the plasmid was inserted into *E. coli* DH5 $\alpha$  cells by electroporation (chapter 5.13.1). The construct was checked by a colony-check PCR and sequencing (chapter 0 and 5.16) (primer can be found in Table 6).

### 5.18.3 Cloning of the paraoxonase *hyPON* of *Hydra vulgaris* (AEP)

The paraoxonase sequence of *Hydra vulgaris* (AEP) (*hyPON*) was cloned into the pET28a(+)-vector. The *hyPON*-sequence was amplified out of cDNA of *Hydra vulgaris* (AEP) (chapter 5.4.1). Restriction sites for *NcoI* and *XhoI* were fused to the sequence by PCR (chapter 5.6.3) and used for cloning into the pET28a(+)-vector (chapter 5.9, 5.7, 5.8, 5.10.1 and 5.11). Electroporation was used to insert the plasmid into *E. coli* DH5 $\alpha$  cells for amplification. The construct was checked by a colony-check PCR and sequencing (chapter 0 and 5.16).

## 5.19 Construction of a reporter for *Curvibacter*'s AHL-receptors *CurR1* and *CurR2*

For the construction of a bacterial reporter for *Curvibacter* sp. AHL-receptors *CurR1* and *CurR2* the plasmid of the AHL-bioreporter pSB401 (Winson et al., 1998) was used as backbone. The *luxRI'* of *Vibrio fischeri* of the pSB401 were cut out with the endonuclease *EcoRI*. To create the *CurR1*- and *CurR2*-reporters (pCP*curR1*, pCP*curR2*) an *EcoRI*-

flanked fragment, consisting of the *curR* open reading frame (ORF) and the beginning of the AHL-synthase *curl* ORF (*curl'*), including the *curl*-promoter, was cloned in the cut pSB401 plasmid. A stop codon terminating the *curl'* was inserted with the used primers. For pCPcurR1, a 1986 bp fragment, ranging from -418 bp of the *curR1* to +152 of *curl1* (Figure 24A), and for the CurR2-reporter (pCPcurR2) a fragment of 1762 bp (-389 bp of the *curR2* to +140 of *curl2* (Figure 24B)), were introduced into the open pSB401. Both plasmids were introduced into *E. coli* DH5 $\alpha$  and could be selected by 10  $\mu$ g/ml tetracycline. The constructs were checked by sequencing (chapter 5.16) (primer can be found in Table 6).

## 5.20 SDS-PAGE and Western blot

For the analysis of a heterologous expression in *E. coli* culture, every hour an aliquot of 10  $\mu$ g protein was taken. The proteins were separated by a tris-glycin-polyacrylamide gel electrophoresis with a 12% separation gel and a 4% stacking gel (Laemmli 1970). Proteins were stained with Roti<sup>®</sup> Blue (Roth). The Semidry blot-System (PepLab) was used to transfer the proteins to a polyvinylidene fluoride (PVDF) membrane. The blotting was performed for 75 min with a current of 130 mA for one SDS-gel or 280 mA for two SDS-gels. Blocking of unspecific antibody bind sides was achieved by incubation in PBT with 3% BSA overnight. The immune detection was performed with a monoclonal mouse anti-His-Tagged Protein antibody (Calbiochem) and a polyclonal sheep-anti-mouse-Ig antibody with a coupled alkaline phosphatase (Millipore). The antibodies were diluted 1:2000 in PBT with 1.5% BSA and incubated for 1 h with the PVDF membrane. For staining the PVDF membrane incubated with NBT / BCIP (Roche), 1:200 diluted in NTMT.

## 5.21 Heterologous expression

### 5.21.1 Heterologous expression of *Curvibacter's* AHL-synthases *Curl1* and *Curl2*

The AHL-synthases from *Curvibacter* sp. (*curl1* and *curl2*) were cloned into the pET22b-vector (chapter 5.18.1) and expressed in *E. coli* Rosetta 2 (DE3) pLysS, as described previously (Gould et al., 2006). *E. coli*, harboring the expression constructs or the control vector, were grown in 5 ml Luria-Bertani (LB) broth supplemented with 100  $\mu$ g/ml ampicillin and 34  $\mu$ g/ml chloramphenicol at 37°C while shaking for 12 to 16 h. Each culture was diluted 1:50 into 50 ml fresh LB broth with 100  $\mu$ g/ml ampicillin and 34  $\mu$ g/ml chloramphenicol. They incubated at 37°C and 250 rpm until an optical density (OD<sub>600</sub>) of 0.6 to 0.8 was reached, afterwards the temperature was lowered to 25°C. Cultures were

induced by addition of 0.5 mM IPTG (isopropyl- $\beta$ -D-thiogalactopyranoside) and incubated for 6 h at 25°C. The success of the overexpression was monitored by SDS-PAGE and Western blot (chapter 5.20).

## 5.22 Generation of transgenic *Hydra vulgaris* (AEP)

### 5.22.1 Hairpin construct for the down regulation of Hydra's paraoxonase HyPON

*Hydra vulgaris* (AEP) polyps with a knock down of the paraoxonase (HyPON) were produced by a hairpin approach (Franzenburg et al., 2012). The sequence for the HyPON were amplified out of cDNA and cloned into the pET28a(+)-vector (chapter 5.18.3). A 545 bp sense fragment of the hyPON was introduced behind the reporter gene egfp into the modified HoTG vector (LigAF1 vector) (Hemmrich et al., 2012) using *Asi*SI and *Eco*RI restriction sites. For the ligation of the antisense fragment connected by a 290 bp linker into the construct both were cut with the endonucleases *Bs*WI and *Eco*RI. The construct was checked by sequencing (chapter 5.16) and purified by a midi-plasmid preparation (chapter 5.14.2) (primer can be found in Table 6).

### 5.22.2 Microinjection of embryonic *Hydra vulgaris* (AEP)

The transfection of the paraoxonase (hyPON) hairpin construct into *Hydra vulgaris* (AEP) embryos via microinjection were done by Jörg Wittlieb (Wittlieb et al., 2006). Constructs (2  $\mu$ g/ $\mu$ l) were injected into *Hydra vulgaris* (AEP) embryos (2- to 8-cell stage), hold by a CellTram Air pump (Eppendorf), using a fine glass needle and the CellTram Vario pump (Eppendorf).

### 5.22.3 Generation of transgenic *Hydra vulgaris* (AEP) lines

Two weeks after microinjection of a hairpin construct into *Hydra vulgaris* (AEP) embryos, first polyps hatched and were screened for a positive eGFP signal. Out of 33 injected embryos one hatchling was positive and due to selection this polyp with a mosaic eGFP signal gave rise to a line with a fully transgenic endoderm and a line with an eGFP signal in the complete ectoderm. Furthermore, a line with no eGFP signal was selected as control line. Consequently, all three Hydra lines are genetically identical. To generate a Hydra line with a transgenic endoderm and ectoderm, the tissue layers of the existing lines were separated (chapter 0) and the correspondent transgenic tissues were transplanted with each other (chapter 5.3). The knock down was tested by quantitative RT-PCR (chapter 5.6.4) (primer can be found in Table 6).



### 5.23 Extraction of *N*-acyl homoserine lactones

Before extraction of *N*-acyl homoserine lactones (AHLs), Hydra polyps were homogenized with a pestle. Bacterial cultures assigned to mass spectrometry were pelletized by centrifugation at 3600 g for 10 min at 18°C; the supernatant were decanted into a 24 ml syringe and passed through a 0.2 µm filter. Samples smaller than 1 ml were extracted with two volumes of acidified ethyl acetate (with 0.01 % acetic acid), while for the extraction of samples with a bigger volume one volume acidified ethyl acetate were used. The samples with a volume up to 1 ml were shaken for 10 min at room temperature (RT), centrifuged for 1 min at 15000 g (RT), bigger volumes were centrifuged for 10 min at 3600 g (18°C). The organic phase containing AHLs was collected for analysis. Extracts from bacterial cultures were completely dried with a rotating evaporator in 50 ml glass flasks and samples were stored at -20°C or -80°C until further use.

### 5.24 Quantification of AHLs with bacterial reporter strains

The detection of *N*-acyl-homoserine lactones (AHLs) was performed with the help of bacterial reporter strains. Dependent on the length of the acyl-side chain of the AHLs three different AHL-bioreporters (pSB536 (C4-HSL), pSB401 (C6-, 3OC6-, C8-, 3OC8-HSL), pSB1075 (3OC10- and 3OC12-HSL) (Swift et al., 1997; Winson et al., 1998)) were used. For the semi-quantification of AHLs, a serial dilution of an AHL standard (Sigma-Aldrich) was performed and treated as the samples. The CurR reporter constructs pCPcurR1 and pCPcurR2 were also analysed by this assay. Extracted AHLs, in 8-well-strips, evaporated overnight under a hood. The next day an overnight culture of the bacterial reporter strain was diluted to an optical density (OD<sub>600</sub>) of 0.1. To every dried AHL probe 200 µl of the diluted bioreporter was added. They incubated for 8 h at 37°C and 220 rpm. After 1 to 2 hours the probes were transferred into a transparent 96-well-plate with flat bottom (Sarstedt) and sealed with PARAFILM<sup>®</sup> M (Eydam). Before measurement, bacterial pellets, which formed during incubation, were resolved by pipetting up and down and air bubbles were removed. The plate reader *Tecan GENios Plus* was used for the measurement of the OD<sub>600</sub> and the bioluminescence. To measure the bioluminescence, the probes were transferred into a white 96-well-plate with flat bottom (Greiner Bio-One). The values of the bioluminescence were correlated with the measured OD<sub>600</sub>; in the following every probe owns a value of relative light units. On the basis of these relative light units and the AHL standard one can determine the AHL concentration in the samples.

## 5.25 Thin-layer chromatography coupled with a bacterial AHL-reporter

Samples extracted with acidified ethyl acetate were resuspended with acidified ethyl acetate and applied to C18 reverse-phase thin-layer chromatography (TLC) sheets (ALUGRAM RP-18W / UV254; 20 x 20 cm; Macherey-Nagel). A mobile phase of methanol/water (60:40, v/v) was used to separate *N*-acyl-homoserine lactones (AHLs) based on the length of their acyl-side chain and the substitution at the third carbon, as described by Shaw et al. (Shaw et al. 1997). AHL standards (Sigma-Aldrich) were prepared in acidified ethyl acetate and also spotted. To detect AHLs, the TLC plate was overlaid with AT soft agar, supplemented with 60 µg/ml spectinomycin, 60 µg/ml X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and  $1 \times 10^7$  CFU/ml *Agrobacterium tumefaciens* NTL4 (pCF218)(pCF372) (Zhu et al. 1998). The plate incubated for 24 h at 29°C under light protected condition; afterwards hydrolysis of X-Gal was documented with the Molecular Imager® Gel Doc™ XR+ (Bio-Rad).

## 5.26 Identification of *N*-acyl-homoserine lactones (AHLs) by mass spectrometry

### 5.26.1 Identification of AHLs by MALDI-TOF mass spectrometry

*N*-acyl-homoserine lactone (AHL) extracts (chapter 5.30.2) were lyophilized, resuspended in 50 µl acetonitrile and 1 µl was spotted onto on a 384-well Opti-TOF MALDI Insert (AB SCIEX, Darmstadt, Germany) with 1 µl CHCA matrix solution (10 mg/ml α-cyano-4-hydroxycinnamic acid in 60% acetonitrile, H<sub>2</sub>O). The MALDI-TOF-MS (matrix assisted laser desorption ionization time of flight mass spectrometry) analysis was performed by Christian Treitz (Institute for Experimental Medicine of the Christian Albrechts University of Kiel) on an AB SCIEX TOF/TOF 5800 mass spectrometer (AB SCIEX, Darmstadt, Germany), accumulating 2000 shots over an *m/z*-range from 100 to 500. The instrument was calibrated with an AHL standard solution (C4-, C6-, C8-, C10- and C12-HSLs) to cover the prospective *m/z* range. MS spectra were manually evaluated for potential AHL degradation products and an inclusion list containing targets for MS/MS acquisition was assembled. Fragment spectra were acquired with a medium CID pressure of  $2 \times 10^{-6}$  Torr, minimum signal-to-noise filter of 10 and a precursor mass tolerance ±200 ppm.

### 5.26.2 Identification of AHLs by LC-ESI-MS

The mass spectrometry analysis was performed by Christian Treitz (Institute for Experimental Medicine of the Christian Albrechts University of Kiel). Ethyl acetate extracts (chapter 5.23) were further purified by solid-phase extraction (SPE) for mass spectrometry analysis as described by Gould et al. (Gould et al., 2006). Each sample was dissolved in 500  $\mu$ l methanol (Roth), 5 ml isooctane-ethyl ether was added and then applied to an activated Sep-Pak silica cartridge (3 cc Vac RC Cartridge with 500 mg Sorbent (Waters)), which were fitted with a 10 ml glass reaction vial and attached to the vacuum manifold. The activation of the cartridges was performed by successive washings with 6 ml of each solvent in the following sequence: isooctane, ethyl ether, acidified ethyl acetate, isooctane and ethyl ether. The SPE cartridge was washed twice with 6 ml of isooctane-ether and then 5 to 8 ml of acidified ethyl acetate were used to elute the AHLs into a glass tube. The purified samples were dried by lyophilization. The samples were dissolved in 50 or 100  $\mu$ l of acetonitrile and transferred to a HPLC vial.

LC-MS analysis was performed with a Dionex Ultimate 3000 HPLC system, equipped with a Dionex Acclaim PepMap100 nano-column (75  $\mu$ m x 15 cm, 3  $\mu$ m, 100 Å, (Thermo Scientific, Germany). The LC was coupled to an LTQ Orbitrap velos mass spectrometer (Thermo Scientific, Germany). Reversed phase HPLC separation was performed with a gradient of Eluents A (Milli-Q water with 0.1% formic acid) and B (80% acetonitrile, 0.1% formic acid). Five microliter of each sample were injected and washed for 5 minutes on a PepMap C18 guard column (300  $\mu$ m x 10 mm; Thermo Scientific, Germany) with 0.1% aqueous TFA at a flow rate of 30  $\mu$ l x min<sup>-1</sup> and then separated over an isocratic gradient of 55% B for 50 min at a flow rate of 250 nl x min<sup>-1</sup>, followed by a sharp increase to 95% eluent B in 1 min and a 5 min washing step. The column was then equilibrated with 5% B for 15 min. UV detection was performed at 214 nm.

HPLC-flow was coupled to the LTQ Orbitrap velos (Thermo Scientific, Germany) using a nanospray ion source with 1.3 kV capillary spray voltage and 210°C capillary temperature with a 30  $\mu$ m PicoTip emitter (New Objective, USA). After a delay time of 5 min, full scans of 50 to 500 m/z range were recorded in positive ion mode with a resolution of 30000 (AGC target 1E6; maximum inject time 500 ms, preview mode for FTMS master scans and Wideband activation were used). Top 5 precursors with a minimum signal intensity of 400 and charge state of 1, were selected from MS/MS acquisition from targets on a parent mass list which included 85 m/z values for 3OH-HSL, 3O-HSL, acyl-HSL and singly unsaturated-AHL molecules with side chain lengths from C4 to C20.

## 5.27 Characterization of bacterial AHL production

Commensal and environmental bacteria of *Hydra vulgaris* (AEP) were cultivated (chapter 5.1.4) for 48 h at 18°C. The cultures were extracted with one volume acidified ethyl acetate (chapter 5.23) and analysed by a TLC combined with the *A. tumefaciens* NTL4 AHL-reporter (chapter 5.25). Samples of *Curvibacter* sp. strain AEP1.3 (96 h) were further analysed by mass spectrometry (chapter 5.26).

## 5.28 Characterization of *Curvibacter*'s AHL-synthases CurI1 and CurI2

The functionality of the AHL-synthases was tested after heterologous expression in *E. coli* (chapter 5.21.1). Bacterial cells were pelletized by centrifugation at 3600 g for 10 min at 18°C, followed by decanting of the supernatant into a 24 ml syringe and passing through a 0.2 µm filter. The culture supernatants were extracted with one volume of acidified ethyl acetate (chapter 5.23) and dried with a rotating evaporator to complete dryness in a 50 ml glass flask. *N*-acyl-homoserine lactones (AHLs) were resuspended with acidified ethyl acetate, separated by a thin-layer chromatography (TLC) and detected with the *A. tumefaciens* NTL4 reporter (chapter 5.25). Furthermore, AHL samples were processed for a mass spectrometric analysis (chapter 5.26).

## 5.29 Characterization of *Curvibacter*'s AHL-receptors CurR1 and CurR2

The functionality of the AHL-receptors CurR1 and CurR2 was analysed with help of the reporter constructs pCPcurR1 and pCPcurR2 (chapter 5.19). The AHL-synthases (CurI1 and CurI2) and the control plasmid (pET22b) were heterologous overexpressed (chapter 5.21.1), extracted with one volume acidified ethyl acetate (chapter 5.23) and concentrated with a rotating evaporator in this way that 3 µl extract correspond to 100 µl overexpression culture. Dilution series of the extract ( $1 \times 10^{-4}$  to  $1 \times 10^{-10}$  l culture) in acidified ethyl acetate were performed. Always six dilution series of the three extracts were analysed by the two CurR reporter constructs (pCPcurR1 and pCPcurR2) with a plate bioassay (chapter 5.24). To decode the CurR activity, dilution series ( $1 \times 10^{-5}$  to  $1 \times 10^{-11}$  M) of all commercially available *N*-acyl-homoserine lactones (AHLs) with oxo- or hydroxy-modification (3OC6-3OC14-HSL, 3OHC8-3OHC14-HSL (Sigma)) were measured in three to five replicates with the plate bioassay (chapter 5.24).

## 5.30 Characterization of quorum quenching

### 5.30.1 Analysis of the quorum quenching activity of Hydra polyps

To investigate the quorum quenching (QQ) activity of Hydra polyps, they incubated for 24 h in 100  $\mu$ l S-medium with 10  $\mu$ M AHLs at 18°C. For every probe 10 polyps (*H. vulgaris* (AEP), *H. oligactis* (strain St. Petersburg)) or, because of their much smaller size, 15 polyps of *H. viridis* (strain A99) were used. For the analysis of transgenic *Hydra vulgaris* (AEP) the experiment was only conducted for 8 h. The remaining AHLs were extracted with acidified ethyl acetate after 0 h, 4 h, 8 h and 24 h (chapter 5.23). Every time point was covered by three replicates. As a control the experiment was conducted in parallel with the same AHL concentration in S-medium. The extracted AHLs were identified and semi-quantified with AHL-bioreporters (chapter 5.24).

### 5.30.2 Analysis of the quorum quenching mechanism of Hydra polyps by mass spectrometry

To identify Hydra's quorum quenching (QQ) mechanism, Hydra polyps were incubated for 24 h in 500  $\mu$ l S-medium with 100  $\mu$ M AHLs at 18°C. For every sample, 50 germfree (chapter 5.1.3) or wild type *H. vulgaris* (AEP) polyps were used. The remaining AHLs were extracted with acidified ethyl acetate after 0 h, 8 h and 24 h (chapter 5.23). Every time point was covered by five to fifteen replicates. As a control the experiment was conducted in parallel with the same AHL concentration in S-medium and Hydra polyps without supplement. The extracted AHLs were analysed by MALDI-TOF mass spectrometry (chapter 5.26.1) and LC-ESI-MS (chapter 5.26.2).

## 5.31 Sample preparation for transmission electron microscopy

Transmission electron microscopy (TEM) was used to visualize *Curvibacter*'s flagella. *Curvibacter* sp. cells of a freshly grown culture were fixated by addition of glutaraldehyde in a final concentration of 3.5% and 20 min incubation at room-temperature. Afterwards cells were pelletized by centrifugation for 10 min at 5000 rpm and washed three times with one volume MilliQ water and centrifugation for 10 min at 5000 rpm. Finally cells were resuspended with 50  $\mu$ l MilliQ water. For contrast 5  $\mu$ l of the cell suspension was spotted on a TEM grid, incubated for 10min at room-temperature before liquid was taken off with filter paper and then 5  $\mu$ l of 1% uranyl acetate was added. After 30 sec liquid was taken off and the grid was applied to TEM, which was performed by Dr. Friederike Anton-Erxleben.

### 5.32 Characterization of *Curvibacter*'s motility

The effect of different *N*-acyl-homoserine lactones (AHLs) on *Curvibacter*'s motility on plate should be investigated. Therefore *Curvibacter* sp. strain AEP1.3 was cultivated in liquid culture (chapter 5.1.4). The overnight culture was used to spot always 1  $\mu$ l on swarming plates, supplemented with 10  $\mu$ M 3OC12-HSL, 10  $\mu$ M 3OHC12-HSL or 1:2000 ethyl acetate. The plates incubated for eight days at 18°C. Between four and seven days the colony area was measured with ImageJ.

### 5.33 Effects of AHLs on bacterial recolonization of Hydra polyps

To analyse the effect of 3-oxo- and 3-hydroxy-HSLs on the bacterial colonization of Hydra polyps, germfree *Hydra vulgaris* (AEP) polyps were recolonized for four days with *Curvibacter* sp. in the presence of 10  $\mu$ M 3OC12-HSL, 10  $\mu$ M 3OHC12.HSL or the AHL solvent acidified ethyl acetate. Always 30 germfree *Hydra vulgaris* (AEP) (chapter 5.1.3) were incubated for 10 h with 5000 CFU/ml of *Curvibacter* sp. (Table 7) in 10 ml S-medium and one of the supplements at 18°C. The concentration of the acidified ethyl acetate was in all samples 1:2000. Afterwards the solutions were exchanged by S-medium with the corresponding supplement twice a day, with a minimum of 8 h in between. The experiment was conducted in triplicates. After 4 days 5 polyps per treatment and replicate were washed twice with 500  $\mu$ l sterile S-medium. The polyps were homogenized with a pestle in 1 ml sterile S-medium and one tenth of a polyp were plated on a R2A agar plate and incubated at 18°C. After three days the bacterial colonies were counted.

### 5.34 Effects of AHLs on bacterial community of Hydra polyps

The effect of 3-oxo- and 3-hydroxy-HSLs on the bacterial community of Hydra polyps should be analysed. Therefore always 15 *Hydra vulgaris* (AEP) polyps were incubated for five days with 5  $\mu$ M 3OC12-HSL, 5  $\mu$ M 3OHC12-HSL or the AHL solvent acidified ethyl acetate in 10 ml S-medium at 18°C. The concentration of the acidified ethyl acetate was in all samples 1:4000. The solutions were exchanged twice a day, with a minimum of 8 h in between. The experiment was conducted in duplicates. After 5 days 3 polyps per treatment and replicate were washed three times with 500  $\mu$ l sterile S-medium. The polyps were homogenized with a pestle in 1 ml sterile S-medium and one tenth and one twentieth of a polyp were plated on a R2A agar plate and incubated at 18°C. After three days the bacterial colonies were counted.

### **5.35 Effects of flagellin on bacterial community of Hydra polyps**

The effect of flagellin on the bacterial community of Hydra polyps should be analysed. Therefore always 16 *Hydra vulgaris* (AEP) polyps were incubated for 24 hours in each 50 µl S-medium with 0.5 , 0.1 or 0.02 µg/ml flagellin of *Salmonella typhimurium* (Sigma) or 1:2000 PBS pH 6.8 as control at 18°C. After 24 h the polyps were washed three times with 500 µl sterile S-medium. The polyps were homogenized with a pestle in 1 ml sterile S-medium and one tenth of a polyp were plated on a R2A agar plate and incubated at 18°C. After three days the bacterial colonies were counted.

## 6 References

Adikesavalu, H., Patra, A., Banerjee, S., Sarkar, A., and Abraham, T.J. (2015). Phenotypic and molecular characterization and pathology of *Flectobacillus roseus* causing flectobacillosis in captive held carp *Labeo rohita* (Ham.) fingerlings. *Aquaculture* 439, 60–65.

Adkins, S., Gan, K.N., Mody, M., and La Du, B.N. (1993). Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. *Am. J. Hum. Genet.* 52, 598–608.

Alagely, A., Krediet, C.J., Ritchie, K.B., and Teplitski, M. (2011). Signaling-mediated cross-talk modulates swarming and biofilm formation in a coral pathogen *Serratia marcescens*. *ISME J.* 5, 1609–1620.

Anderson, K. V, Bokla, L., and Nüsslein-Volhard, C. (1985a). Establishment of dorsal-ventral polarity in the *Drosophila* embryo: the induction of polarity by the Toll gene product. *Cell* 42, 791–798.

Anderson, K. V, Jürgens, G., and Nüsslein-Volhard, C. (1985b). Establishment of dorsal-ventral polarity in the *Drosophila* embryo: Genetic studies on the role of the Toll gene product. *Cell* 42, 779–789.

Antunes, L.C.S., Visca, P., and Towner, K.J. (2014). *Acinetobacter baumannii*: evolution of a global pathogen. *Pathog. Dis.* 71, 292–301.

Athman, R., and Philpott, D. (2004). Innate immunity via Toll-like receptors and Nod proteins. *Curr. Opin. Microbiol.* 7, 25–32.

Augustin, R., Anton-Erxleben, F., Jungnickel, S., Hemmrich, G., Spudy, B., Podschun, R., and Bosch, T.C.G. (2009). Activity of the novel peptide arminin against multiresistant human pathogens shows the considerable potential of phylogenetically ancient organisms as drug sources. *Antimicrob. Agents Chemother.* 53, 5245–5250.

Backhed, F., Ding, H., Wang, T., Hooper, L. V, Koh, G.Y., Nagy, A., Semenkovich, C.F., and Gordon, J.I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. U. S. A.* 101, 15718–15723.

Bäckhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D. a, and Gordon, J.I. (2005). Host-bacterial mutualism in the human intestine. *Science* 307, 1915–1920.

Barott, K.L., Rodriguez-Brito, B., Janouškovec, J., Marhaver, K.L., Smith, J.E., Keeling, P., and Rohwer, F.L. (2011). Microbial diversity associated with four functional groups of benthic reef algae and the reef-building coral *Montastraea annularis*. *Environ. Microbiol.* 13, 1192–1204.

Bar-Rogovsky, H., Hugenmatter, A., and Tawfik, D.S. (2013). The evolutionary origins of detoxifying enzymes: The mammalian serum paraoxonases (PONs) relate to bacterial homoserine lactonases. *J. Biol. Chem.* 288, 23914–23927.

Behmer, S.T. (2009). Animal behaviour: feeding the superorganism. *Curr. Biol.* 19, R366–R368.



- Bell, G. (1998). Model Metaorganism. A review by Graham Bell. *Science* (80- ).
- De Bentzmann, S., and Plésiat, P. (2011). The *Pseudomonas aeruginosa* opportunistic pathogen and human infections. *Environ. Microbiol.* *13*, 1655–1665.
- Beutler, B. (2004). Inferences, questions and possibilities in Toll-like receptor signalling. *Nature* *430*, 257–263.
- Bevins, C.L., and Salzman, N.H. (2011a). Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nat. Rev. Microbiol.* *9*, 356–368.
- Bevins, C.L., and Salzman, N.H. (2011b). The potter's wheel: the host's role in sculpting its microbiota. *Cell. Mol. Life Sci.* *68*, 3675–3685.
- Biagi, E., Candela, M., Fairweather-Tait, S., Franceschi, C., and Brigidi, P. (2012). Ageing of the human metaorganism : the microbial counterpart. *Age* (Omaha).
- Bijtenhoorn, P., Mayerhofer, H., Müller-Dieckmann, J., Utpatel, C., Schipper, C., Hornung, C., Szesny, M., Grond, S., Thürmer, A., Brzuszkiewicz, E., et al. (2011). A novel metagenomic short-chain dehydrogenase/reductase attenuates *Pseudomonas aeruginosa* biofilm formation and virulence on *Caenorhabditis elegans*. *PLoS One* *6*, 1–15.
- Bode, H.R., Heimfeld, S., Chow, M.A., and Huang, L.W. (1987). Gland Cells Arise by Differentiation from Interstitial Cells in *Hydra attenuata*. *Dev. Biol.* *122*, 577–585.
- Bosch, T.C.G. (2003). Ancient signals: peptides and the interpretation of positional information in ancestral metazoans. *Comp. Biochem. Physiol. Part B* *136*, 185–196.
- Bosch, T.C.G. (2007a). Why polyps regenerate and we don't: towards a cellular and molecular framework for *Hydra* regeneration. *Dev. Biol.* *303*, 421–433.
- Bosch, T.C.G. (2007b). Symmetry breaking in stem cells of the basal metazoan *Hydra*. In *Asymmetric Cell Division*, pp. 61–78.
- Bosch, T.C.G. (2009). *Hydra* and the evolution of stem cells. *Bioessays* *31*, 478–486.
- Bosch, T.C.G. (2012). Understanding complex host-microbe interactions in *Hydra*. *Gut Microbes* *3*, 345–351.
- Bosch, T.C.G., and David, C.N. (1986). Male and female stem cells and sex reversal in *Hydra* polyps. *Proc. Natl. Acad. Sci. USA* *83*, 9478–9482.
- Bosch, T.C.G., and David, C.N. (1987). Stem cells of *Hydra magnipapillata* can differentiate into somatic cells and germ line cells. *Dev. Biol.* *121*, 182–191.
- Bosch, T.C.G., and McFall-Ngai, M.J. (2011). Metaorganisms as the new frontier. *Zoology* *114*, 185–190.
- Bosch, T.C.G., Augustin, R., Anton-Erxleben, F., Fraune, S., Hemmrich, G., Zill, H., Rosenstiel, P., Jacobs, G., Schreiber, S., Leippe, M., et al. (2009). Uncovering the evolutionary history of innate immunity: the simple metazoan *Hydra* uses epithelial cells for host defence. *Dev. Comp. Immunol.* *33*, 559–569.

- Böttger, A., Doxey, A.C., Hess, M.W., Pfaller, K., Salvenmoser, W., Deutzmann, R., Geissner, A., Pauly, B., Altstätter, J., Münder, S., 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, 1–17.
- Brameyer, S., and Heermann, R. (2015). Specificity of Signal-Binding via Non-AHL LuxR-Type Receptors. *PLoS One* 10, 1–15.
- Brandl, K., Plitas, G., Schnabl, B., DeMatteo, R.P., and Pamer, E.G. (2007). MyD88-mediated signals induce the bactericidal lectin RegIII gamma and protect mice against intestinal *Listeria monocytogenes* infection. *J. Exp. Med.* 204, 1891–1900.
- Brucker, R.M., and Bordenstein, S.R. (2012). Speciation by symbiosis. *Trends Ecol. Evol.* 27, 443–451.
- Brucker, R.M., and Bordenstein, S.R. (2013). The Hologenomic Basis of Speciation: Gut Bacteria Cause Hybrid Lethality in the Genus *Nasonia*. *Science* (80-. ). 341, 667–669.
- Campbell, R.D. (1967). Tissue Dynamics of Steady State Growth in *Hydra littoralis*. *Dev. Biol.* 15, 487–502.
- Campbell, R.D. (1987). A new species of *Hydra* (Cnidaria, Hydrozoa) from North- America with comments on species clusters within the genus. *Zool. J. Linn. Soc.* 91, 253–263.
- Carlier, A., Uroz, S., Smadja, B., Fray, R., Latour, X., Dessaux, Y., and Faure, D. (2003). The Ti Plasmid of *Agrobacterium tumefaciens* Harbors an attM -Paralogous Gene , *aiiB* , Also Encoding N -Acyl Homoserine Lactonase Activity. *Appl. Environ. Microbiol.* 69, 4989–4993.
- Cash, H.L., Whitham, C. V, Behrendt, C.L., and Hooper, L. V (2006). Symbiotic Bacteria Direct Expression of an Intestinal Bactericidal Lectin. *Science* (80-. ). 313, 1126–1130.
- Certner, R.H., and Vollmer, S. V. (2015). Evidence for Autoinduction and Quorum Sensing in White Band Disease-Causing Microbes on *Acropora cervicornis*. *Sci. Rep.* 5, 11134.
- Cha, C., Gao, P., Chen, Y., Shaw, P.D., and Farrand, S.K. (1998a). Production of Acyl-Homoserine Lactone Quorum-Sensing Signals by Gram-Negative Plant-Associated Bacteria. *Am. Phytopathol. Soc.* 11, 1119–1129.
- Cha, C., Gao, P., Chen, Y.C., Shaw, P.D., and Farrand, S.K. (1998b). Production of acyl-homoserine lactone quorum-sensing signals by gram-negative plant-associated bacteria. *Mol. Plant. Microbe. Interact.* 11, 1119–1129.
- Chan, K.-G., Atkinson, S., Mathee, K., Sam, C.-K., Chhabra, S.R., Cámara, M., Koh, C.-L., and Williams, P. (2011). Characterization of N-acylhomoserine lactone-degrading bacteria associated with the *Zingiber officinale* (ginger) rhizosphere: co-existence of quorum quenching and quorum sensing in *Acinetobacter* and *Burkholderia*. *BMC Microbiol.* 11, 1–13.
- Chapman, J.A., Kirkness, E.F., Simakov, O., Hampson, S.E., Mitros, T., Weinmaier, T., Rattei, T., Balasubramanian, P.G., Borman, J., Busam, D., et al. (2010). The dynamic genome of *Hydra*. *Nature* 464, 592–596.

- Chen, C.-N., Chen, C.-J., Liao, C.-T., and Lee, C.-Y. (2009). A probable aculeacin A acylase from the *Ralstonia solanacearum* GMI1000 is N-acyl-homoserine lactone acylase with quorum-quenching activity. *BMC Microbiol.* *9*, 1–11.
- Choi, Y., Park, H.Y., Park, S.J., Park, S.J., Kim, S.K., Ha, C., Im, S.J., and Lee, J.H. (2011). Growth phase-differential quorum sensing regulation of anthranilate metabolism in *Pseudomonas aeruginosa*. *Mol. Cells* *32*, 57–65.
- Chowdhary, P.K., Keshavan, N., Nguyen, H.Q., Peterson, J. a, González, J.E., and Haines, D.C. (2007). *Bacillus megaterium* CYP102A1 oxidation of acyl homoserine lactones and acyl homoserines. *Biochemistry* *46*, 14429–14437.
- Chu, Y.Y., Nega, M., Wölfle, M., Plener, L., Grond, S., Jung, K., and Götz, F. (2013). A New Class of Quorum Quenching Molecules from *Staphylococcus* Species Affects Communication and Growth of Gram-Negative Bacteria. *PLoS Pathog.* *9*, 1–13.
- Chun, C.K., Ozer, E.A., Welsh, M.J., Zabner, J., and Greenberg, E.P. (2004). Inactivation of a *Pseudomonas aeruginosa* quorum-sensing signal by human airway epithelia. *Proc. Natl. Acad. Sci. USA* *101*, 3587–3590.
- Churchill, M.E.A., and Chen, L. (2011). Structural Basis of Acyl-homoserine Lactone-Dependent Signaling. *Chem. Rev.* *111*, 68–85.
- Clarke, T.B., Davis, K.M., Lysenko, E.S., Zhou, A.Y., Yu, Y., and Weiser, J.N. (2010). Recognition of Peptidoglycan from the Microbiota by Nod1 Enhances Systemic Innate Immunity. *Nat. Med.* *16*, 228–231.
- Cole, T.B., Beyer, R.P., Bammler, T.K., Park, S.S., Farin, F.M., Costa, L.G., and Furlong, C.E. (2011). Repeated developmental exposure of mice to chlorpyrifos oxon is associated with paraoxonase 1 (PON1)-modulated effects on cerebellar gene expression. *Toxicol. Sci.* *123*, 155–169.
- Collins, A.G., Schuchert, P., Marques, A.C., Jankowski, T., Medina, M., and Schierwater, B. (2006). Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. *Syst. Biol.* *55*, 97–115.
- Collins, S.M., Surette, M., and Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. *Nat. Rev. Microbiol.* *10*, 735–742.
- Costa, L.G., Laat, R. de, Dao, K., Pellacani, C., Cole, T.B., and Furlong, C.E. (2014). Paraoxonase-2 (PON2) in brain and its potential role in neuroprotection. *Neurotoxicology* *43*, 3–9.
- Cullender, T.C., Chassaing, B., Janzon, A., Kumar, K., Muller, C.E., Werner, J.J., Angenent, L.T., Bell, M.E., Hay, A.G., Peterson, D. a, et al. (2013). Innate and adaptive immunity interact to quench microbiome flagellar motility in the gut. *Cell Host Microbe* *14*, 571–581.
- Cvitkovitch, D.G., Li, Y.H., and Ellen, R.P. (2003). Quorum sensing and biofilm formation in streptococcal infections. *J. Clin. Invest.* *112*, 1626–1632.

- d'Angelo-Picard, C., Faure, D., Penot, I., and Dessaux, Y. (2005). Diversity of N-acyl homoserine lactone-producing and -degrading bacteria in soil and tobacco rhizosphere. *Environ. Microbiol.* 7, 1796–1808.
- Darch, S.E., West, S. a., Winzer, K., and Diggle, S.P. (2012). Density-dependent fitness benefits in quorum-sensing bacterial populations. *Proc. Natl. Acad. Sci. USA* 109, 8259–8263.
- David, C.N., and Murphy, S. (1977). Characterization of interstitial stem cells in hydra by cloning. *Dev. Biol.* 58, 372–383.
- Davis, L.E., and Haynes, J.F. (1968). An ultrastructural examination of the mesoglea of Hydra. *Zeitschrift Für Zellforsch.* 92, 149–158.
- Dessaux, Y., Chapelle, E., and Faure, D. (2010). Quorum Sensing and Quorum Quenching in Soil Ecosystem. In *Biocommunication in Soil Microorganisms*, G. Witzany, ed. (Springer Berlin Heidelberg), pp. 339–367.
- Dickschat, J.S. (2010). Quorum sensing and bacterial biofilms. *Nat. Prod. Rep.* 27, 343–369.
- Domazet-Lošo, T., Klimovich, A., Anokhin, B., Anton-Erxleben, F., Hamm, M.J., Lange, C., and Bosch, T.C.G. (2014). Naturally occurring tumours in the basal metazoan Hydra. *Nat. Commun.* 5, 1–8.
- Dong, Y., Gusti, A.R., Zhang, Q., Xu, J., and Zhang, L. (2002). Identification of Quorum-Quenching N -Acyl Homoserine Lactonases from Bacillus Species Identification of Quorum-Quenching N -Acyl Homoserine Lactonases from Bacillus Species. *Appl. Environ. Microbiol.* 68, 1754–1759.
- Dong, Y.H., Xu, J.L., Li, X.Z., and Zhang, L.H. (2000). AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3526–3531.
- Dong, Y.H., Wang, L.H., Xu, J.L., Zhang, H.B., Zhang, X.F., and Zhang, L.H. (2001). Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. *Nature* 411, 813–817.
- Draganov, D.I., and La Du, B.N. (2004). Pharmacogenetics of paraoxonases: a brief review. *Naunyn. Schmiedebergs. Arch. Pharmacol.* 369, 78–88.
- Draganov, D.I., Teiber, J.F., Speelman, A., Osawa, Y., Sunahara, R., and La Du, B.N. (2005). Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities. *J. Lipid Res.* 46, 1239–1247.
- Dworjanyn, S. a., De Nys, R., and Steinberg, P.D. (2006). Chemically mediated antifouling in the red alga *Delisea pulchra*. *Mar. Ecol. Prog. Ser.* 318, 153–163.
- Engebrecht, J., Neelson, K., and Silverman, M. (1983). Bacterial Bioluminescence : Isolation and Genetic Analysis of Functions from *Vibrio fischeri*. *Cell* 32, 773–781.
- Epp, L.G., Tardent, P., and Bänninger, R. (1979). Isolation and Observation of Tissue Layers in *Hydra attenuata* Pall. (Cnidaria, Hydrozoa). *Trans. Am. Microsc. Soc.* 98, 392–400.

- Falagas, M.E., Karveli, E. a, Siempos, I.I., and Vardakas, K.Z. (2008). Acinetobacter infections: a growing threat for critically ill patients. *Epidemiol. Infect.* *136*, 1009–1019.
- Fan, J., Qian, G., Chen, T., Zhao, Y., Liu, F., Walcott, R.R., and Hu, B. (2010). The acyl-homoserine lactone (AHL)-type quorum sensing system affects growth rate, swimming motility and virulence in *Acidovorax avenae* subsp. *citrulli*. *World J. Microbiol. Biotechnol.* *27*, 1155–1166.
- Federle, M.J., and Bassler, B.L. (2003). Interspecies communication in bacteria. *J Clin Invest* *112*, 1291–1299.
- Fekete, A., Frommberger, M., Rothballer, M., Li, X., Englmann, M., Fekete, J., Hartmann, A., Eberl, L., and Schmitt-Kopplin, P. (2007). Identification of bacterial N-acylhomoserine lactones (AHLs) with a combination of ultra-performance liquid chromatography (UPLC), ultra-high-resolution mass spectrometry, and in-situ biosensors. *Anal. Bioanal. Chem.* *387*, 455–467.
- Fenchel, T. (2002). Microbial behavior in a heterogeneous world. *Science (80-. )*. *296*, 1068–1071.
- Feng, L., Wu, Z., and Yu, X. (2013). Quorum sensing in water and wastewater treatment biofilms. *J. Environ. Biol.* *34*, 437–444.
- Fetzner, S. (2015). Quorum quenching enzymes. *J. Biotechnol.* *201*, 2–14.
- Frank, D.N., St Amand, A.L., Feldman, R. a, Boedeker, E.C., Harpaz, N., and Pace, N.R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl. Acad. Sci. USA* *104*, 13780–13785.
- Franzenburg, S., Fraune, S., Künzel, S., Baines, J.F., Domazet-Lošo, T., and Bosch, T.C.G. (2012). MyD88-deficient Hydra reveal an ancient function of TLR signaling in sensing bacterial colonizers. *Proc. Natl. Acad. Sci. USA*.
- Franzenburg, S., Walter, J., Künzel, S., Wang, J., Baines, J.F., Bosch, T.C.G., and Fraune, S. (2013a). Distinct antimicrobial peptide expression determines host species-specific bacterial associations. *Proc. Natl. Acad. Sci. USA* *110*, 1–9.
- Franzenburg, S., Fraune, S., Altrock, P.M., Künzel, S., Baines, J.F., Traulsen, A., and Bosch, T.C.G. (2013b). Bacterial colonization of Hydra hatchlings follows a robust temporal pattern. *ISME J.* *7*, 781–790.
- Fraune, S. (2008). Towards understanding a holobiont: Host-microbe interactions in Hydra. Dr. Thesis 104.
- Fraune, S., and Bosch, T.C.G. (2007). Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. *Proc. Natl. Acad. Sci. USA* *104*, 13146–13151.
- Fraune, S., and Bosch, T.C.G. (2010). Why bacteria matter in animal development and evolution. *Bioessays* *32*, 571–580.
- Fraune, S., Abe, Y., and Bosch, T.C.G. (2009). Disturbing epithelial homeostasis in the metazoan Hydra leads to drastic changes in associated microbiota. *Environ. Microbiol.* *11*, 2361–2369.

- Fraune, S., Augustin, R., Anton-Erxleben, F., Wittlieb, J., Gelhaus, C., Klimovich, V.B., Samoilovich, M.P., and Bosch, T.C.G. (2010). In an early branching metazoan, bacterial colonization of the embryo is controlled by maternal antimicrobial peptides. *Proc. Natl. Acad. Sci. USA* *107*, 18067–18072.
- Fraune, S., Franzenburg, S., Augustin, R., and Bosch, T.C.G. (2011). Das Prinzip Metaorganismus. *Biospektrum* *17*, 634–636.
- Fraune, S., Anton-erxleben, F., Knop, M., Schro, K., Willoweit-ohl, D., and Bosch, T.C.G. (2014). Bacteria - bacteria interactions within the microbiota of the ancestral metazoan Hydra contribute to fungal resistance. *ISME J.* 1–14.
- Fujii, A., Seki, M., Higashiguchi, M., Tachibana, I., Kumanogoh, A., and Tomono, K. (2014). Community-acquired, hospital-acquired, and healthcare-associated pneumonia caused by *Pseudomonas aeruginosa*. *Respir. Med. Case Reports* *12*, 30–33.
- Fukata, M., Vamadevan, A.S., and Abreu, M.T. (2009). Toll-like receptors (TLRs) and Nod-like receptors (NLRs) in inflammatory disorders. *Semin. Immunol.* *21*, 242–253.
- Fuqua, C., and Winans, S.C. (1996). Conserved cis-acting promoter elements are required for density-dependent transcription of *Agrobacterium tumefaciens* conjugal transfer genes. *J. Bacteriol.* *178*, 435–440.
- Fuqua, W.C., and Winans, S.C. (1994). A LuxR-LuxI type regulatory system activates *Agrobacterium Ti* plasmid conjugal transfer in the presence of a plant tumor metabolite. *J. Bacteriol.* *176*, 2796–2806.
- Fuqua, C., Parsek, M.R., and Greenberg, E.P. (2001). Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. *Annu. Rev. Genet.* *35*, 439–468.
- Gao, X., and Zhang, P. (2007). Transgenic RNA interference in mice. *Physiology (Bethesda)*. *22*, 161–166.
- Garrett, W.S. (2015). Cancer and the microbiota. *Science (80-. )*. *348*, 80–86.
- Gierer, A., and Meinhardt, H. (1972). A theory of biological pattern formation. *Kybernetik* *12*, 30–39.
- Gill, S.R., Pop, M., Deboy, R.T., Eckburg, P.B., Turnbaugh, P.J., Samuel, B.S., Gordon, J.I., Relman, D. a, Fraser-Liggett, C.M., and Nelson, K.E. (2006). Metagenomic analysis of the human distal gut microbiome. *Science* *312*, 1355–1359.
- Girardin, S.E., Boneca, I.G., Viala, J., Chamaillard, M., Labigne, A., Thomas, G., Philpott, D.J., and Sansonetti, P.J. (2003). Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J. Biol. Chem.* *278*, 8869–8872.
- Golberg, K., Eltzov, E., Shnit-Orland, M., Marks, R.S., and Kushmaro, A. (2011). Characterization of quorum sensing signals in coral-associated bacteria. *Microb. Ecol.* *61*, 783–792.
- Gonzalez, A., Stombaugh, J., Lozupone, C., Turnbaugh, P.J., Gordon, J.I., and Knight, R. (2011). The mind-body-microbial continuum. *Dialogues Clin. Neurosci.* *13*, 55–62.

- Gorokhova, E., Rivetti, C., Furuhausen, S., Edlund, A., Ek, K., and Breitholtz, M. (2015). Bacteria-mediated effects of antibiotics on *Daphnia* nutrition. *Environ. Sci. Technol.* *49*, 5779–5787.
- Gould, T.A., Herman, J.P., Krank, J., Robert, C., Churchill, M.E.A., and Murphy, R.C. (2006). Specificity of Acyl-Homoserine Lactone Synthases Examined by Mass Spectrometry. *J. Bacteriol.*
- Grasis, J. a., Lachnit, T., Anton-Erxleben, F., Lim, Y.W., Schmieder, R., Fraune, S., Franzenburg, S., Insua, S., Machado, G., Haynes, M., et al. (2014). Species-Specific Viromes in the Ancestral Holobiont Hydra. *PLoS One* *9*, 1–13.
- Green, S.J., Michel, F.C., Hadar, Y., and Minz, D. (2007). Contrasting patterns of seed and root colonization by bacteria from the genus *Chryseobacterium* and from the family Oxalobacteraceae. *ISME J.* *1*, 291–299.
- Gustafsson, B.E. (1959). Vitamin K deficiency in germfree rats. *Ann. N. Y. Acad. Sci.* *78*, 166–174.
- Hanzelka, B.L., Stevens, a M., Parsek, M.R., Crone, T.J., and Greenberg, E.P. (1997). Mutation analysis of the *Vibrio fischeri* LuxI polypeptide:critical regions of an autoinducer synthase. *J. Bacteriol.* *179*, 4882–4887.
- Harder, T., Campbell, A.H., Egan, S., and Steinberg, P.D. (2012). Chemical mediation of ternary interactions between marine holobionts and their environment as exemplified by the red alga *Delisea pulchra*. *J. Chem. Ecol.* *38*, 442–450.
- Hartmann, A., and Schikora, A. (2012). Quorum sensing of bacteria and trans-kingdom interactions of N-acyl homoserine lactones with eukaryotes. *J. Chem. Ecol.* *38*, 704–713.
- Hartmann, A., Rothballer, M., Hense, B. a, and Schröder, P. (2014). Bacterial quorum sensing compounds are important modulators of microbe-plant interactions. *Front. Plant Sci.* *5*, 131.
- Hassett, C., Richter, R.J., Humbert, R., Chapline, C., Crabb, J.W., Omiecinski, C.J., and Furlong, C.E. (1991). Characterization of cDNA clones encoding rabbit and human serum paraoxonase: the mature protein retains its signal sequence. *Biochemistry* *30*, 10141–10149.
- Hayashi, F., Smith, K.D., Ozinsky, a, Hawn, T.R., Yi, E.C., Goodlett, D.R., Eng, J.K., Akira, S., Underhill, D.M., and Aderem, a (2001). The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* *410*, 1099–1103.
- Hemrich, G., Anokhin, B., Zacharias, H., and Bosch, T.C.G. (2007). Molecular phylogenetics in Hydra, a classical model in evolutionary developmental biology. *Mol. Phylogenet. Evol.* *44*, 281–290.
- Hemrich, G., Khalturin, K., Boehm, A.-M., Puchert, M., Anton-Erxleben, F., Wittlieb, J., Klostermeier, U.C., Rosenstiel, P., Oberg, H.-H., Domazet-Lošo, T., 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. *Mol. Biol. Evol.* *29*, 3267–3280.
- Hentzer, M., Riedel, K., Rasmussen, T.B., Heydorn, A., Andersen, J.B., Parsek, M.R., Rice, S. a., Eberl, L., Molin, S., Høiby, N., et al. (2002). Inhibition of quorum sensing in

*Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology* 148, 87–102.

Hoang, T.T., Sullivan, S.A., Cusick, J.K., and Schweizer, H.P. (2002).  $\beta$ -Ketoacyl acyl carrier protein reductase (FabG) activity of the fatty acid biosynthetic pathway is a determining factor of 3-oxo-homoserine lactone acyl chain lengths. *Microbiology* 148, 3849–3856.

Hoffmann, T.W., Pham, H.-P., Bridonneau, C., Aubry, C., Lamas, B., Martin-Gallausiaux, C., Moroldo, M., Rainteau, D., Lapaque, N., Six, A., et al. (2015). Microorganisms linked to inflammatory bowel disease-associated dysbiosis differentially impact host physiology in gnotobiotic mice. *ISME J*.

Holstein, T.W., and Emschermann, P. (1995). *Süßwasserfauna von Mitteleuropa, Cnidaria: Hydrozoa/Kamptozoa* (Stuttgart, Jena, New York: Gustav Fischer Verlag).

Holstein, T.W., Hess, M.W., and Salvenmoser, W. (2010). Preparation techniques for transmission electron microscopy of Hydra. (Elsevier Inc.).

Hongoh, Y., Deevong, P., Inoue, T., Moriya, S., Trakulnaleamsai, S., Ohkuma, M., Noparatnaraporn, N., and Kudo, T. (2005). Intra- and Interspecific Comparisons of Bacterial Diversity and Community Structure Support Coevolution of Gut Microbiota and Termite Host Intra- and Interspecific Comparisons of Bacterial Diversity and Community Structure Support Coevolution of Gut Micro. *Appl. Environ. Microbiol.* 71, 6590–6599.

How, K.Y., Hong, K.-W., Sam, C.-K., Koh, C.-L., Yin, W.-F., and Chan, K.-G. (2015). Unravelling the genome of long chain N-acylhomoserine lactone-producing *Acinetobacter* sp. strain GG2 and identification of its quorum sensing synthase gene. *Front. Microbiol.* 6, 1–13.

Hsu, J., Chang, S.J., and Franz, A.H. (2006). MALDI-TOF and ESI-MS analysis of oligosaccharides labeled with a new multifunctional oligosaccharide tag. *J. Am. Soc. Mass Spectrom.* 17, 194–204.

Huang, J.J., Han, J., Zhang, L., Jared, R., and Leadbetter, J.R. (2003). Utilization of Acyl-Homoserine Lactone Quorum Signals for Growth by a Soil Pseudomonad and *Pseudomonas* Utilization of Acyl-Homoserine Lactone Quorum Signals for Growth by a Soil Pseudomonad and *Pseudomonas aeruginosa* PAO1. *Appl. Environ. Microbiol.*

Huang, J.J., Petersen, A., Whiteley, M., Leadbetter, R., Huang, J.J., Petersen, A., Whiteley, M., and Leadbetter, J.R. (2006). Identification of QuiP, the Product of Gene PA1032, as the Second Acyl-Homoserine Lactone Acylase of *Pseudomonas*. *Appl. Environ. Microbiol.* 72, 1190–1197.

Huang, W., Lin, Y., Yi, S., Liu, P., Shen, J., Shao, Z., and Liu, Z. (2012). QsdH, a Novel AHL Lactonase in the RND-Type Inner Membrane of Marine Pseudoalteromonas byunsanensis Strain 1A01261. *PLoS One* 7, 1–12.

Hufnagel, L.A., and Myhal, M.L. (1977). Observations on a spirochaete symbiotic in Hydra. *Trans Amer Micros Soc* 96, 406–411.

Inohara, N., Ogura, Y., Fontalba, A., Gutierrez, O., Pons, F., Crespo, J., Fukase, K., Inamura, S., Kusumoto, S., Hashimoto, M., et al. (2003). Host recognition of bacterial



- muramyl dipeptide mediated through NOD2: Implications for Crohn's disease. *J. Biol. Chem.* *278*, 5509–5512.
- Van Iten, H., de Moraes Leme, Juliana Guimarães Simões, Marcello Carlos Marques, A., and Collins G., A. (2006). Reassessment of the phylogenetic position of conulariids (?Ediacaran-Triassic) within the subphylum medusozoa (phylum cnidaria). *J. Syst. Palaeontol.* *4*, 109–118.
- Ji, G., Beavis, R., and Novick, R.P. (1997). Bacterial interference caused by autoinducing peptide variants. *Science* (80-. ). *276*, 2027–2030.
- Jimenez, P.N., Koch, G., Thompson, J. a, Xavier, K.B., Cool, R.H., and Quax, W.J. (2012). The multiple signaling systems regulating virulence in *Pseudomonas aeruginosa*. *Microbiol. Mol. Biol. Rev.* *76*, 46–65.
- Johnson, J.W., Fisher, J.F., and Mobashery, S. (2013). Bacterial cell-wall recycling. *Ann. N. Y. Acad. Sci.* *1277*, 54–75.
- Jung, S., Dingley, A.J., Augustin, R., Anton-Erxleben, F., Stanisak, M., Gelhaus, C., Gutschmann, T., Hammer, M.U., Podschun, R., Bonvin, A.M., et al. (2009). Hydramacin-1, structure and antibacterial activity of a protein from the basal metazoan Hydra. *J. Biol. Chem.* *284*, 1896–1905.
- Kalia, V.C. (2015). Quorum Sensing vs Quorum Quenching: A Battle with No End in Sight.
- Kaplan, H.B., and Greenberg, E.P. (1985). Diffusion of Autoinducer Is Involved in Regulation of the *Vibrio fischeri* Luminescence System. *Microbiology* *163*, 1210–1214.
- Khalturin, K., Hemmrich, G., Fraune, S., Augustin, R., and Bosch, T.C.G. (2009). More than just orphans: are taxonomically-restricted genes important in evolution? *Trends Genet.* *25*, 404–413.
- Kim, H., and Farrand, S.K. (1998). Opine catabolic loci from *Agrobacterium* plasmids confer chemotaxis to their cognate substrates. *Mol. Plant. Microbe. Interact.* *11*, 131–143.
- Kobayashi, M., Shinohara, M., Sakoh, C., Kataoka, M., and Shimizu, S. (1998). Lactone-ring-cleaving enzyme: genetic analysis, novel RNA editing, and evolutionary implications. *Proc. Natl. Acad. Sci. U. S. A.* *95*, 12787–12792.
- Koch, B., Liljefors, T., Persson, T., Nielsen, J., Kjelleberg, S., and Givskov, M. (2005). The LuxR receptor: the sites of interaction with quorum-sensing signals and inhibitors. *Microbiology* *151*, 3589–3602.
- Koch, G., Jimenez, P.N., Cool, R.H., and Quax, W.J. (2014). *Deinococcus radiodurans* can interfere with quorum sensing by expressing an AHL-acylase and an AHL-lactonase. *FEMS Microbiol. Lett.* *356*, 62–70.
- Krysciak, D., Schmeisser, C., Preuss, S., Riethausen, J., Quitschau, M., Grond, S., and Streit, W.R. (2011). Involvement of multiple loci in quorum quenching of autoinducer I molecules in the nitrogen-fixing symbiont *Rhizobium* (*Sinorhizobium*) sp. strain NGR234. *Appl. Environ. Microbiol.* *77*, 5089–5099.
- Krysciak, D., Grote, J., Rodriguez Orbegoso, M., Utpatel, C., Förstner, K.U., Li, L., Schmeisser, C., Krishnan, H.B., and Streit, W.R. (2014). RNA-seq in the broad host range

- strain *Sinorhizobium fredii* NGR234 identifies a large set of genes linked to quorum sensing-dependent regulation in the background of a *tral* and *ngri* deletion mutant. *Appl. Environ. Microbiol.* *80*, 5655–5671.
- Kurokawa, K., Itoh, T., Kuwahara, T., Oshima, K., Toh, H., Toyoda, A., Takami, H., Morita, H., Sharma, V.K., Srivastava, T.P., et al. (2007). Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. *DNA Res.* *14*, 169–181.
- Lange, C., Hemmrich, G., Klostermeier, U.C., López-Quintero, J.A., Miller, D.J., Rahn, T., Weiss, Y., Bosch, T.C.G., and Rosenstiel, P. (2011). Defining the origins of the NOD-like receptor system at the base of animal evolution. *Mol. Biol. Evol.* *28*, 1687–1702.
- LaSarre, B., and Federle, M.J. (2013). Exploiting quorum sensing to confuse bacterial pathogens. *Microbiol. Mol. Biol. Rev.* *77*, 73–111.
- Lazazzera, B. a. (2000). Quorum sensing and starvation: Signals for entry into stationary phase. *Curr. Opin. Microbiol.* *3*, 177–182.
- Leadbetter, J.R., and Greenberg, E.P. (2000). Metabolism of acyl-homoserine lactone quorum-sensing signals by *Variovorax paradoxus*. *J. Bacteriol.* *182*, 6921–6926.
- Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.M., and Hoffmann, J.A. (1996). The dorsoventral regulatory gene cassette *spatzle/Toll/Cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* *86*, 973–983.
- Lenhoff, H.M., and Brown, R.D. (1970). Mass Culture of *Hydra*: an Improved Method and Its Application to other aquatic invertebrates. *Lab. Anim.* *4*, 139–154.
- Letran, S.E., Lee, S.-J., Atif, S.M., Flores-Langarica, A., Uematsu, S., Akira, S., Cunningham, A.F., and McSorley, S.J. (2011). TLR5-deficient mice lack basal inflammatory and metabolic defects but exhibit impaired CD4 T cell responses to a flagellated pathogen. *J. Immunol.* *186*, 5406–5412.
- Ley, R.E., Turnbaugh, P.J., Klein, S., and Gordon, J.I. (2006). Microbial ecology: human gut microbes associated with obesity. *Nature* *444*, 1022–1023.
- Li, X., Pietschke, C., Fraune, S., Altrock, P.M., Bosch, T.C.G., and Traulsen, A. (2015). Which games are growing bacterial populations playing? *J. R. Soc. Interface* *12*, 1–10.
- Liao, Y., Smyth, G.K., and Shi, W. (2013). The Subread aligner: Fast, accurate and scalable read mapping by seed-and-vote. *Nucleic Acids Res.* *41*, 1–17.
- Liao, Y., Smyth, G.K., and Shi, W. (2014). FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* *30*, 923–930.
- Lin, Y.H., Xu, J.L., Hu, J., Wang, L.H., Leong Ong, S., Renton Leadbetter, J., and Zhang, L.H. (2003). Acyl-homoserine lactone acylase from *Ralstonia* strain XJ12B represents a novel and potent class of quorum-quenching enzymes. *Mol. Microbiol.* *47*, 849–860.
- Lo, H.H., and Chang, S.M. (2014). Identification, characterization, and biofilm formation of clinical *Chryseobacterium gleum* isolates. *Diagn. Microbiol. Infect. Dis.* *79*, 298–302.

- Lodes, M.J., Cong, Y., Elson, C.O., Mohamath, R., Landers, C.J., Targan, S.R., Fort, M., and Hershberg, R.M. (2004). Bacterial flagellin is a dominant antigen in Crohn disease. *J. Clin. Invest.* *113*, 1296–1306.
- Lofgren, E.T., Cole, S.R., Weber, D.J., Anderson, D.J., and Moehring, R.W. (2014). Hospital-Acquired *Clostridium difficile* Infections Estimating All- Cause Mortality and Length of Stay. *Epidemiology* *25*, 570–575.
- Luo, W., Friedman, M.S., Shedden, K., Hankenson, K.D., and Woolf, P.J. (2009). GAGE: generally applicable gene set enrichment for pathway analysis. *BMC Bioinformatics* *10*, 1–17.
- Luo, Z.Q., Clemente, T.E., and Farrand, S.K. (2001). Construction of a derivative of *Agrobacterium tumefaciens* C58 that does not mutate to tetracycline resistance. *Mol. Plant. Microbe. Interact.* *14*, 98–103.
- Lupton, J.R. (2004). Diet Induced Changes in the Colonic Environment and Colorectal Cancer Microbial Degradation Products Influence Colon Cancer Risk: *J. Nutr.* *134*, 479–482.
- Lyon, G.J., Wright, J.S., Christopoulos, A., Novick, R.P., and Muir, T.W. (2002). Reversible and specific extracellular antagonism of receptor-histidine kinase signaling. *J. Biol. Chem.* *277*, 6247–6253.
- Mackness, M., and Mackness, B. (2015). Human paraoxonase-1 ( PON1 ): Gene structure and expression , promiscuous activities and multiple physiological roles. *Gene* *1*, 12–21.
- Macnab, R.M. (2003). How bacteria assemble flagella. *Annu. Rev. Microbiol.* *57*, 77–100.
- Madhaiyan, M., Poonguzhali, S., Saravanan, V.S., Hari, K., Lee, K.C., and Lee, J.S. (2013). *Duganella sacchari* sp. nov. and *Duganella radialis* sp. nov., two novel species isolated from rhizosphere of field-grown sugar cane. *Int. J. Syst. Evol. Microbiol.* *63*, 1126–1131.
- Manefield, M., Rasmussen, T.B., Hentzer, M., Andersen, J.B., Steinberg, P., Kjelleberg, S., and Givskov, M. (2002). Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. *Mircobiology* *148*, 1119–1127.
- Markov, a. V., Lazebny, O.E., Goryacheva, I.I., Antipin, M.I., and Kulikov, a. M. (2009). Symbiotic bacteria affect mating choice in *Drosophila melanogaster*. *Anim. Behav.* *77*, 1011–1017.
- De Martel, C., Ferlay, J., Franceschi, S., Vignat, J., Bray, F., Forman, D., and Plummer, M. (2012). Global burden of cancers attributable to infections in 2008: A review and synthetic analysis. *Lancet Oncol.* *13*, 607–615.
- Martin, V.J., Littlefield, C.L., Archer, W.E., and Bode, H.R. (1997). Embryogenesis in *Hydra*. *Biol. Bull.* *192*, 345–363.
- Martínez, D.E., Iñiguez, A.R., Percell, K.M., Willner, J.B., Signorovitch, J., and Campbell, R.D. (2010). Phylogeny and biogeography of *Hydra* (Cnidaria: Hydridae) using mitochondrial and nuclear DNA sequences. *Mol. Phylogenet. Evol.* *57*, 403–410.

- Martínez-García, E., Nickel, P.I., Chavarría, M., and de Lorenzo, V. (2014). The metabolic cost of flagellar motion in *Pseudomonas putida* KT2440. *Environ. Microbiol.* *16*, 291–303.
- Mathesius, U., Mulders, S., Gao, M., Teplitski, M., Caetano-anolle, G., Rolfe, B.G., and Bauer, W.D. (2003). Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. *Proc. Natl. Acad. Sci. USA* *100*, 1444–1449.
- Maximilian, R., De Nys, R., Holmström, C., Gram, L., Givskov, M., Crass, K., Kjelleberg, S.A., and Steinberg, P.D. (1998). Chemical mediation of bacterial surface colonisation by secondary metabolites from the red alga *Delisea pulchra*. *Aquat. Microb. Ecol.* *15*, 233–246.
- McCole, D.F., and Barrett, K.E. (2003). Epithelial transport and gut barrier function in colitis. *Curr. Opin. Gastroenterol.* *19*, 578–582.
- McFall-Ngai, M.J., Hadfield, M.G., Bosch, T.C.G., Carey, H. V, Domazet-Lošo, T., Douglas, A.E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S.F., et al. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl. Acad. Sci. USA* *110*, 3229–3236.
- McKenzie, V.J., Bowers, R.M., Fierer, N., Knight, R., and Lauber, C.L. (2012). Co-habiting amphibian species harbor unique skin bacterial communities in wild populations. *ISME J.* *6*, 588–596.
- Mei, G.-Y.Y., Yan, X.-X.X., Turak, A., Luo, Z.-Q.Q., and Zhang, L.-Q.Q. (2010). AidH, an alpha/beta-hydrolase fold family member from an *Ochrobactrum* sp. strain, is a novel N-acylhomoserine lactonase. *Appl. Environ. Microbiol.* *76*, 4933–4942.
- Meinhardt, H., and Gierer, A. (1974). Applications of a theory of biological pattern formation based on lateral inhibition. *J. Cell Sci.* *15*, 321–346.
- Meinhardt, H., and Gierer, A. (2000). Pattern formation by local self-activation and lateral inhibition. *Bioessays* *22*, 753–760.
- Miller, D.J., Hemmrich, G., Ball, E.E., Hayward, D.C., Khalturin, K., Funayama, N., Agata, K., and Bosch, T.C.G. (2007). The innate immune repertoire in cnidaria-ancestral complexity and stochastic gene loss. *Genome Biol.* *8*, R59.1–R59.13.
- Miller, S.D., Haddock, S.H.D., Elvidge, C.D., and Lee, T.F. (2005). Detection of a bioluminescent milky sea from space. *Proc. Natl. Acad. Sci. U. S. A.* *102*, 14181–14184.
- Millikan, D.S., and Ruby, E.G. (2003). FlrA , a sigma<sup>54</sup> -Dependent Transcriptional Activator in *Vibrio fischeri* , Is Required for Motility and Symbiotic Light-Organ Colonization. *J. Bacteriol.* *185*, 3547–3557.
- Mohamed, N.M., Cicirelli, E.M., Kan, J., Chen, F., Fuqua, C., and Hill, R.T. (2008). Diversity and quorum-sensing signal production of Proteobacteria associated with marine sponges. *Environ. Microbiol.* *10*, 75–86.
- Nakatsuji, T., and Gallo, R.L. (2012). Antimicrobial Peptides: Old Molecules with New Ideas. *J. Invest. Dermatol.* *132*, 887–895.
- Ng, W.L., and Bassler, B.L. (2009). Bacterial quorum-sensing network architectures. *Annu. Rev. Genet.* *43*, 197–222.

- Niu, C., Clemmer, K.M., Bonomo, R. a., and Rather, P.N. (2008). Isolation and characterization of an autoinducer synthase from *Acinetobacter baumannii*. *J. Bacteriol.* *190*, 3386–3392.
- O'Toole, R., Lundberg, S., Fredriksson, S.Å., Jansson, A., Nilsson, B., and Wolf-Watz, H. (1999). The chemotactic response of *Vibrio anguillarum* to fish intestinal mucus is mediated by a combination of multiple mucus components. *J. Bacteriol.* *181*, 4308–4317.
- Ochman, H., Worobey, M., Kuo, C.H., Ndjango, J.B., Peeters, M., Hahn, B.H., and Hugenholtz, P. (2010). Evolutionary relationships of wild hominids recapitulated by gut microbial communities. *PLoS Biol* *8*.
- Ott, B.M., Rickards, A., Gehrke, L., and Rio, R.V.M. (2015). Characterization of shed medicinal leech mucus reveals a diverse microbiota. *Front. Microbiol.* *5*, 1–10.
- Ottemann, K.M., and Miller, J.F. (1997). Roles for motility in bacterial – host interactions. *Mol. Microbiol.* *24*, 1109–1117.
- Pandya, S., Iyer, P., Gaitonde, V., Parekh, T., and Desai, A. (1999). Chemotaxis of *Rhizobium* sp.S2 towards *Cajanus cajan* root exudate and its major components. *Curr. Microbiol.* *38*, 205–209.
- Park, S.Y., Lee, S.J., Oh, T.K., Oh, J.W., Koo, B.T., Yum, D.Y., and Lee, J.K. (2003). AhlD, an N-acylhomoserine lactonase in *Arthrobacter* sp., and predicted homologues in other bacteria. *Microbiology* *149*, 1541–1550.
- Parsek, M.R., Schaefer, a L., and Greenberg, E.P. (1997). Analysis of random and site-directed mutations in rhII, a *Pseudomonas aeruginosa* gene encoding an acylhomoserine lactone synthase. *Mol. Microbiol.* *26*, 301–310.
- Patankar, A. V., and González, J.E. (2009). Orphan LuxR regulators of quorum sensing: Review article. *FEMS Microbiol. Rev.* *33*, 739–756.
- Paulsen, I.T., Press, C.M., Ravel, J., Kobayashi, D.Y., Myers, G.S. a, Mavrodi, D. V, DeBoy, R.T., Seshadri, R., Ren, Q., Madupu, R., et al. (2005). Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. *Nat. Biotechnol.* *23*, 873–878.
- Pehl, M.J., da Jamieson, W., Kong, K., Forbester, J.L., Fredendall, R.J., Gregory, G. a., McFarland, J.E., Healy, J.M., and Orwin, P.M. (2012). Genes that influence swarming motility and biofilm formation in *variovorax paradoxus* EPS. *PLoS One* *7*, 1–12.
- Petnicki-Ocwieja, T., Hrnčir, T., Liu, Y.-J., Biswas, A., Hudcovic, T., Tlaskalova-Hogenova, H., and Kobayashi, K.S. (2009). Nod2 is required for the regulation of commensal microbiota in the intestine. *Proc. Natl. Acad. Sci. U. S. A.* *106*, 15813–15818.
- Philpott, D.J., and Girardin, S.E. (2004). The role of Toll-like receptors and Nod proteins in bacterial infection. *Mol. Immunol.* *41*, 1099–1108.
- Piel, J. (2010). Biosynthesis of polyketides by trans-AT polyketide synthases. *Nat. Prod. Rep.* *27*, 996–1047.

- Piper, K.R., Beck von Bodman, S., and Farrand, S.K. (1993). Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid transfer by autoinduction. *Nature* 362, 448–450.
- Poltorak, A., He, X., Smirnova, I., Liu, M.Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., et al. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282, 2085–2088.
- Primo-Parmo, S.L., Sorenson, R.C., Treiber, J., and La Du, B.N. (1996). The Human Serum Paraoxonase / Arylesterase Gene ( PON1 ) Is One Member of a Multigene Family. *Genomics* 498–507.
- Rainey, P.B. (1999). Adaptation of *Pseudomonas fluorescens* to the plant rhizosphere. *Environ. Microbiol.* 1, 243–257.
- Ransome, E., Munn, C.B., Halliday, N., Cámara, M., and Tait, K. (2014). Diverse profiles of N-acyl-homoserine lactone molecules found in cnidarians. *FEMS Microbiol. Ecol.* 87, 315–329.
- Reddy, S.T., Wadleigh, D.J., Grijalva, V.R., Ng, C., Hama, S., Gangopadhyay, A., Shih, D.M., Lusic, A.J., Navab, M., and Fogelman, A.M. (2001). Human Paraoxonase-3 Is an HDL-Associated Enzyme With Biological Activity Similar to Paraoxonase-1 Protein but Is Not Regulated by Oxidized Lipids. *Arterioscler. Thromb. Vasc. Biol.* 21, 542–547.
- Rehm, B.H.A. (2008). *Pseudomonas: Model Organism, Pathogen, Cell Factory* (WILEY-VCH).
- Rehman, A., Sina, C., Gavrilova, O., Häslér, R., Ott, S., Baines, J.F., Schreiber, S., and Rosenstiel, P. (2011). Nod2 is essential for temporal development of intestinal microbial communities. *Gut* 60, 1354–1362.
- Reshef, L., Koren, O., Loya, Y., Zilber-Rosenberg, I., and Rosenberg, E. (2006). The Coral Probiotic Hypothesis. *Environ. Microbiol.* 8, 2068–2073.
- Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2009). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26, 139–140.
- Rohwer, F.L., Seguritan, V., Azam, F., and Knowlton, N. (2002). Diversity and distribution of coral-associated bacteria. *Mar. Ecol. Prog. Ser.* 243, 1–10.
- Rosenberg, E., Koren, O., Reshef, L., Efrony, R., and Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. *Nat. Rev. Microbiol.* 5, 355–362.
- Rosenberg, E., Sharon, G., and Zilber-Rosenberg, I. (2009). The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework. *Env. Microbiol.*
- Rosenstiel, P., Jacobs, G., Till, a., and Schreiber, S. (2008). NOD-like receptors: Ancient sentinels of the innate immune system. *Cell. Mol. Life Sci.* 65, 1361–1377.
- Ruby, E.G. (1996). Lessons from a cooperative, bacterial-animal association: the *Vibrio fischeri*-*Euprymna scolopes* light organ symbiosis. *Annu Rev Microbiol* 50, 591–624.

- Ruby, E.G., and Asato, L.M. (1993). Growth and flagellation of *Vibrio fischeri* during initiation of the sepiolid squid light organ symbiosis. *Arch. Microbiol.* *159*, 160–167.
- Rungrassamee, W., Klanchui, A., Maibunkaew, S., Chaiyapechara, S., Jiravanichpaisal, P., and Karoonuthaisiri, N. (2014). Characterization of intestinal bacteria in wild and domesticated adult black tiger shrimp (*Penaeus monodon*). *PLoS One* *9*, 1–11.
- Rutherford, S.T., and Bassler, B.L. (2012). Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harb. Perspect. Med.* *2*, 1–26.
- Salzman, N.H., Hung, K., Haribhai, D., Chu, H., Karlsson, J., Amir, E., Tegatz, P., Barman, M., Hayward, M., Stoel, M., et al. (2010). Enteric defensins are essential regulators of intestinal microbial ecology. *Nat. Immunol.* *11*, 76–82.
- Sanders, C.J., Yu, Y., Moore, D. a, Williams, I.R., and Gewirtz, A.T. (2006). Humoral immune response to flagellin requires T cells and activation of innate immunity. *J. Immunol.* *177*, 2810–2818.
- Sartor, R.B., and Mazmanian, S.K. (2012). Intestinal Microbes in Inflammatory Bowel Diseases. *Am. J. Gastroenterol. Suppl.* *1*, 15–21.
- Schaefer, A.L., Val, D.L., Hanzelka, B.L., Cronan Jr., J.E., and Greenberg, E.P. (1996a). Generation of cell-to-cell signals in quorum sensing: acyl homoserine lactone synthase activity of a purified *Vibrio fischeri* LuxI protein. *Proc Natl Acad Sci U S A* *93*, 9505–9509.
- Schaefer, A.L., Hanzelka, B.L., Eberhard, A., and Greenberg, E.P. (1996b). Quorum sensing in *Vibrio fischeri*: Probing autoinducer-LuxR interactions with autoinducer analogs. *J. Bacteriol.* *178*, 2897–2901.
- Scharf, M.E., Karl, Z.J., Sethi, A., and Boucias, D.G. (2011). Multiple levels of synergistic collaboration in termite lignocellulose digestion. *PLoS One* *6*, 1–7.
- Schauder, S., and Bassler, B.L. (2001). The languages of bacteria. *Genes Dev.* *15*, 1468–1480.
- Scheffe, J.H., Lehmann, K.E., Buschmann, I.R., Unger, T., and Funke-Kaiser, H. (2006). Quantitative real-time RT-PCR data analysis: current concepts and the novel “gene expression’s CT difference” formula. *J. Mol. Med.* *84*, 901–910.
- Scheurwater, E., Reid, C.W., and Clarke, A.J. (2008). Lytic transglycosylases: Bacterial space-making autolysins. *Int. J. Biochem. Cell Biol.* *40*, 586–591.
- Schlaeppli, K., Dombrowski, N., Oter, R.G., Ver Loren van Themaat, E., and Schulze-Lefert, P. (2014). Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives. *Proc. Natl. Acad. Sci. U. S. A.* *111*, 585–592.
- Schulze, P. (1917). Neue Beitrage zu einer Monographie der Gattung Hydra. *Arch. F. Biontologie* *4*, 33–119. .
- Schwentner, M., and Bosch, T.C.G. (2015). Revisiting the age, evolutionary history and species level diversity of the genus Hydra (Cnidaria: Hydrozoa). *Mol. Phylogenet. Evol.* *91*, 41–55.
- Seeley, T.D. (1989). The Honey Bee Colony as a Superorganism. *Am. Sci.* *77*, 546–553.

- Shai, Y. (1999). Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by  $\alpha$ -helical antimicrobial and cell non-selective membrane-lytic peptides. *Biochim. Biophys. Acta* 1462, 55–70.
- Shaw, P.D., Ping, G., Daly, S.L., Cha, C., Cronan Jr., J.E., Rinehart, K.L., and Farrand, S.K. (1997). Detecting and characterizing N-acyl-homoserine lactone signal molecules by thin-layer chromatography. *Proc. Natl. Acad. Sci. U. S. A.* 94, 6036–6041.
- Shepherd, R.W., and Lindow, S.E. (2009). Two dissimilar N-acyl-homoserine lactone acylases of *Pseudomonas syringae* influence colony and biofilm morphology. *Appl. Environ. Microbiol.* 75, 45–53.
- Sheu, S.Y., Chiu, T.F., Cho, N.T., Chou, J.H., Sheu, D.S., Arun, a. B., Young, C.C., Chen, C.A., Wang, J.T., and Chen, W.M. (2009). *Flectobacillus roseus* sp. nov., isolated from freshwater in Taiwan. *Int. J. Syst. Evol. Microbiol.* 59, 2546–2551.
- Shimizu, H., Takaku, Y., Zhang, X., and Fujisawa, T. (2007). The aboral pore of hydra: evidence that the digestive tract of hydra is a tube not a sac. *Dev. Genes Evol.* 217, 563–568.
- Shiner, E.K., Rumbaugh, K.P., and Williams, S.C. (2005). Inter-kingdom signaling: deciphering the language of acyl homoserine lactones. *FEMS Microbiol Rev* 29, 935–947.
- Simanski, M., Babucke, S., Eberl, L., and Harder, J. (2012). Paraoxonase 2 acts as a quorum sensing-quenching factor in human keratinocytes. *J. Invest. Dermatol.* 132, 2296–2299.
- Sio, C.F., Otten, L.G., Cool, R.H., Diggle, S.P., Braun, P.G., Bos, R., Daykin, M., Ca, M., Williams, P., and Quax, W.J. (2006). Quorum Quenching by an N -Acyl-Homoserine Lactone Acylase from *Pseudomonas aeruginosa* PAO1. *Infect. Immun.* 74, 1673–1682.
- Skerker, J.M., Prasol, M.S., Perchuk, B.S., Biondi, E.G., and Laub, M.T. (2005). Two-component signal transduction pathways regulating growth and cell cycle progression in a bacterium: a system-level analysis. *PLoS Biol.* 3, 1770–1788.
- Smith, R.S., and Iglewski, B.H. (2003). *P. aeruginosa* quorum-sensing systems and virulence. *Curr. Opin. Microbiol.* 6, 56–60.
- Smith, K., McCoy, K.D., and Macpherson, A.J. (2007). Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin. Immunol.* 19, 59–69.
- Solomon, J.M., Lazazzera, B. a, and Grossman, a D. (1996). Purification and characterization of an extracellular peptide factor that affects two different developmental pathways in *Bacillus subtilis*. *Genes Dev* 10, 2014–2024.
- Sommer, F., and Bäckhed, F. (2013). The gut microbiota--masters of host development and physiology. *Nat. Rev. Microbiol.* 11, 227–238.
- Stock, J.B., Ninfa, A.J., and Stock, A.M. (1989). Protein phosphorylation and regulation of adaptive responses in bacteria. *Microbiol Rev.* 53, 450–490.
- Stoltz, D.A., Ozer, E.A., Ng, C.J., Yu, J.M., Reddy, S.T., Lusic, A.J., Bourquard, N., Parsek, M.R., Zabner, J., and Shih, D.M. (2007). Paraoxonase-2 deficiency enhances



*Pseudomonas aeruginosa* quorum sensing in murine tracheal epithelia. *Am J Physiol Lung Cell Mol Physiol* 292, L852–L860.

Swift, S., Karlyshev, A. V., Fish, L., Durant, E.L., Winson, M.K., Chhabra, S.R., Williams, P., Macintyre, S., and Stewart, G.S. (1997). Quorum sensing in *Aeromonas hydrophila* and *Aeromonas salmonicida*: identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179, 5271–5281.

Tasteyre, a., Barc, M.C., Collignon, A., Boureau, H., and Karjalainen, T. (2001). Role of FliC and FliD flagellar proteins of *Clostridium difficile* in adherence and gut colonization. *Infect. Immun.* 69, 7937–7940.

Taylor, M.W., Schupp, P.J., Baillie, H.J., Charlton, T.S., Nys, R. De, Kjelleberg, S., and Steinberg, P.D. (2004). Evidence for Acyl Homoserine Lactone Signal Production in Bacteria Associated with Marine Sponges. *Appl. Environ. Microbiol.* 70, 4387–4389.

Tempé, J., Petit, A., Holsters, M., Montagu, M., and Schell, J. (1977). Thermosensitive step associated with transfer of the Ti plasmid during conjugation: Possible relation to transformation in crown gall. *Proc. Natl. Acad. Sci. USA* 74, 2848–2849.

Teplitski, M., Mathesius, U., and Rumbaugh, K.P. (2011). Perception and Degradation of N -Acyl Homoserine Lactone Quorum Sensing Signals by Mammalian and Plant Cells. *Chem. Rev.* 111, 100–116.

Teta, R., Gurgui, M., Helfrich, E.J.N., Künne, S., Schneider, A., Van Echten-Deckert, G., Mangoni, A., and Piel, J. (2010). Genome mining reveals Trans-AT polyketide synthase directed antibiotic biosynthesis in the bacterial phylum bacteroidetes. *ChemBioChem* 11, 2506–2512.

Tout, J., Thomas C Jeffries, K.P., Tyson, G.W., Webster, N.S., Garren, M., Stocker, R., Ralph, P.J., and Seymour, J.R. (2015). Chemotaxis by natural populations of coral reef bacteria. *ISME J.* 9, 1764–1777.

Tremaroli, V., and Bäckhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. *Nature* 489, 242–249.

Trembley, A. (1744). Mémoires, pour servir à l'histoire d'un genre de polypes d'eau douce, à bras en forme de cornes (Leiden: Jean Verbeeck, Herman Verbeeck).

Trindade-Silva, A.E., Rua, C.P.J., Andrade, B.G.N., Vicente, A.C.P., Silva, G.G.Z., Berlinck, R.G.S., and Thompson, F.L. (2013). Polyketide synthase gene diversity within the microbiome of the sponge *Arenosclera brasiliensis*, endemic to the southern Atlantic Ocean. *Appl. Environ. Microbiol.* 79, 1598–1605.

Turnbaugh, P.J., Ley, R.E., Mahowald, M. a, Magrini, V., Mardis, E.R., and Gordon, J.I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444, 1027–1031.

Uroz, S., Chhabra, S.R., Cámara, M., Williams, P., Oger, P., and Dessaux, Y. (2005). N-Acylhomoserine lactone quorum-sensing molecules are modified and degraded by *Rhodococcus erythropolis* W2 by both amidolytic and novel oxidoreductase activities. *Microbiology* 151, 3313–3322.

- Uroz, S., Oger, P.M., Chapelle, E., Adeline, M.T., Faure, D., and Dessaux, Y. (2008). A *Rhodococcus* *qsqA*-encoded enzyme defines a novel class of large-spectrum quorum-quenching lactonases. *Appl. Environ. Microbiol.* *74*, 1357–1366.
- Vaishnava, S., Yamamoto, M., Severson, K.M., Ruhn, K. a, Yu, X., Koren, O., Ley, R.E., Wakeland, E.K., and Hooper, L. V (2011). The antibacterial lectin RegIII $\gamma$  promotes the spatial segregation of microbiota and host in the intestine. *Science* *334*, 255–258.
- Vandewalle, A. (2008). Toll-like Receptors and Renal Bacterial Infections. *Chang Gung Med J.* *31*, 525–537.
- Vega Thurber, R., Willner-Hall, D., Rodriguez-Mueller, B., Desnues, C., Edwards, R.A., Angly, F., Dinsdale, E., Kelly, L., and Rohwer, F. (2009). Metagenomic analysis of stressed coral holobionts. *Environ. Microbiol.* *11*, 2148–2163.
- Verberkmoes, N.C., Russell, A.L., Shah, M., Godzik, A., Rosenquist, M., Halfvarson, J., Lefsrud, M.G., Apajalahti, J., Tysk, C., Hettich, R.L., et al. (2009). Shotgun metaproteomics of the human distal gut microbiota. *ISME J.* *3*, 179–189.
- Verma, S.C., and Miyashiro, T. (2013). Quorum sensing in the squid-Vibrio symbiosis. *Int. J. Mol. Sci.* *14*, 16386–16401.
- Van der Waaij, L. a, Limburg, P.C., Mesander, G., and van der Waaij, D. (1996). In vivo IgA coating of anaerobic bacteria in human faeces. *Gut* *38*, 348–354.
- Wadhams, G.H., and Armitage, J.P. (2004). Making sense of it all: bacterial chemotaxis. *Nat. Rev. Mol. Cell Biol.* *5*, 1024–1037.
- Wagner, C.L., Taylor, S.N., and Johnson, D. (2008). Host Factors in Amniotic Fluid and Breast Milk that Contribute to Gut Maturation. *Clin. Rev. Allergy Immunol.* *34*, 191–204.
- Wagner, V.E., Bushnell, D., Passador, L., Brooks, a. I., and Iglewski, B.H. (2003). Microarray Analysis of *Pseudomonas aeruginosa* Quorum-Sensing Regulons: Effects of Growth Phase and Environment. *J. Bacteriol.* *185*, 2080–2095.
- Wahjudi, M., Papaioannou, E., Hendrawati, O., van Assen, A.H.G., van Merkerk, R., Cool, R.H., Poelarends, G.J., and Quax, W.J. (2011). PA0305 of *Pseudomonas aeruginosa* is a quorum quenching acylhomoserine lactone acylase belonging to the Ntn hydrolase superfamily. *Microbiology* *157*, 2042–2055.
- Wang, H., Cai, T., Weng, M., Zhou, J., Cao, H., Zhong, Z., and Zhu, J. (2006). Conditional production of acyl-homoserine lactone-type quorum-sensing signals in clinical isolates of enterobacteria. *J. Med. Microbiol.* *55*, 1751–1753.
- Wang, L., Rothmund, D., Curd, H., and Reeves, P.R. (2003). Species-Wide Variation in the *Escherichia coli* Flagellin (H-Antigen) Gene. *J. Bacteriol.* *185*, 2936–2943.
- Wang, W.Z., Morohoshi, T., Ikenoya, M., Someya, N., and Ikeda, T. (2010). AiiM, a Novel Class of N-Acylhomoserine Lactonase from the Leaf-Associated Bacterium *Microbacterium testaceum*. *Appl. Environ. Microbiol.* *76*, 2524–2530.
- Wang, W.Z., Morohoshi, T., Someya, N., and Ikeda, T. (2012). Aidc, a Novel N-Acylhomoserine Lactonase from the Potato Root-Associated Cytophaga-Flavobacteria-

- Bacteroides (CFB) Group Bacterium *Chryseobacterium* sp. Strain strb126. *Appl. Environ. Microbiol.* **78**, 7985–7992.
- Weber, T., Blin, K., Duddela, S., Krug, D., Kim, H.U., Bruccoleri, R., Lee, S.Y., Fischbach, M. a., Muller, R., Wohlleben, W., et al. (2015). antiSMASH 3.0--a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res.* **43**, 237–243.
- WHO (2014). *ANTIMICROBIAL RESISTANCE Global Report on Surveillance 2014*.
- Williams, P. (2007). Quorum sensing, communication and cross-kingdom signalling in the bacterial world. *Microbiology* **153**, 3923–3938.
- Winson, M.K., Camara, M., Latifi, A., Foglino, M., Chhabra, S.R., Daykin, M., Bally, M., Chapon, V., Salmond, G.P., and Bycroft, B.W. (1995). Multiple N-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 9427–9431.
- Winson, M.K., Swift, S., Fish, L., Throup, J.P., Jorgensen, F., Chhabra, S.R., Bycroft, B.W., Williams, P., Stewart, G.S.A.B., Jørgensen, F., et al. (1998). Construction and analysis of luxCDABE -based plasmid sensors for investigating N-acyl homoserine lactone-mediated quorum sensing. *FEMS Microbiol Lett* **163**, 185–192.
- Wittlieb, J., Khalturin, K., Lohmann, J.U., Anton-Erxleben, F., and Bosch, T.C.G. (2006). Transgenic Hydra allow in vivo tracking of individual stem cells during morphogenesis. *Proc. Natl. Acad. Sci. USA* **103**, 6208–6211.
- Yang, Q., and Defoirdt, T. (2015). Quorum sensing positively regulates flagellar motility in pathogenic *Vibrio harveyi*. *Environ. Microbiol.* **17**, 960–968.
- Yates, E. a., Philipp, B., Buckley, C., Atkinson, S., Chhabra, S.R., Sockett, R.E., Goldner, M., Dessaux, Y., Cámara, M., Smith, H., et al. (2002). N-acylhomoserine lactones undergo lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. *Infect. Immun.* **70**, 5635–5646.
- Young, M.D., Wakefield, M.J., Smyth, G.K., and Oshlack, A. (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biol.* **11**, R14.
- Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature* **415**, 389–395.
- Zhang, H.-B., Wang, L.-H., and Zhang, L.-H. (2002). Genetic control of quorum-sensing signal turnover in *Agrobacterium tumefaciens*. *Proc. Natl. Acad. Sci. USA* **99**, 4638–4643.
- Zhu, J., and Winans, S.C. (1999). Autoinducer binding by the quorum-sensing regulator TraR increases affinity for target promoters in vitro and decreases TraR turnover rates in whole cells. *Proc. Natl. Acad. Sci. USA* **96**, 4832–4837.
- Zhu, J., and Winans, S.C. (2001). The quorum-sensing transcriptional regulator TraR requires its cognate signaling ligand for protein folding, protease resistance, and dimerization. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 1507–1512.

Zilber-Rosenberg, I., and Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *Fems Microbiol. Rev.* 32, 723–735.

Zimmer, B.L., May, A.L., Bhedi, C.D., Dearth, S.P., Prevatte, C.W., Pratte, Z., Campagna, S.R., and Richardson, L.L. (2014). Quorum Sensing Signal Production and Microbial Interactions in a Polymicrobial Disease of Corals and the Coral Surface Mucopolysaccharide Layer. *PLoS One* 9, 1–16.

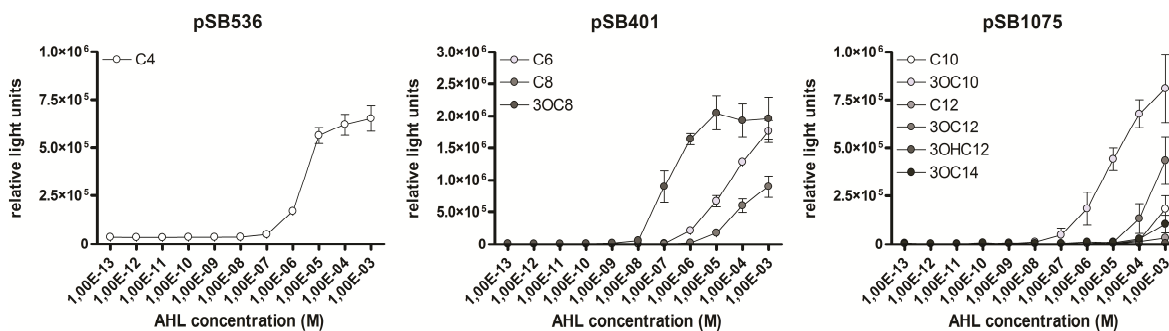
## 7 List of Publications

**Parts of the present thesis have been published previously:**

Li X, **Pietschke C**, Fraune S, Altrock P M, Bosch T C G and Traulsen A (2015) Which games are growing bacterial populations playing? *J. R. Soc. Interface* 12: 1–10.

## 8 Appendix

### 8.1 AHL-bioreporters



**Figure 35: Sensitivity of AHL-recognition by the employed AHL-bioreporters**

The AHL-bioreporter pSB536 (Swift et al., 1997) was used for the detection of C4-HSLs. While bioreporter pSB401 (Winson et al., 1995) possess the highest sensitivity towards mid-length AHLs, as C6-, C8- and 3OC8-HSLs. For detection of long-chain AHLs the bioreporter pSB1075 (Winson et al., 1995) was taken. The pSB1075 has a high sensitivity for 3-oxo-HSLs, but unmodified- and 3-hydroxy-HSLs cannot be detected (chapter 4.1.2). For all measurements commercially available AHLs were used, which were extracted with acidified ethyl acetate (chapter 5.23).

### 8.2 Sequence of Hydra's paraoxonase hyPON

#### 8.2.1 cDNA sequence of Hydra's paraoxonase hyPON

The hyPON cDNA sequence from the transcriptome of *Hydra vulgaris* (AEP) can be found in the following. The section, marked in red, indicates the coding sequence, while the underline denotes the sequence of the signal peptide.

```
>db454_haep_celera_v02_6197 length:1612 numreads:170 (ext:59 wt:29
ecto:31 endo:6 nanos:12 pa:13 lps:11 flag:9)
GGCGCGGGACGGAAAAAACACATACTGAACTTTTTAGTCCCTGTGTAATAAGATTAACTTTAG
ATATATTTTATTTTTAAACACGAACTTACTCTAACCTGCATGAAAGTCATTTGAAGCTCTAAAAAT
GATAAATTTCAAAGCATTGTTGTAAGTGTGTTGCTGCTATCCTTATACCCAAAATTTACTTATG
GTTAACAAGTAACGCTGTGGGAGTTTCAGTTTTATAATGTCCTATCCTGGAATGTGTAATCTATTCC
TGGTATTGTCTGCGGTCTGAAAAAATTACAGTGACTTCCGATGGACTTGCTTTTTTTACTAGTGG
AATGAAAGGTGCATCAAATTTGTGATCATCGTTATCTGCATGGTCGATTTTATTTATTTGATTTTAA
AGATCCAGAAAAAGGAGCAACAGAAGTTTTTTTGAAGAGTAACAGTATATTACCCAGTAGTTTTAG
TCCCACGGGATGGACTTGTGTTGAAGATTTAAAAAATAATTTAATTAAGTCTGGTGATCAATCA
TGCTAATCATAGCGAGAGTGTGAAAGTTTTTATTTTTTAAACGTAGTGAACCTTCTTATTTGGATCA
TGTAATAACTTTAACTGATAAAAAGTTTGTGTTGCTTAAATGATATTGCTGTTATAAATGAAAATAT
ATTTTATGTTACTAATTATCTGAAGTACTGTCAATATAACGAATTTGCTTCATTGCTTTTTGAAAT
TGGTTTTAAAAATGCCACATCTAATATAGTTTACTATAATGAAGGAAGCACTTTAGTTGCAGCAGA
CGGTACTCCTCATTAACGGTATAGCCATAAGTAAAGATTTCTCCATTGTTTTTGTATCTTCAAC
TGCAAATAATACTTTATCTGTTTTTAAACGCGAAGCTAAATCAGGAAAACTTTTACTTCATAATGC
AATTGATATTTTATATAGTCTGACAATGTTTACATTGATCATCAAAGTGGTTTTTTATATGTTGC
AGTCTTAAAAAAACCACTTCATTTCTTCTTGGCAGTAAGAACTTAACTCTCAACACTGGAAGTGG
GATAAAAATTTGCTGACTCTAATCAATGAAAAATGCTGATGTAAGTGAAGTTTTTTCATGATAA
TGGTCAGCTTATTCAAAGTGCAAGCATGATAGTTTTTTTACAAAATCAATATTTAATTGGTTCCGT
```

TTATAACACATTGGTTTACTGCCGCACAGATAATATCTAAATGATATTATTTGTGCACATAAATCT  
TATTGTTAAATGAGATTTTATCTTCAACTCTTTTTTCGCCCTTCTCCTTAAACCTTCCATGCCTTT  
TTAAAAAAAAGAAAAAAGTTTATATTTTTTCAAAGAAATTTGTTTTCTAGGAAATAATCTGATTTT  
TTTCTGAAAAAGTTTTCTTAGATACACATTTGTTAAATGCTCTTTTAATACTCTTTGTGTGTGTGT  
GTGTGTGCGTGTGCGCGCGCGTGTGTGTGGTGTGTGTGTCAACTTAACACTCTTTTATAACTTTAA  
AGTTTAATGTTGTATTTTTCTTACGTATTTTATAAATCACCAGCTCGTTTTTTTTATAAAATAAGAT  
CTTTTTAATACCAAAAAAAAAAAAAAAAAA

## 8.2.2 Protein sequence of Hydra's paraoxonase HyPON

The protein sequence of the HyPON was generated with help of DNAMAN out of the cDNA sequence from the transcriptome of *Hydra vulgaris* (AEP). The sequence can be found in the following. The signal peptide is marked by an underline.

MINFKSIVVTVLSAILIPKIYLWLTSNAVGVQFYNVYPGMCKSIPGIVCGSEKITVTS DGLAFFTS  
GMKGASNCDHRYLHGRIYLFDFKDPEKGATELFLKSNSILPSSFSPHGMDLFEDLKNNLIKLLVIN  
HANHSESVEVFIFKRSEPSYLDHVKTLTDKKFVCLNDIAVINENIFYVTNYLKYCQYNEFASLLFE  
IGLKMPTSNIVYYNEGSTLVAADGYSSLNGIAISKDFSIVFVSSTANNLTSVFKREAKSGKLLLLHN  
AIDISYSPDNVYIDHQTGFLYVAVLKKPLHFFLAVRNLTLNTGTGIKIVPDSNQWKNADVTEVFHD  
NGQLIQSASMIVFYKNQYLIQSVYNTLVYCRDNI

## 8.3 Curvibacter's quorum sensing system

### 8.3.1 Quorum sensing operon - AHL-synthase curI and AHL-receptor curR1

Operon consisting of the AHL-synthase curI and the AHL-receptor curR1 of *Curvibacter* sp. CurI is marked in red, while curR1 is indicted with a green mark. Binding sides for the primer AEP1.3\_luxR1\_F and AEP1.3\_luxR1\_R, which were used for the CurR1-reporter pCPcurR1, are marked with boxes.

GCAAAAGACCGACTACCAAGCGGCCCTTTCCAAAGGTTACGCCGAGGTATATGCCTGATGGCAAGCG  
TTTGATGTTGGCGGCAGAGGATTATCCAAAACGACCCAACGACGCTGATTTCTTTGTAACGCACG]A  
AGAACC GGGAGGTCATTT CGAACCGACTACACGGGAAAGTTCGGCAGAAACATGACGGTTGT CAT  
TGAAGGTCTCCGAGACCCCGTACGGCCGTACTTTCCTACGCTCAAGAAGGTGGAGAAAGACGGCCA  
AGTCCTTTGGGAGCCGGTGGACGATCACATGTGGTTGATCGTACGGGATGTCTCCAGAGACGGGAA  
GCTTGCTGTCTATGACGACAGAGGTATCGGAATGTGTTTCCGTTATTTAGATCAGCCGCTTCGTCA  
TGAAGAGTGCTTTTCCAGAGCATCCTTTGGTTTGTATTGCTGTTGTGCGGGCCGACCCCGCACCCC  
GCACCCCGCCGGTTCGCGGGTGGACGGGGCTGTGCTCTCGTGATGGTTCACGGGCTTGGAGCGCGGT  
ATGAAAAGTTGGCAAGAAGATTTACTTTGACCTTGGCTTGGCAAGGCAACGTGTGAACGTGAGATT  
TTTAGACGAGTTGAAGAGGCTGCTTTAGCGCTTGGGTTCGAACATGTGGCCTACGGTTTTAGTCCG  
CCCACGCCGTT CAGTAGACCGAAAATTACACTGCTGAACAGCTACCCTCTGTCTGTGGCAAGAGCCG  
TATGCCAAGCGGATACTTCAAGTCGATCCAACCGTCTGCTCGCGGACGTGTTCTCAAGAGCCG  
ATTGTCTGGACAGACCAAGTTTTTGCGAATGCACAGAGTCTTTGGGATGAAGCCAGAGAGCACGGA  
CTGAGGGTTCGGCTGGGCACAGTCCAGTCTGGATGTCTTGGGTTCCGGGAGGCATGCTTACGCTGTCA  
AGGTCTTGCAGTTTTTTAAATCCCAGGAGCTTGAAGCCAAACAACAAAAAATGCGGTGGCTCGTT  
CAGGTGGCTCACCTAGCGTTTTTCGCGCTTGCTTCGGTGTAATCAAACAAATGGATCGCACAGGCTC

TCAGCCCGGGAGTTGGAGGTCTTGAAGTGGTCTGCCGACGGGAAGTCGGCGCAGGATATAGCGGAC  
 ATTCTGAACGTATCAAAGAACACGGTGGACTTTTACATCAAGAACTCCGTGGCTAAAATGCAGGTA  
 GCCAATAAGACAGCGGCAGTGGTACGCGCTGCATTGCTCGGGTTACTCTATTGACGCACCGTGTTT  
 GCGTTCTCCGGTAGTAATGCTTATCTCTGAGTGCAGCAGTGTATGTAGACCCGAGGAAAAGTGGG  
 CACGCGAAAATGCATGTTTCCGAATCGGGCCAAGTTCGAGCGACAGTCGACGATCTCTTGAGTGTG  
 TATTTTTTTTACCATTTTTTCCGGCTTAGATATTGAAGTTGGCGATCCTTCAAAGGCTGCGGTGCT  
 CCAACCGCTATCTCTGGATTACAGAATGGGTTGGTCACTCTTTGTTTCAGATCTCCATCGGTTGG  
 GATTGGCATTGGAAGTGAAGGAGACATCGTTTCGCTTGATGCGAGCCGATTGCCCTCGAACTAAT  
 GTTCAGCTTATCAATTCAGATGGGCGGGATTTCGCTGGGAACAAAATCTTCAGATTCTCGGGACG  
 ATTGTTGACCGTTACCTTGGGCGATCTCGGTAGAGACCCATTTTTCTTCCATCAGACGACGGGCTC  
 GTATCGATGTGACGAGTTTTCTCCCCGAGCATTCCCCGTTCCGCCGAGGGGCGACCTCTTCTA  
 GAGTGCGGCGCGTCCGACCATCTGACGCGCACTCAGTGCACCTCATCTCTTGGTCTGCTGCGGA  
 TTGTTTTCGCCGCCGTGAACATGAACCTACTTTTTTTTAGTAGGTTACACCACCCCAAACCTTGAACA  
 CAATAACCCCAATTGTAAAGGGGTGGGTATATGCATTGCGTTGCTGGTAGGCGAGAGGAATTGCCG  
 CCGCACCTGATGGAAGCGGTGGGGCGCTATCGTCACAAGGTTTTTGTGAGTACCTTGGTTGGTCA  
 TTGCGATCCTCGGGAGAGGTTGAGGCAGACGAGTTGATGGGCCTGACGCTGTATACGCTCTCCTCG  
 CAAGACAAGCATGGTACGTTAACGGCGTAGCGCGCCTATTGCCGACAACGGTTCCTTACTTGTTA  
 GAGAAAGTATTTCCATCGCTTTGGGGTGGACTTGAGTTGCCGCGCAGCGCTGAAGTATGGGAGTTG  
 TCGAGATTTGCTGCTGTGATTTTGATACTTGCGCATCCCTGGCAAATCAGGCTTCAAGTACAC  
 GCGGCAAGTCTATTTCAACAGGTGGTTCCGATTGCAGCAGAGCATGGAGCCCGCACTCTGATTACC  
 GTATCGCCGGTCCGAATGGAGCGTCTACTCCGAGTCAACGGATTTTCGAGCTATACGAGCAGGGGTT  
 CCTGCACAACAGGGCGGGTGCTCTGTTGTTGCGCTCAGTATTTCCCTCAGTGAATACATGGAGGTG  
 TGGCAATGAACCGTTCAAACCTAACTCGCTGGTACGCGTCTGTAACCCTCAGACCAATCGCGGAC  
 TGCTCATCCCGATTGATCATGGCCTTACGTGCGGTGAGATGGACGGTCTCCTCCACTCGTCGATGC  
 CGGCACGCTGGTTGAGTCACCCAGCGATCACGGCGGTGGTGGCGCATAAGGGTATGGTGCCTCGG

### 8.3.2 AHL-synthase *curl1* and AHL-receptor *curR1* - cDNA sequence

>AEP\_03680 *lasI\_2* Acyl-homoserine-lactone synthase  
 ATGCATTGCGTTGCTGGTAGGCGAGAGGAATTGCCGCCGACCTGATGGAAGCGGTGGGGCGCTAT  
 CGTCACAAGGTTTTTGTGAGTACCTTGGTTGGTCAATTGCGATCCTCGGGAGAGGTTGAGGCAGAC  
 GAGTTCGATGGGCCTGACGCTGTATACGCTCTCCTCGCAAGACAAGCATGGTACGTTAACGGCGTA  
 GCGCGCTATTGCCGACAACGGTTCTTACTTGTAGAGAAAGTATTTCCATCGCTTTGGGGTGGAA  
 CTTGAGTTGCCGCGCAGCGCTGAAGTATGGGAGTTGTGAGATTTGCTGCTGTGATTTTGATACT  
 TGCGCATCCCTGGCAAATCAGGCTTCAAGTACGCGGCAAGTCTATTTCAACAGGTGGTTCCGG  
 ATTGCAGCAGAGCATGGAGCCCGCACTCTGATTACCGTATCGCCGGTCCGAATGGAGCGTCTACTC  
 CGAGTCAACGGATTTTCGAGCTATACGAGCAGGGGTTCTTGCACAACAGGGCGGGTGCTCTGTTGTT  
 GCGCTCAGTATTTCCCTCAGTGAATACATGGAGGTGTGGCAATGA

>AEP\_03679 *sdiA\_2* Regulatory protein *SdiA*  
 ATGAAAAGTTGGCAAGAAGATTTACTTTGACCTTGCCTTGGCAAGGCAACGTGTGAACGTGAGATT  
 TTTAGACGAGTTGAAGAGGCTGCTTTAGCGCTTGGGTTCGAACATGTGGCCTACGGTTTTTCAAGTGC  
 CCCACGCCGTTCAAGTAGACCCGAAAATTACACTGCTGAACAGCTACCTCTGTCTGTTGGCAAGAGCGC  
 TATGCCAAAGCGGGATACCTTCAAGTGCATCCAACCGTTCGCTCGCGGACGTCGTTCTCAAGAGCCG  
 ATTGTCTGGACAGACCAAGTTTTTTCGAAATGCACAGAGTCTTTGGGATGAAGCCAGAGAGCACGGA  
 CTGAGGGTCCGGTGGGCACAGTCCAGTCTGGATGTCTTGGGTTCCGGGAGGCATGCTTACGCTGTCA  
 AGGTCTTGCAGTTTTTTAAATCCCAGGAGCTTGAAGCCAAACAACAAAAAATGCGGTGGCTCGTT  
 CAGGTGGCTCACCTAGCGTTTTTCGCGCTTGCTTCCGGTGAATCAAACAATGGATCGCACAGGCTC  
 TCAGCCCGGGAGTTGGAGGTCTTGAAGTGGTCTGCCGACGGGAAGTCGGCGCAGGATATAGCGGAC  
 ATTCTGAACGTATCAAAGAACACGGTGGACTTTTACATCAAGAACTCCGTGGCTAAAATGCAGGTA  
 GCCAATAAGACAGCGGCAGTGGTACGCGCTGCATTGCTCGGGTTACTCTATTGA



### 8.3.3 AHL-synthase *curl1* and AHL-receptor *curR1* – amino acid sequence

>AEP\_03680 *lasI\_2* Acyl-homoserine-lactone synthase  
MHCVAGRREELPPLHMEAVGRYRHKVFVSHLWGLRSSGEVEADEFDGPDAVYVSSQDKHGHVNGV  
ARLLPTTVPYLLKVFPSLWGGLELPRSAEVWELSRFAAVDFDTCASLANQASASHAAASLFQQVVR  
IAAEHGARTLITVSPVGMERLLRVNGFRAIRAGVPAQQGGCSVVALSIPLESEYMEVWQ\*

>AEP\_03679 *sdiA\_2* Regulatory protein *SdiA*  
MKSWQEDLLDLASGKATCEREIFRRVEEAALALGFEHVAYGFQSPFPFRPKITLLNSYPLSWQER  
YAQAGYLQVDPTVARGRRSQEPVWTDQVFANAQSLWDEAREHGLRVGWAQSSLDVLDGSGGMLTSL  
RSCEFLNPQELEAKQQKMRWLQVAHLAFSRLLRNQTNGSHRLSARELEVLRKWSADGKSAQDIAD  
ILNVSKNTVDFHIKNSVAKMQVANKTAAVVRAALLGLLY\*

### 8.3.4 Quorum sensing operon - AHL-synthase *curl2* and AHL-receptor *curR2*

Operon consisting of the AHL-synthase *curl2* and the AHL-receptor *curR2* of *Curvibacter* sp. *Curl2* is marked in red, while *curR2* is indicated with a green mark. Binding sites for the primer AEP1.3\_luxR2\_F and AEP1.3\_luxR2\_R, which were used for the *CurR2*-reporter pCP*curR2*, are marked with boxes.

GAAAAACGGTATGACAGCATCTAAATCTGGAAACGGCAAGCCCTTGGCGCAGCGTTAGGCTGCA  
GTCTGGGCATTTATTCTGGCGAAGGCCTCAATGTGGCGGTCAAATCGCCGCACTTCTTGAGACTCG  
AAACCTGTCTTGATAAGCAGGTACCGATGGCCAATATTGGATTTCGAAATGTCAGATTTTGTAAACC  
ACCATTTTTAATAGGTGACAGCTATCGCGAAAGATTTTTTGGAACTTAGCTACCAAGATTGGTAGG  
TTTCAAAGCCACCCAAGTGGCGTCCAATAGCGCAGTTACCTCTGAATCAGTCTCTTGTTGAGGG  
CGAGGGAACCGCCCTTCTAGCGATTACATACTTGCCGAATTTGTTCCACTGGATGCTTGGCAGGAG  
CATCAGGTATTCATTGGCGGAAAATTCTGTCAAACCTGCTCTGCATAAAAATGCATGTCGGCCATAC  
AGATTGGTCTACCGATCGCACAGGGAATGGGGGGTAGTTATGCAGAGCTGGTTTCGAGCAATTGAT  
TTGCAATCTGGACAGCACCAAGGATGAAAAGGAAATTTATGCCTATATCGCAGCAGCGGCACGTGA  
CCTCGGTTTTGAATACTGCGGTTTTGGCCTGCAGTTCCCTTATCCGCTGACCAGCAAACGGGTGGT  
TCTCCTGAATAACTACCCCAAGTCTGGCAAGAGCATTACCAAAAACAAAACACTACGTGAACCAAGA  
CCCTACCGTACTGCAAGGGCGCAGGAGCAATACGCCATTCCTCTGGAACGACGGATTGTTTCAGCGA  
TGCCACCGATATGTGGGAAGAGGCGCAGGCCATGGCCTGAGTCAGGGGTGGTCTCAGTCCAGTTT  
GGATGGATCGGGAGCCGCCAGCTTGCTCACGCTGGCACGCTCCAACGACAAGATTTCTGCGCTGGA  
GTTGCAGGCCAACGAAAGCCGCATGCGTTGGCTGGTGTGCGTGCACACCTGTGCTCTCCAACGT  
CTACCAGTCGGACCTGCGCGCGCAATTTAATCCCAACCTTACGGAGCGAGAGCTCGAGGTCTTGCG  
CTGGAGTGCAGATGGCAAGTCAGCGGGAGAGATTTCTGACATTTCTGTCCATCTCCAAAACACTGT  
GGACTTTACATCAAAAATGCAGTGCACAAGCTCCGAACCTCCAACAAGACAGCCGCCGTAGTGGC  
TGCCGTGATGATGGGCTGTTGTTCTGATGCTCGCCTTTGGTGAAAAGAGCCTTCCCATGAAACTT  
ATTGAAAATGGTCTCGTATTGGTCGGAATCCCCGAACTGATGCAGGTCCCTTTCAAGCATCTGGTG  
ACATCTCTGGACGAAGATAAGGACAACAGCCTGTCCAATTGCGGCCGTGCTGCCACCATCACGGGC  
TATACGGAGTGGTTTTTCGGAAACACAACCTACCTGACCGTGGGATGGGATGGGTGATCGATGAA  
CGCGCCCAAGGCAGCGCGCTTGGCGTTTTGGGCTCGCCGCGCACCAATGTCAATCTTGTGGCAAC  
GATGGTGGGCATTGCCGTGGGAGGCAAGTCTGGAAAAATTGGGGCAGCTGATGGATGCCCTGCTT  
CCTTGGCAGCAAACCGTCCATGCCTTTGAAGTGCCCCAACCGGTAGCTCAAATCGAGCATCTTCT  
CCCCTGAAAACATCTTGTGACCCTGTAAAAATAGTAGTTACAAGAAAGAAGAGCTTCGATGTA  
ATACCGTCCAACAAGATGGATGGGGAATTTCTGTTGATTTTCAGGGCAGGAACGGTAAATAGCTTTTC  
GAGTGAATATTTTTAGGGGCTTTTCGCAATACCGGCATCGCGTTTTTTGTGGAGATGCTGGGCTGGGA  
GTTGCAATGCGAGCACGGCATTGAAACGGACCAGTTCGACCGTAACGACACGGTCTATGTGGTGGC  
CCACGACGACGCATCTGAAATCATTGGATTAGGGCGCTTGCTGCCAACCAAGCCTTACTTGCT  
GGGAGAAGTCTTCCCTGAGCTTCTCAATGGCAAGACACCGCCAGCTCACCAGATGTGTGGGAGCT  
CTCCCGTTTCGACGAGTGGACTTCAATGCCAACGCGACTAGCGCCCTGGGGCAGTCTCCAGCGA  
GATTGCGATAGAGCTGTTGCACCAATCCATTGCGACCGCCCGGGCCCATGGTCCAAGGAATTGAT

TACGGTCTCCCATTTGGGAATTGAACGTCTGTTGCGCCGCGCTGGATTCAAGGCGCATCGCGCTGG  
 CCCGCCAAGATGTACGGACGCCATGCAGTATTTGCCAATGTCATTGACTGCTTGGCATAGAAACT  
 GAACTTACAAAAGAAGCCCGCACGAATCCGGGCTGAACCTTCGAAGATGGCCTCGTTCAGGGAGCT  
 AAAACGAGGGGCATACGCCCATCGGATATTTTAGAAGACTTGAGGCCAAACCATTGAGACCGTAAG

### 8.3.5 AHL-synthase *curl2* and AHL-receptor *curR2* - cDNA sequence

>AEP\_02249 *lasI\_1* Acyl-homoserine-lactone synthase  
 ATGGGGAATTTTCGTGGATTTTCAGGGCAGGAACGGTAAATAGCTTTTCGAGTGAATATTTTCAGGGG  
 CTTTCGCAATACCGGCATCGCGTTTTTTGTGGAGATGCTGGGCTGGGAGTTGCAATGCGAGCACGGC  
 ATTGAAACGGACCAGTTTCGACCGTAACGACACGGTCTATGTGGTGGCCACGACGACGCATCTGAA  
 ATCATTGGATTAGGGCGCTTGCTGCCAACCACCAAGCCTTACTTGCTGGGAGAAGTCTTCCCTGAG  
 CTTCTCAATGGCAAGACACCGCCAGCTCACCAGATGTGTGGGAGCTCTCCCGGTTTCGAGCAGTG  
 GACTTCAATGCCAACGCGACTAGCGCCCTGGGGCAGTTCTCCAGCGAGATTGCGATAGAGCTGTTG  
 CACCAATCCATTGCGACCGCCCGGGCCATGGTGCCAAGGAATTGATTACGGTCTCCCATTTGGGA  
 ATTGAACGTCTGTTGCGCCGCGCTGGATTCAAGGCGCATCGCGCTGGGCCGCCAAGATGTACGGA  
 CGCCATGCAGTATTTGCCAATGTCATTGACTGCTTGGCATAG

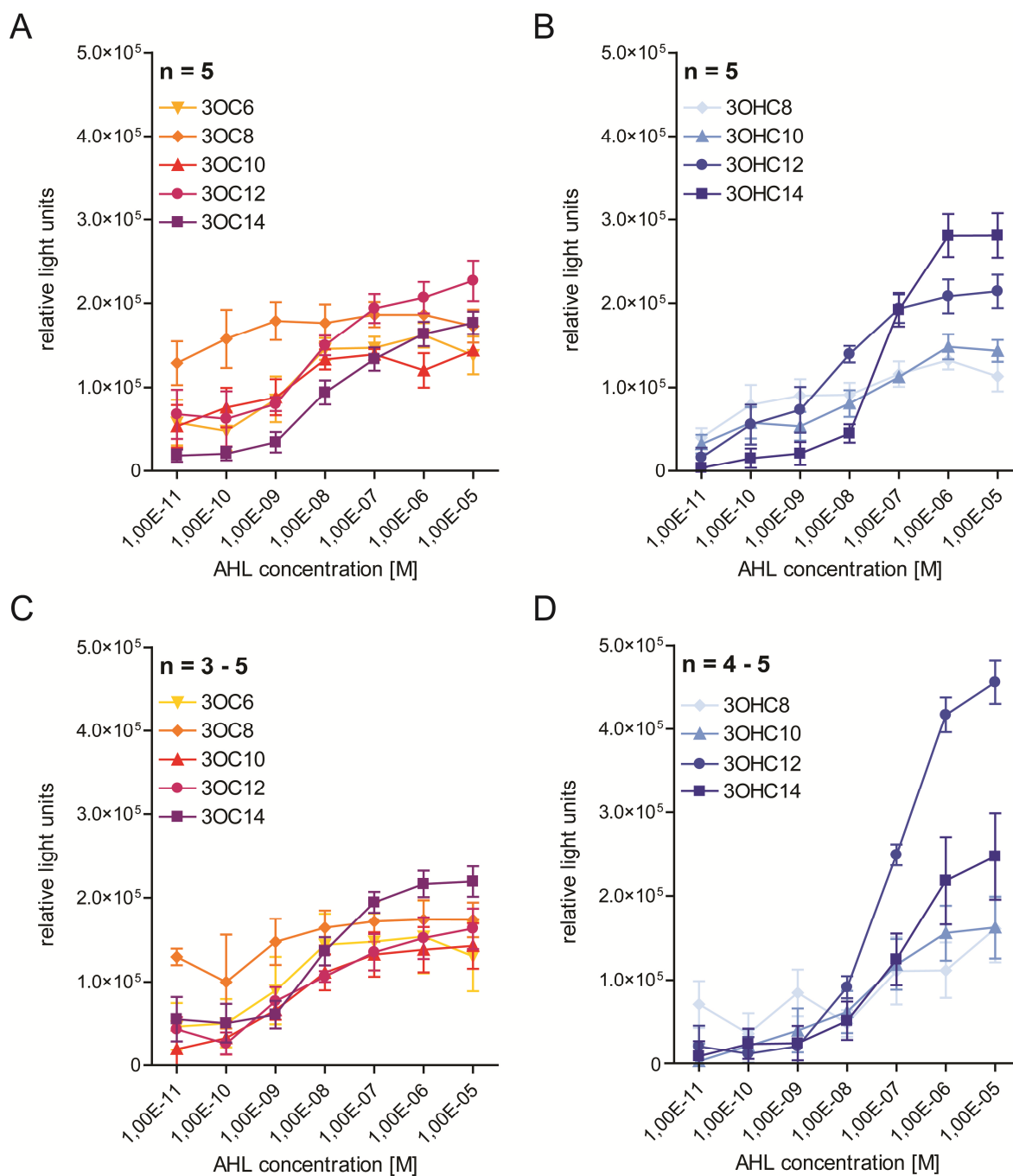
>AEP\_02251 *sdiA\_1* Regulatory protein *SdiA*  
 ATGCAGAGCTGGTTCGAGCAATTGATTTGCAATCTGGACAGCACCAAGGATGAAAAGGAAATTTAT  
 GCCTATATCGCAGCAGCGGCACGTGACCTCGGTTTTGAATACTGCGCGTTTTGGCCTGCAGTTCCCT  
 TATCCGCTGACCAGCAAACGGGTGGTTCTCCTGAATAACTACCCCAAGTCTGGCAAGAGCATTAC  
 CAAAACAAAACACTACGTGAACCAAGACCCTACCGTACTGCAAGGGCGCAGGAGCAATACGCCATT  
 CTCTGGAACGACGGATTGTTTCAGCGATGCCACCGATATGTGGGAAGAGGCGCAGGCCCATGGCCTG  
 AGTCAGGGGTGGTCTCAGTCCAGTTTGGATGGATCGGGAGCCGCCAGCTTGCTCACGCTGGCACGC  
 TCCAACGACAAGATTTCTGCGCTGGAGTTGCAGGCCAACGAAAGCCGCATGCGTTGGCTGGTGTGCG  
 GTCGCACACCTGTGCTCTCCAACGTCTACCAGTCGGACCTGCGCGCGCAATTTAATCCCAACCTT  
 ACGGAGCGAGAGCTCGAGGTCTTGCCTGGAGTGCAGATGGCAAGTCAGCGGGAGAGATTTCTGAC  
 ATTCTGTCCATCTCCAAAAACACTGTGGACTTTCACATCAAAAATGCAGTGCACAAGCTCCGAACC  
 TCCAACAAGACAGCCCGCGTAGTGCGTGCCGTGATGATGGGCCTGTTGTTCTGA

### 8.3.6 AHL-synthase *curl2* and AHL-receptor *curR2* – amino acid sequence

>AEP\_02249 *lasI\_1* Acyl-homoserine-lactone synthase  
 MGNFVDFRAGTVNSFSSEYFQGLSQYRHRVFEMLGWELQCEHGIETDQFDRNDTVYVVAHDDASE  
 I IGLGRLLPPTTKPYLLGEVFPPELLNGKTPPSSPDVWELSRFAAVDFNANATSALGQFSSEIAIELL  
 HQSIATARAHAKEKITVSPGLGIERLLRRAGFKAHRAGPPKMYGRHAVFANVIDCLA\*

>AEP\_02251 *sdiA\_1* Regulatory protein *SdiA*  
 MQSWFEQLICNLDSTKDEKEIYAYIAAAARDLGFYCAFGLOFPYPLTSKRVVLLNNYPQVWQEHY  
 QKQNYVNQDPTVLQGRSNTPLWNDGLFSDATDMWEEAQAHGLSQWSQSSLDGSGAASLLTLAR  
 SNDKISALELQANESRMRWLVSVAHLSLSNVYQSDLRAQFNPNLTERELEVLRWSADGKSAGEISD  
 ILSISKNTVDFHIKNAVHKLRTSNKTAAVVRAVMMGLLF\*

## 8.4 Recognition of AHLs by the AHL-receptors CurR1 and CurR2



**Figure 36: AHL response of the AHL-receptor reporter constructs pCPcurR1 and pCPcurR2**  
 For characterization of the AHL-specificity of the CurR-receptors, dilution series ( $10^{-5}$ - $10^{-11}$  M) of commercially available 3-oxo- and 3-hydroxy-HSLs (3OC6-C14, 3OHC8-C14) were measured with pCPcurR1 (A,B) and pCPcurR2 (C, D) in a plate assay.

## 8.5 Transcriptome statistics

Table 15: Overall statistics of the analysed *Curvibacter* sp. transcriptomes.

sample number	condition	number of reads	unique mapping	all mapping
1	D1	9709184	80.4	90.2
2	D2	10034646	79.0	90.0
3	D3	12830930	79.5	89.8
4	D4	10677771	78.1	88.6
5	D5	11055096	80.3	90.2
6	E1	12416134	81.8	89.3
7	E2	8603225	79.9	91.0
8	E3	10909520	79.7	87.6
9	E4	21957314	77.9	88.4
10	E5	12639194	77.2	88.0
11	F1	11988092	78.8	89.6
12	F2	11357462	77.0	87.6
13	F3	11092341	77.2	87.5
14	F4	9898320	78.0	89.4
15	F5	10483339	77.4	89.1

*Curvibacter* sp. was incubated for 4 h with 1:2000 acidified ethyl acetate (D), 10  $\mu$ M 3OC12-HSL (E) or 10  $\mu$ M 3OHC12-HSL (F) (n=5).

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## 10 Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation nach den 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. Teile dieser Arbeit wurden bereits veröffentlicht.

Kiel, den 24. September 2015

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Cleo Pietschke