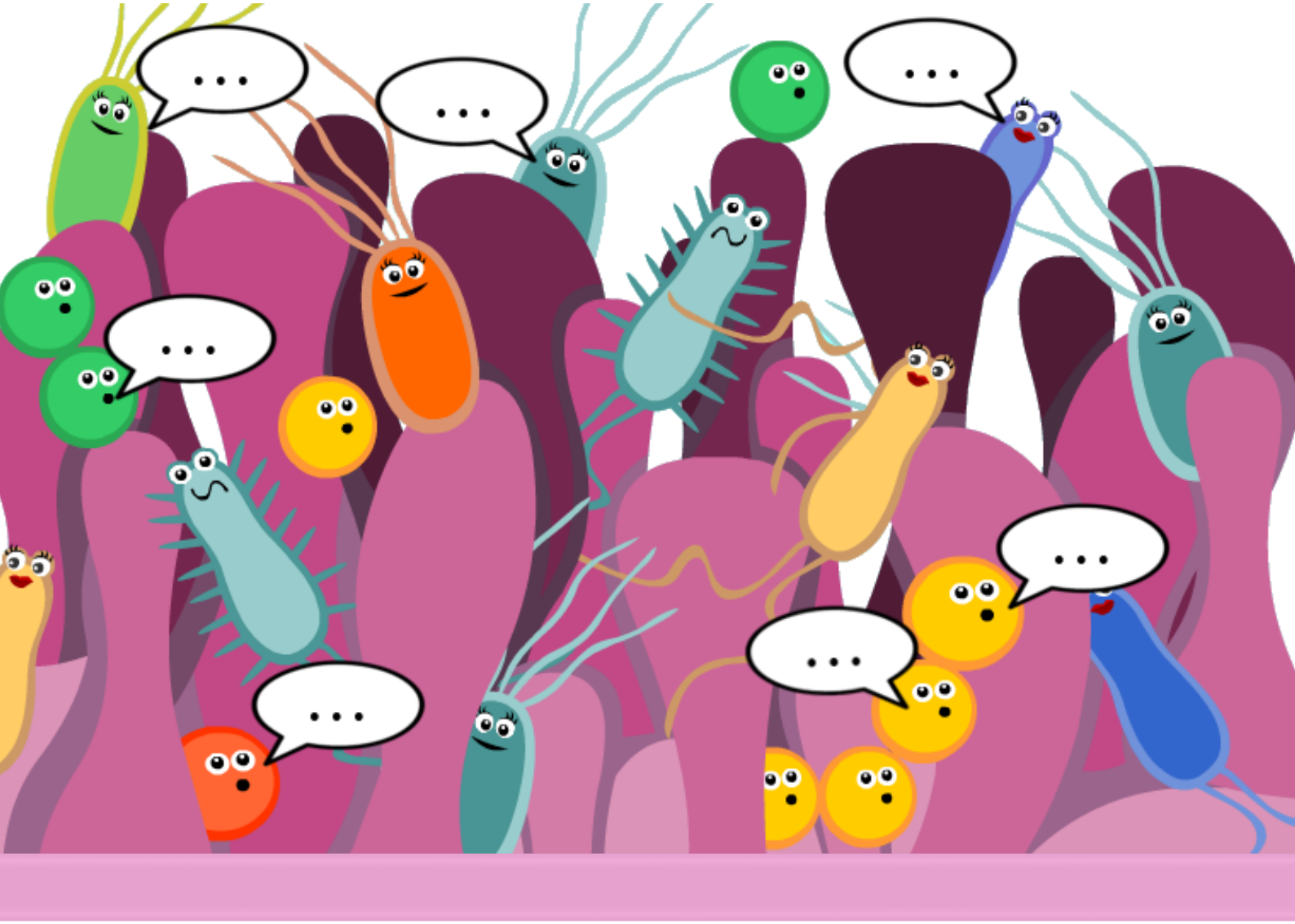
Quorum sensing

The microbial social network

Wouter Lenferink
ENBI-VAKWERK



Quorum sensing: the microbial social network

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Zwolle, 02-06-2016

Voorwoord

Voor u ligt mijn vakwerkstuk over communicatie tussen bacteriën. Vanaf de start van deze opleiding heb ik mij als student geprofileerd in de kennis van het biochemische en microscopische. De oorsprong van deze interesse stamt echter al af van de middelbare school. Middels dit vakwerkstuk tracht ik mijn vaardigheden te laten zien in het vergaren van kennis rondom celbiologische processen. Het onderwerp van dit vakwerkstuk sluit tevens aan bij mijn ambitie om verder te studeren in de microbiologie. Naast een ode aan de wereld van micro-organismen, wil ik met dit vakwerkstuk ook mijn passie en gedrevenheid op dit gebied laten merken. Om tot het werkstuk te komen dat nu voor u ligt heb ik veel steun en enthousiasme mogen ontvangen van mijn begeleider Teresa Pedro Gomes. Daarnaast wil ik mijn dank uitspreken naar het docententeam van de opleiding LVO biologie op het Windesheim voor hun onuitputbare inzet in het onderwijs dat hier gegeven wordt.

In de sfeer van dit werkstuk en om nadruk te leggen op het belang van micro-organismen in onze wereld, sluit ik dit voorwoord af met de woorden van Louis Pasteur.

“Messieurs, c’est les microbes qui auront le dernier mot.” – Louis Pasteur
Heren, het zijn de microben die het laatste woord hebben.

Wouter Lenferink, Almelo, 20-05-2016

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Abstract

Accumulating evidence dismisses the idea that bacteria are solitary bugs, living a completely independent existence. Instead, this definition is being replaced by the notion that bacteria are intimately communicating organisms holding tight relations to one another. One such process through which this is achieved is called quorum sensing, a cell-density-dependent way of controlling gene expression in communities of single- and mixed cell populations. This discovery and the on-going investigations towards this phenomenon also reveal appealing alternatives to microbial control and culturing of novel microorganisms. This review aims to elucidate the mechanisms underlying this process of quorum sensing and its implications on present-day knowledge on microbial cell-cell signalling. Here, several model organisms are displayed with their cognate signalling systems, such as the LuxI/LuxR system in *Vibrio fisheri*, Agr system in *Staphylococcus aureus* and AI-2 system in *Vibrio harveyi*. In general, bacterial communication is achieved by secreting small, signalling molecules or by actively transporting peptide signals into the environment. This signal is received by all bacteria which share the cognate receptor to that signal. Quorum sensing controls many natural processes such as virulence, bioluminescence and biofilm formation, making this process an important area of investigation due to its impact on different fields.

Introduction

Since Antoni van Leeuwenhoek's discovery of the vastness that entangles the world of microorganisms, almost 350 years ago, many steps have been taken in characterising and elucidating the systems that drive these microscopic bugs. It wasn't however until the 1960's and 70's that communication amongst microorganisms was discovered (Bassler & Losick, 2006). This process, called quorum sensing, changed the definition that characterised microorganisms as free-living, independent unicellular organisms that only respond to external stimuli (Greenberg, 2003; Reading & Sperandio, 2006). The term quorum sensing itself was first used by E.P. Greenberg in 1994 when reviewing a family of cell density-responsive proteins that serve a regulatory function within cells (Fuqua, Winans, & Greenberg, 1994). The discovery of these complex communicational systems that were only thought to belong to "higher organisms" changed the view on bacteriology. Bacteria would as of now be described as intimately communicating organisms which can hold tight relations to one another.

Quorum sensing essentially governs the communication between bacteria through chemical signalling molecules (Bassler & Losick, 2006; Bassler, 2002). Produced molecules are excreted into the environment and sensed by nearby microorganisms. The term quorum sensing comes from this ‘sensing’ as well as its effect on certain microbial behaviours only productive in larger cell densities, a quorum (Bandara, Lam, Jin, & Samaranayake, 2012; Bassler, 2002). Examples of these behaviours include but are not limited to bioluminescence, virulence, sporulation and mating (Bassler, 2002). The process of quorum sensing becomes even more complex when taking interspecies and interkingdom signalling into consideration. These kinds of signalling cover communication between different species and communication between bacteria and other taxonomic kingdoms, respectively (Pacheco & Sperandio, 2009). It was also observed that quorum sensing systems differ in gram-positive and –negative bacteria. Evolutionary this contributed to a wider spectrum of signalling molecules and regulatory systems, distributed over an even wider spectrum of bacterial species (Lerat & Moran, 2004).

One of the most relevant implications to man, caused by quorum sensing is biofilm formation. Communication amongst species of bacteria directly affects population cell density, formation, maturation and dissolution of biofilms (Bandara et al., 2012; Parsek & Greenberg, 2005). In turn, biofilms pose an increasing risk to clinical health and safety, as well as sewage systems by forming infectious or corrosion inducing populations on medical equipment and concrete sewer pipes (Costerton et al., 2003; Donlan, 2001; Vincke, Boon, & Verstraete, 2001). The combination of quorum sensing and biofilm studies even received the name ‘sociomicrobiology’ as a new science (Parsek & Greenberg, 2005).

It is clear then, that quorum sensing is an important field of investigation. The present literature study aims to review the latest perspectives on quorum sensing and to a lesser extent, the current state of sociomicrobiology. Elucidating how quorum sensing facilitates the communication between bacteria in a microbial population through a set of sub-questions. These questions include how quorum sensing differs between gram-positive and –negative bacteria, what distinctions interspecies and intraspecies signalling harbour, how quorum sensing can be inhibited and the influences of quorum sensing on higher organisms. To understand the mechanisms by which this takes place, this study will first review several fundamental properties of bacteria.

Chapter I – Bacteria
Look who's talking

Considering the recent advances of the last 150 years mainly due to the techniques involved in studying microorganisms, microbiology is one of the most rapidly evolving fields of biology to date (Madigan, Martinko, Bender, Buckley, & Stahl, 2012). Whilst microscopic life had been suspected for a long time, its discovery had to await the invention of the microscope. This endeavour was taken on by the Dutch draper and lens maker Antoni van Leeuwenhoek, whom, in 1676, discovered the existence of bacteria (Madigan et al., 2012). This made him the first microscopist. This first chapter will address some basic principles that have been uncovered since that discovery and which prove equally fundamental to quorum sensing.

1. The bacterial cell

Whilst being one of the simplest forms of life, bacterial cells are able to take on a wide variety of shapes, sizes and functions. Cell shapes and sizes, like the round-shaped cocci or long-stretched rods have little to no effect on quorum sensing. Vice versa however, quorum sensing can have significant effects on cell shape and size (Patzelt et al., 2013). The key to quorum sensing system diversity are the different structures that make up the bacterial cell. As such, the most significant differences in quorum sensing systems can be observed between bacteria with different cell wall structures, grouped into gram-positive and gram-negative bacteria (Bassler & Losick, 2006).

1.1. Cell structure, membranes and cell walls

Without describing the bacterial cell to the full depth of its complexity, Figure 1 gives a representative overview. Most significant to the current literature study are the layers that cover the bacterial cell and its single, circular chromosome. In some species, this chromosome is linear, as are eukaryotic chromosomes (Madigan et al., 2012). The plasma membrane of bacteria is composed of a phospholipid bilayer with many integral (membrane embedded) and peripheral (not membrane embedded) proteins. These proteins serve in various fundamental processes including but not limited to movement, transport, recognition and signalling (Madigan et al., 2012; Tortora, Funke, & Case, 2011). Moving outward from the plasma membrane, the bacterial cell is covered by a cell wall and (optionally) a capsule (Figure 1). The cell wall of bacteria contains a layer of peptidoglycan. The thickness of this peptidoglycan layer is dependent on the group to which a bacterium belongs. In distinguishing two groups of bacteria, Hans Christian Gram developed a staining technique that divided bacteria into two major groups based on colour: gram-positive (purple) and gram-negative (pink; Madigan et al., 2012). The different colours after staining the two groups can be explained based on the composition of their cell walls. In this staining method, a wash with ethanol clears the purple dye from gram-negative cells, but not from their thick peptidoglycan walled counterparts (Madigan et al., 2012). This is only one of the many characteristic differences between the cell wall structures of these two groups.

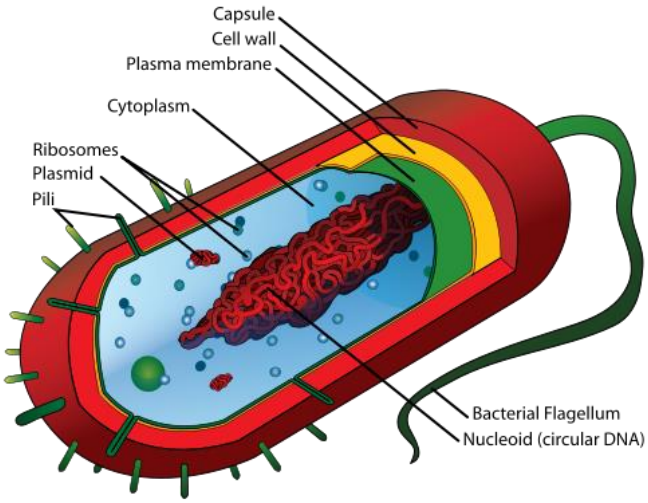

Figure 1. Schematic diagram of a typical prokaryotic cell (Villareal, 2008).

Gram-positive cell walls

Bacteria that stain purple during Gram staining are classified as gram-positive. The cell walls of this group of microbes are distinguishable from that of gram-negative microbes by their thick and extensive network of peptidoglycan "cable" structures, covering the plasma membrane (Figure 2; Madigan et al., 2012). Characteristic for gram-negative bacteria, the peptidoglycan wall has teichoic acids anchored to it, which render the gram-positive cell surface with a negative electrical charge (Madigan et al., 2012; Tortora et al., 2011). In total, about 90 % of the gram-positive cell wall consists out of peptidoglycan and can either form a singular layer surrounding the plasma membrane or, in some species, multiple layers stacked upon one another.

Gram-negative cell walls

The cell walls of gram-negative cells contain only a small fraction of peptidoglycan. As can be seen in Figure 2, a second membrane (the outer membrane) takes up most space in gram-negative cell walls. The portion of peptidoglycan in gram-negative bacteria is located between the plasma membrane and the outer membrane. This region called the periplasm, is of gel-like consistency and holds many proteins that cannot enter the cell or leave through the outer membrane (Madigan et al., 2012). Perhaps the most characteristic feature of gram-negative cells is the polysaccharide chains that extend from the outer membrane. These chains are embedded in the lipids of the outer membrane and is thus collectively called the lipopolysaccharide complex or LPS layer (Madigan et al., 2012).

In gram-negative bacteria, this LPS layer is often the cause of toxicity in many gram-negative species. Such as the common species of *Salmonella* and *Escherichia* (Madigan et al., 2012; Tortora et al., 2011). As different quorum sensing systems are discussed, this study will also show that some systems aid in the formation of endotoxins. These endotoxins, as the name implies, are toxic compounds associated with the LPS layer (Madigan et al., 2012).

Other cell-surface structures

Moving outward from the cell wall, several other structures can be observed. Although of not much importance in the current study, these structures will be reviewed briefly. First, bacteria can be surrounded by a layer of sticky or slimy material called capsules and slime layers (Madigan et al., 2012). One major function of these outer components is attachment, formation and maturation of biofilms (Madigan et al., 2012; Tortora et al., 2011). The relationship between quorum sensing and biofilms will also be elaborated on in the current study. Lastly, most bacteria have complex outer membrane structures called pili and fimbriae which serve an extra role in attachment to surfaces, genetic exchange and twitching motility (Madigan et al., 2012).

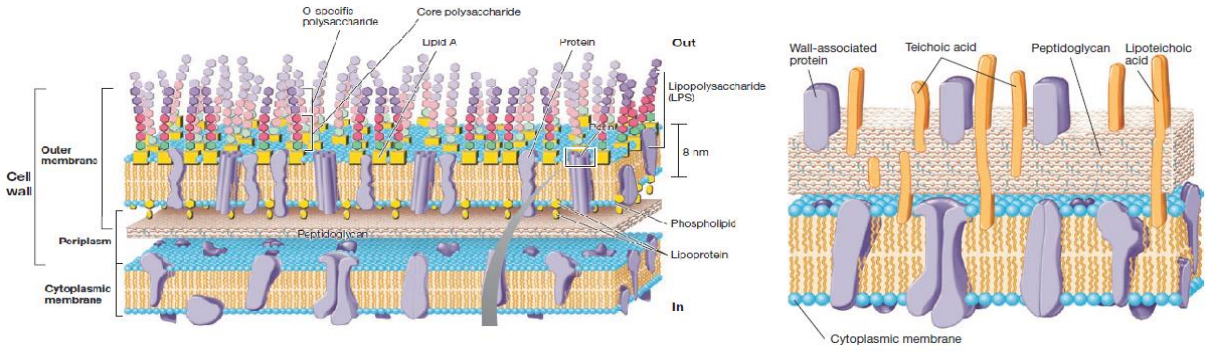

Figure 2. Schematic diagram of the membrane and cell wall of gram-negative (left) and gram-positive (right) bacteria (Madigan et al., 2012).

1.2. The microbial genome and its regulation

In review, the morphology and physiological abilities of bacteria are governed by the proteins they contain. Even more important to prokaryotic life than to eukaryotes is the ability to control which proteins are synthesised and degraded (Lodish et al., 2013). It is fundamental to the survival of bacterial species that cells can adapt to rapidly changing environmental conditions or otherwise respond to outside stimuli. This interaction with the environment is regulated on the level of gene expression.

The bacterial genome

In contrast to the human genome which contains between 20,000 to 25,000 genes, the genome of bacteria only contains 1,400 to 10,000 genes (Lodish et al., 2013). Another big difference is that bacterial genomes are arranged in a single, circular chromosome. The lack of a nucleus membrane is perhaps one of the most significant differences, leaving the genome exposed to the cytoplasm. Because of this feature, bacteria are able to translate proteins from RNA transcripts as soon as the RNA transcript is polymerised from the template DNA strand (Madigan et al., 2012). In contrast, eukaryotic RNA transcripts need to be exported from the nucleus prior to translating them into the protein they encode (Lodish et al., 2013).

Gene regulation

In bacteria, most genes are co-ordinately regulated through operon structures. These structures include several genes linked together, ensuring that when transcription is initiated, all genes in the operon are transcribed (Lodish et al., 2013). Several regulatory components can be distinguished in the bacterial operon as can be seen in Figure 3. First, the promoter sequence (yellow) functions in binding the RNA polymerase protein which transcribes the template DNA into RNA transcripts. Secondly, the operator (orange) sequence functions in preventing transcription from taking place. In most cases, when a repressor protein binds the operator site, it physically blocks RNA polymerase from binding the promoter site. Finally, the activator sequence (green) strengthens the binding intensity of RNA polymerase to the promoter site (Lodish et al., 2013; Madigan et al., 2012). Proteins that function in the negative and positive control of gene expression are collectively called transcription factors. In quorum sensing, these proteins play a major role in the regulation of gene expression through cell-to-cell signalling pathways. Whilst these components are relatively close to the promoter region, another regulatory component called an enhancer or silencer (depending on its function) can be located several kilobases away from the promoter region and still affect transcription (Lodish et al., 2013).

2. Bacterial perception

Animals and other higher organisms generally perceive their environment using sensory organs. If an animal sees (senses) immediate danger such as a falling object, it will adjust its movement to dodge this object. If the animal did not avoid this object, it would lead to his death. Similarly, bacteria need to respond to environmental stimuli. Naturally, bacteria do not have eyes or any of the other complex sensory organs that animals have. Bacteria can, however, adjust their metabolism and movement based on environmental signals such as temperature, pH, oxygen and nutrient availability (Madigan et al., 2012). Quorum sensing

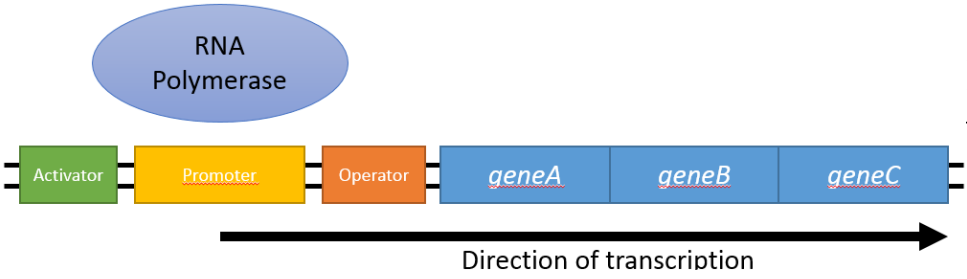

Figure 3. Diagram of a representative, fictional bacterial operon. This figure was inspired by the E. coli lac operon as depicted by Lodish et al (2013).

even adds another dimension in which bacterial cells can respond to external stimuli based on cell-density. It is safe to say that although bacteria have no complex organs to perceive their environment, they do have other elegant systems to effectuate an effective response to external stimuli. In most cases, the way bacteria perceive these environmental stimuli and act on them is by means of signal transduction (Madigan et al., 2012). In essence, quorum sensing is an elaborate signal transduction pathway as will be explained later in this review. The following paragraphs display several fundamental systems through which bacteria perceive their environment.

2.1. Two-component regulatory system

In some cases, chemical environmental signals such as small molecules can diffuse through the plasma membrane and directly interact with selected proteins. As a result, these proteins undergo a conformational change which alters the proteins function (Madigan et al., 2012). Such molecules, called effectors, can be responsible for a change in gene transcription by activating or deactivating transcription factors. However, the most common signal transduction system is the two-component regulatory (2CR) system, which derives its name from the interactions between two system parts that transduce the environmental signal (Madigan et al., 2012). The 2CR systems are of special importance in gram-positive quorum sensing.

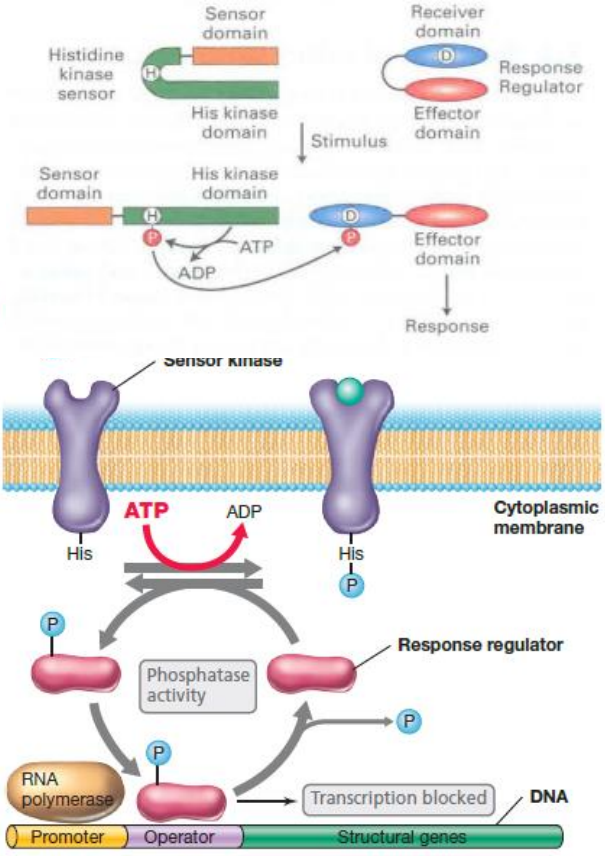

Figure 4. (top) Diagram of the two-component regulatory proteins undergoing a conformational change in the presence of an environmental stimulus (Lodish et al., 2013). (bottom) The control of gene expression by a two-component regulatory system (Madigan et al., 2012).

In a basic 2CR model, signals are usually received on the outside of the cell by a protein embedded in the plasma. Upon receiving an environmental signal, a sensor kinase protein activates a response regulator protein. In turn, this protein directly or indirectly alters expression of target genes (Figure 4; Lodish et al., 2013; Madigan et al., 2012).

Under circumstances where no external stimulus is present, both the histidine kinase sensor and the response regulator components are in an inactive conformational state. However, when a stimulus presents itself to the sensor domain of the kinase sensor, it undergoes a conformational change. This change exposes a conserved histidine on the transmitter domain, which is phosphorylated at the cost of ATP. Following this event, the phosphate group is then transferred to aspartic acid within the regulatory domain of the response regulator. In turn, this causes a conformational change of the response regulator which activates the effector domain of the response regulator. The active response regulator can then affect the expression of genes by binding the DNA until the phosphate group is cleaved off by phosphatases (Lodish et al., 2013; Madigan et al., 2012). In this case, phosphatases serve as a feedback loop in the regulatory system. If the stimulus is no longer present,

phosphatases will complete dephosphorylating response regulators and reset the system (Madigan et al., 2012).

2.2. Chemotaxis

Another effect that is initiated by external stimuli is bacterial motility. Indeed, bacteria possess structures, called flagella (Figure 1), which promote the movement towards or away from a stimulus (Madigan et al., 2012). The flagellum is a tail-like structure that extends from the outside of a bacterium. It aids in motility by rotating at different speeds and in different directions.

The movement towards or away from a chemical stimulus is called chemotaxis. Regulation of chemotaxis-affiliated genes takes place by systems related to 2CR systems (Madigan et al., 2012). As such, one might speculate about the involvement of quorum sensing when investigating the movement behaviour of bacteria. As it turns out, quorum sensing is indeed involved in the flagella-driven movement of so-called swarmer cells (Daniels, Vanderleyden, & Michiels, 2004). In their investigation, Daniels et al (2004) showed that swarmer cells (hyperflagellated, elongated, multinucleated cells) are affected by quorum sensing. The function of quorum sensing induced motility, is most likely linked to the dispersion of bacterial cells in biofilms at a point where too many cells inhabit a single niche (Daniels et al., 2004). Less favourable conditions may lead to quorum sensing induced swarming of bacterial populations, in general. Other forms of taxis (motility), might include thermotaxis, phototaxis, magnetotaxis or gravitaxis (Madigan et al., 2012).

2.3. Quorum sensing

Quorum sensing is the main subject of this review. As the name implies, a quorum (sufficient number of cells) is sensed (perceived) by the bacterial cell. The exact means by which quorum sensing takes place will form the core of this investigation and will be further discussed in chapter II. Quorum sensing is another form by which bacteria perceive their environment. More specifically, it is meant to identify the number of same-species cells and different-species cells that are present in the environment. Briefly, quorum sensing acts by means of chemical signals that effectuate a response when a high enough concentration of that signal is reached (Bandara et al., 2012; Bassler & Losick, 2006; Madigan et al., 2012). By means of altering the expression of certain genes, quorum sensing is involved in virulence, biofilm formation, sporulation, maturation, motility and more. In microbiological research, quorum sensing and biofilm formation are two topics that are inseparably connected to one another (Parsek & Greenberg, 2005). It is also said that most bacteria in nature live in biofilm communities (Tortora et al., 2011). The importance of biofilm formation and dissolution is crucial in quorum sensing and will be discussed next.

3. Biofilms

The different stages of biofilm development are partially regulated by quorum sensing. Biofilms are multicellular structures that exist on surfaces colonised by bacteria. Most biofilms contain multiple species of bacteria, enclosed in an adhesive matrix (Madigan et al., 2012). The matrix is usually a mixture of polysaccharides, proteins and nucleic acids. The most complex biofilms have internal structures such as small water canals (Figure 5), air ducts and spatial arrangement of bacteria that serve their own purpose within the biofilm (Greenberg, 2003; Madigan et al., 2012).

There are several reasons for bacteria to form biofilms, first of which is self-defense (Madigan et al., 2012). When arranged as a biofilm, bacteria are able to withstand greater physical forces. It has also been shown that biofilm-associated pathogens cause more trouble in infectious diseases (Greenberg, 2003). The latter is explained due to biofilms being more resistant to phagocytosis by protozoans, the immune system of hosts and to toxins such as antibiotics (Donlan, 2001; Madigan et al., 2012). A related advantage of biofilms is that it allows bacteria to remain within a preferred niche by attaching to nutrient rich surfaces (Madigan et al., 2012). Thirdly, bacteria often live in closely associated communities with one another as is required in active cell-to-cell communication (Greenberg, 2003; Madigan et al., 2012). This tight-knit relation also allows for more efficient co-metabolism and genetic exchange between bacteria in a competent state (Madigan et al., 2012).

3.1. Biofilm formation and maturation

Recall the name sociomicrobiology, the investigation into microbial group behaviour, which is nowhere as apparent as it is in biofilm-associated processes (Parsek & Greenberg, 2005). The formation of biofilms requires firmly coordinated microbial associations. As such, various signalling techniques such as quorum sensing, are used to assemble these multicellular structures (Costerton et al., 2003; Davies et al., 1998; Madigan et al., 2012). In examining the effects of quorum sensing on biofilm formation, the lifecycle of a biofilm can roughly be separated into four stages – attachment, colonisation, development and dispersal (Figure 5; Madigan et al., 2012; Parsek & Greenberg, 2005). Cell-to-cell signalling has effects separately on each of the four stages (Bandara et al., 2012).

Attachment

Adhesion to a surface is the first step in biofilm formation. Suspended, planktonic cells exhibit extracellular structures such as pili, flagella and cell surface proteins that are involved in attaching to a suitable surface – the biofilm substrate (Madigan et al., 2012; Parsek & Greenberg, 2005). When cells attach to a substrate, a signal is transduced to express biofilm-specific genes (Madigan et al., 2012). These genes encode for proteins that synthesise intercellular signalling molecules and initiate matrix formation. In *Staphylococcus aureus,* a normally functioning quorum sensing system called the accessory gene regulator (Agr), prevents the attachment phase by repressing surface adhesion proteins (Parsek & Greenberg, 2005). Mutants of Agr seem to adhere more readily to both biotic and abiotic substrates because the aforementioned suppression of adhesion proteins no longer takes place. Upon attachment, cells often lose the proteins that are responsible for motility in their planktonic state (Madigan et al., 2012). In general, rougher and more hydrophobic materials form better substrates for biofilms (Donlan, 2001). This is due to the electrostatic and hydrophobic interactions that bacteria engage in during biofilm attachment.

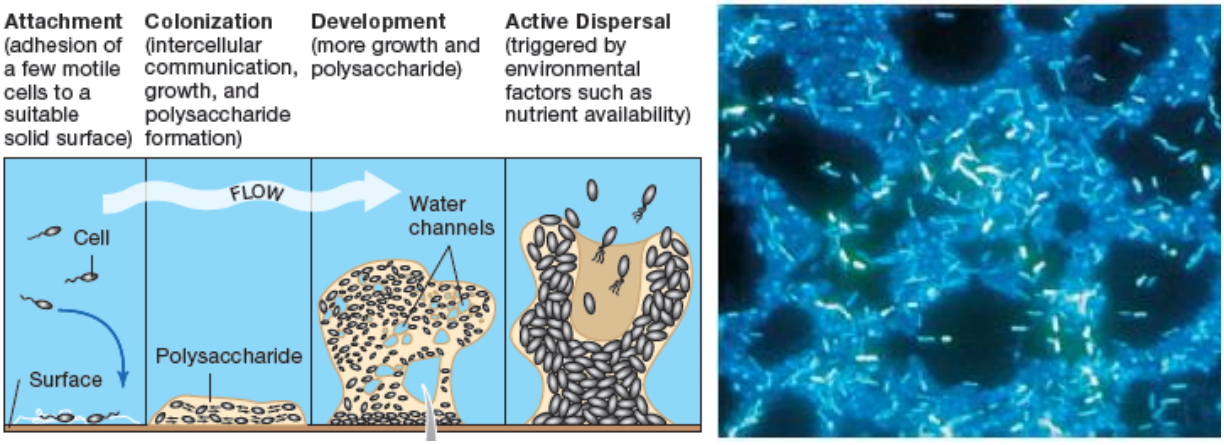

Figure 5. (left) The four stages of biofilm formation and maturation. (right) Photomicrograph of a biofilm on stainless steel coloured with DAPI. Water channels are seen as black gaps between the DAPI-stained cells (Madigan et al., 2012).

Colonisation and development

As the biofilm matures it must be noted that it is not a fixed structure. Cells that are present in the biofilm are able to disperse randomly throughout the biofilm lifecycle (Donlan, 2001; Madigan et al., 2012). The biofilm actively changes morphology over time and space (Donlan, 2001). They can grow variably from flat, homogenous biofilms to highly structured biofilms such as is portrayed in Figure 5. Factors that affect this development include motility, extracellular matrix production and rhamnolipid production (Parsek & Greenberg, 2005). Considering these factors are partly controlled by quorum sensing systems, it is possible to speculate about the contribution of quorum sensing in biofilm formation. Again, research showed that mutations in a specific quorum sensing pathway of *Burkholderia cepacia* resulted in biofilms arrested at stages of microcolony growth (Huber et al., 2001). The wild-type strain, however, showed no deviations and formed robust biofilms. Another study towards biofilm formation in *Pseudomonas aeruginosa* suggests intercellular signalling to affect biofilm formation as well (Davies et al., 1998). Parsek and Greenberg (2005) do question the ease at which scientists attribute abnormalities in biofilm formation to quorum sensing mutations. They state it is not surprising that mutations in global regulatory systems induce abnormalities in biofilm phenotypes.

Dispersal

The final step in a biofilm lifecycle is dispersion. Most significant to humanity in a medical context, the dispersion is where cells detach from a biofilm and are able to colonise, and subsequently, infect another area (Donlan, 2001). This may lead to systemic bacterial infection. Dispersion is yet another stage of biofilm growth that is actively controlled by quorum sensing. This makes sense, considering that growing cell numbers whilst nutrients are depleting call for active dispersal. There is also evidence for the involvement of quorum sensing in biofilm dispersal (Parsek & Greenberg, 2005). In *Rhodobacter sphaeroides* for example, a mutation in the community escape response (*cer*) gene caused a defect in aggregate dispersion, resulting in hyperaggregation of cells. This response is regulated by quorum sensing.

Suffice it to say, quorum sensing has a great influence on biofilms. And in turn, biofilms have a great influence on us. Studying the involvement of quorum sensing can lead to more effective treatments in clinical practices (Balaban, Gov, Bitler, & Boelaert, 2003; Costerton et al., 2003). Unravelling the systems of quorum sensing may also lead to advances in biotechnological applications, co-culturing of organisms and controlling microbial damage to infrastructure (March & Bentley, 2004; Vincke et al., 2001). Nonetheless, some scepticism is still in order towards making conclusions on the effects of quorum sensing on biofilm formation.

Chapter II – Quorum sensing
Exposing bacterial communication

The following chapter will form the core of this review. Throughout this chapter, several quorum sensing systems will be elaborated on, along with the scientific and evolutionary history of quorums sensing in prokaryotes.

1. Quorum sensing basics

Before diving into the biochemical pathways that make up the tremendous diversity of quorum sensing systems, first, the basics are covered. Here, a definition is given to the chemical signals that make up quorum sensing molecules. Furthermore, three model systems are discussed along with some hints towards the diversity of systems and chemical signalling molecules.

1.1. Chemical signals

Communication between bacteria is not accomplished through the exchange of words. Instead, bacteria communicate using chemical signalling molecules called autoinducers (AI) – signifying these molecules cause autoinduction. The process of autoinduction is one in which small signal molecules are produced in larger amounts as population size increases (Madigan et al., 2012). Only when a certain threshold is reached, these AI molecules initiate transcription of genes that would serve little to no purpose in population sizes below the threshold. Furthermore in autoinduction, AI molecules positively regulate the operons responsible for synthesising the corresponding AI (Daniels et al., 2004). As such, when the threshold is reached, both the desired quorum effect as well as a dramatic increase in signalling molecules can be observed simultaneously.

Among both gram-positive and gram-negative bacteria, there is a vast variety of available AI molecules, called quorum sensing molecules (QSMs). In gram-negative bacteria, acylated homoserine lactone molecules (AHLs) form the most widespread group of QSMs (Bandara et al., 2012). These molecules are synthesised by AHL synthases and contain a variable acyl side-chain. This side-chain can roughly be classified into short (4-8 carbon atoms) and long (10-18 carbon atoms) side-chain moieties (Figure 6). Many different AHLs are made to account for their specificity and the diversity of AHL networks (Bandara et al., 2012; Daniels et al., 2004). Each AHL of different length is synthesised by a different AHL synthase in a different bacteria species. Consequently, each AHL of different length interacts with a receptor protein that is specific for that AHL (Daniels et al., 2004). This does not only apply to AHLs but also to many other QSMs up to now (Reading & Sperandio, 2006). QSMs made by one species of bacteria, may not at all be recognised by another. In a way, bacteria have developed their own species-specific language of communication. However, quorum sensing is not restricted to intraspecies signalling – the communication between bacteria within the same species – as will be reviewed later in this investigation.

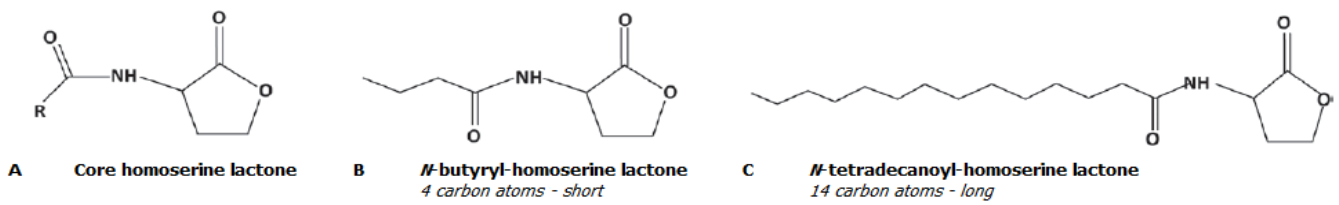

Figure 6. (A) chemical structures of the core AHL molecule, (B) a short AHL molecule and (C) a long AHL molecule. The corresponding amount of carbon atoms are mentioned below the chemical structure. This image was adapted from Bandara et al (2012).

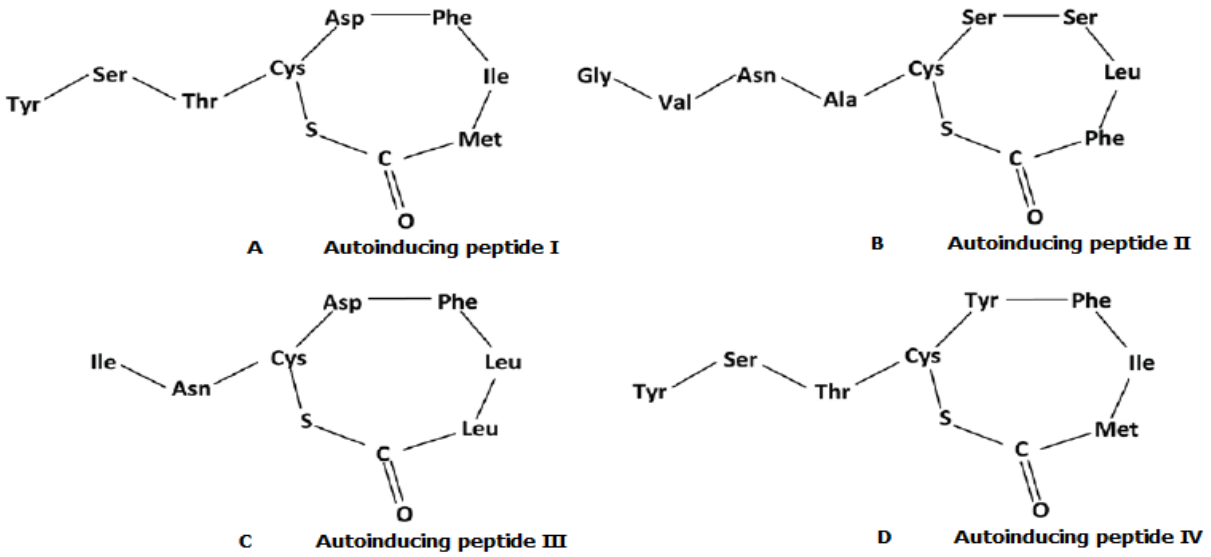

Figure 7. Amino acid sequence and structure of autoinducing peptides in S. aureus groups I to IV, respectively. This image was adapted from Bandara et al (2012).

In gram-positive bacteria, completely different molecules are observed that facilitate quorum sensing. In contrast to the relatively small AHL molecules, gram-positive bacteria exhibit a widespread use of autoinducing peptides (AIPs; Bandara et al., 2012; Kleerebezem, Quadri, Kuipers, & de Vos, 1997). These AIPs are oligopeptides which usually consist out of 5 to 17 amino acids with the added possibility of several side-chain modifications. Figure 7 pictures several AIPs from *S. aureus* groups I to IV alone. Considering this diversity, one can imagine the scale of different signalling molecules that are employed in quorum sensing not only by bacteria but archaea and eukaryotes as well. One crucial difference of peptide QSMs is that, in contrast to smaller AHL molecules, peptide QSMs cannot freely cross the plasma membrane. Peptide quorum sensing systems rely on 2CR pathways to indirectly regulate gene expression. Because of this, QSMs are not internalised or have to be actively transported across the membrane. Consequently, gram-negative bacteria often rely on more components in their pathways.

1.2. Model systems

In this review, quorum sensing has been divided into two main system groups: AHL communication utilised by gram-negative bacteria and AIP communication utilised by gram-positive bacteria. Two model systems of the sorts will be discussed to gain a basic understanding of the biochemical pathways that underlie all quorum sensing systems in bacteria. Finally, a third model system will also be discussed which bridges communication between gram-positive and gram-negative bacteria – interspecies signalling.

The LuxI/LuxR signalling system

The first quorum sensing system to be described is the LuxI/LuxR system (Nealson, Platt, & Hastings, 1970). The system was discovered in species of *Vibrio,* which contain marine bacteria that colonise the luminescent organs of *Euprymna scolopes* squids (Suchetha et al., 2015). With homologs to the LuxI/LuxR proteins found in many other species of bacteria, this system is widely used as a model for quorum sensing in gram-negative bacteria (Bassler, 1999). In *V. fisheri*, the LuxI/LuxR system controls bioluminescence – the production of light – by regulating the *luxICDABEG* operon (Fuqua et al., 1994).

Figure 8 displays a general diagram of the LuxI/LuxR system and shows the major components that make up this pathway. The signal synthase protein LuxI (AHL synthase), AHL signal molecules, the signal receptor protein LuxR and lastly the target genes (Bassler, 1999). In a growing population of bacteria, LuxI synthesises

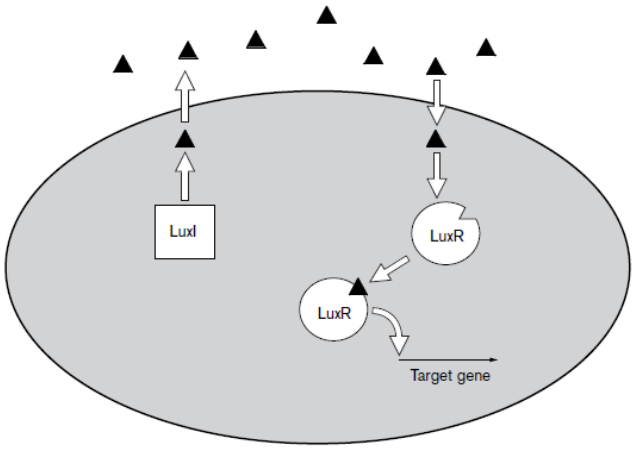

Figure 8. Schematic diagram of LuxI/LuxR quorum sensing. LuxI signal synthase is represented by a square, the AHL signals are represented by triangles, LuxR signal receptors are shown as circles. Take note that AHL signals freely diffuse across the membrane. This image was adapted from Bassler (1999).

AHLs at low levels. Synthesis of AHLs occurs by the transfer of a fatty acid chain from an acylated acyl-carrier protein, onto S-adenosylmethionine. This reaction yields both a 5'-methylthioadenosine and an AHL molecule. The AHL molecule then freely diffuses across the plasma membrane and is released into the environment (Bandara et al., 2012). Considering a growing population, as more cells are formed, more AHLs are released into the environment. At a certain threshold concentration of signal molecules, AHL is able to diffuse back into the cell where it interacts with the LuxR signal receptor. Activated LuxR proteins additionally act as positive transcription factors and upregulate the expression of LuxR-targeted genes (Bandara et al., 2012). In *Vibrio fisheri*, LuxR activates the *luxCDABEGH* genes encoding the subunits for a protein responsible for bioluminescence – luciferase (Bandara et al., 2012). Another gene that is targeted in the activated LuxI/LuxR system is the gene encoding LuxI, leading to an overproduction of LuxI proteins and consequently, to an explosive production of AHL signal molecules (Bandara et al., 2012).

Control of bioluminescence through the LuxI/LuxR system serves a perceptive purpose. As one can imagine, a single, or several bacteria, will not create a potent luminescent signal that is visible to the naked eye. Therefore, bacteria make use of quorum sensing as a means of measuring their population size before initiating a costly process such as bioluminescence.

The Agr signalling system

One of the most well-studied signalling systems in gram-positive bacteria is the Agr system in *S. aureus* (Bandara et al., 2012; Reading & Sperandio, 2006). Already described briefly in the attachment phase of biofilms, *S. aureus* makes a potent pathogen in humans and is the leading cause of several potentially fatal infections (Yarwood, Bartels, Volper, & Greenberg, 2004). The *agr* locus encodes two RNA transcripts, RNA II and RNA III. Whilst RNA III encodes all the components that are required in AIP quorum sensing, RNA II is used to synthesise proteins involved in toxin and protease secretion (Bandara et al., 2012; Reading & Sperandio, 2006). When population density is low, the population of bacteria mainly express proteins involved in colonisation and attachment. However, as population density rises, the Agr system brings about a change in the expression pattern. This change causes *S. aureus* to express more proteins affiliated with the RNA II and III loci (Reading & Sperandio, 2006). One such excreted toxin, affiliated with the Agr system, is a superantigen called toxic shock syndrome toxin 1 (TSST-1), which causes a strong immunological response and is potentially fatal (Bandara et al., 2012).

As was mentioned in the previous paragraph, *S. aureus* AIP molecules know a variety of four groups. Each group corresponds with an *S. aureus* subspecies. For practical purposes, the portrayed model of the Agr signalling system will only contain one AIP molecule. Notably, AIP groups I to IV are specific to the extent

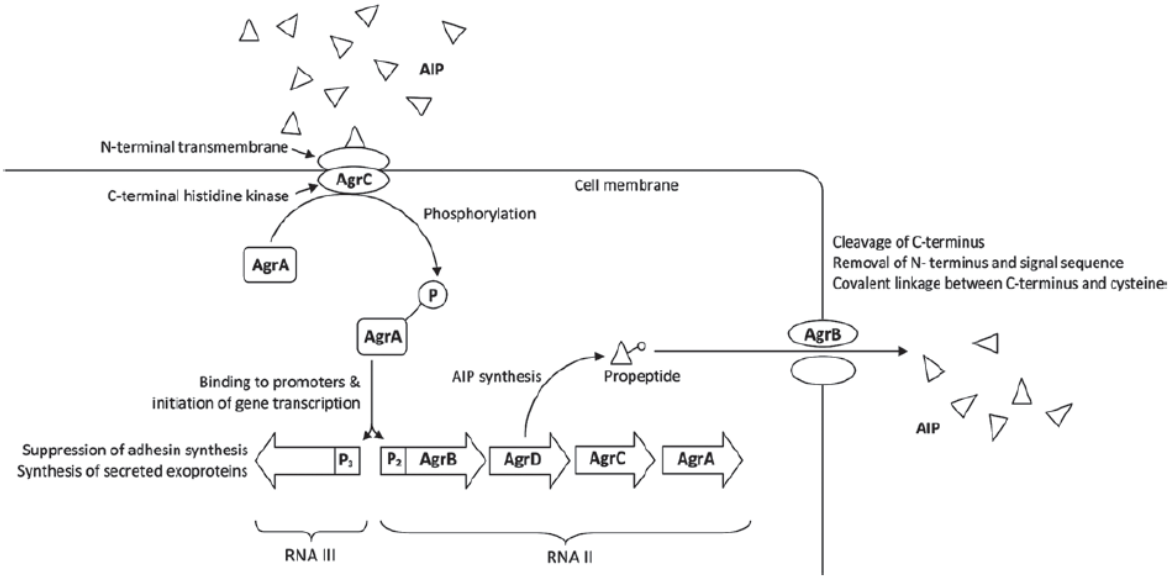

Figure 9. Diagram of the S. aureus agr signalling system. Here the synthesis of AIPs from propeptides is shown along with the 2CR transduction-mediated regulation of gene expression in the RNA II and III loci. This image was adapted from Bandara et al (2012).

that their receptors will not recognise their noncognate AIP groups and are competitively bound by their noncognate AIP groups (Reading & Sperandio, 2006).

In the Agr system (Figure 9), propeptides are synthesised directly from the *agrD* gene at a constant level (Bandara et al., 2012). These propeptides need to be actively transported outside the cell, upon which their C-terminus is cleaved by the membrane-bound AgrB protein. The N-terminus is also cleaved by a signal peptidase called SpsB (not shown). Before the mature AIP is ready, a covalent bond is formed between the cleaved C-terminus and cysteine, forming a thiolactone ring with a free N-terminal tail as can be seen in Figure 7 (Bandara et al., 2012). AIPs in the environment bind to a signal receptor AgrC, encoded by the *agrC* gene. The signal receptor consists out of two domains, similar to that in 2CR transduction histidine kinase sensors. The N-terminal transmembrane sensor detects environmental AIP. When bound to AIP and the sensor domain, the AgrC C-terminal histidine kinase domain phosphorylates an AgrA response regulator, encoded by the *agrA* gene. In turn, phosphorylated (activated) AgrA binds promoter regions on the RNA II and RNA III loci, activating transcription of the corresponding genes (Bandara et al., 2012; Yarwood et al., 2004).

Earlier in this investigation, it was stated that Agr quorum sensing prevents the attachment phase in biofilm forming *S. aureus* (Parsek & Greenberg, 2005). Another study by Yarwood et al (2003) concluded that the effect of Agr quorum sensing on biofilm formation depended on the circumstances in which the experiments were conducted. As it turns out, Agr quorum sensing is indirectly connected to *S. aureus* biofilm formation by acting as a posttranscriptional repressor of the virulence gene repressor *Rot*. Bandara et al (2012) conclude that, as a result, this suppresses the adhesion proteins that aid in *S. aureus* colonisation.

The LuxS signalling system

The third system that is discussed marks an exception to the Gram-affiliated divide that was superimposed on the previous two model systems. It was discovered that a molecule called autoinducer 2 (AI-2) was produced by over 55 species of bacteria (Xavier & Bassler, 2003). Remarkably, these 55 species included

both gram-positive and gram-negative bacteria. More so, AI-2 production appears to be identical in all species that secrete this signalling molecule.

Another species of *Vibrio*, *V. harveyi,* harbours the LuxS signalling system that makes use of AI-2 to induce bioluminescence (Bassler & Losick, 2006; Xavier & Bassler, 2003). Surprisingly, AI-2 from several other species was also able to induce bioluminescence in *V. harveyi* and turned out to control phenotypes in multiple other species of bacteria as well (Bandara et al., 2012; Xavier & Bassler, 2003). As such, AI-2 was named the "universal signalling molecule". In *V. harveyi*, AI-2 is produced by a protein called LuxS, encoded by the *luxS* gene, through a series of biochemical reactions. In the periplasm, AI-2 is bound by the LuxP autoinducer-specific binding protein. The AI-2/LuxP complex interacts with a membrane bound LuxQ sensor kinase. Through a pathway of phosphate transfers, activated LuxQ dephosphorylates LuxO. In phosphorylated (active) form, LuxO suppresses LuxR and transcription of the luciferase operon is not upregulated. This means that, when LuxQ activation leads to inactivation of LuxO, LuxR is no longer suppressed and higher transcription of the luciferase operon takes place (Xavier & Bassler, 2003).

1.3. Quorum sensing families

So far, two model systems have been discussed because of the intensive study conducted on these systems to date. A third model system was also introduced, to form a bridge between gram-positive and gram-negative bacteria. AHL and AIP quorum sensing systems are inseparably linked to gram-negative and gram-positive bacteria, respectively. It is from their respective model systems that "families" of quorum sensing associated proteins were named. In AHL quorum sensing, the most abundant proteins are homologues to either the LuxI signal synthase or the LuxR receptor protein (Bassler & Losick, 2006; Bassler, 1999; Reading & Sperandio, 2006). In AIP quorum sensing, signals are exported by proteins of the ATP-binding cassette transporter (ABC transporters) protein superfamily. The signal reception proteins belong to superfamilies of sensor kinase proteins and response regulator proteins (Bassler, 1999). LuxS, the third model system, consists out of proteins that originate from both (super-) families. When this review refers to families or homologues related to LuxS, it means that the quorum sensing system as whole bears resemblance to the LuxS system.

The emergence of these families of QSMs and systems are preceded by a long evolutionary history, as will be discussed next.

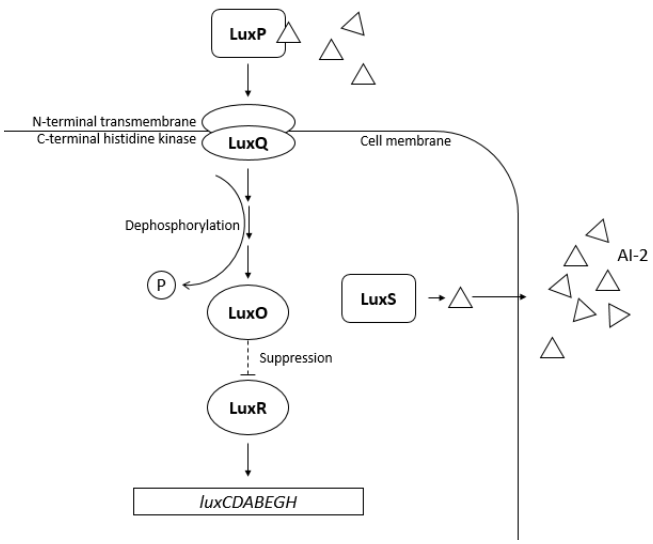

Figure 10. Diagram of the V. harveyi LuxS signalling system. Reception of the AI-2 signal by LuxP and LuxQ trigger a cascade of dephosphorylation through LuxU (not depicted) and LuxO. In a dephosphorylated state, LuxO is inactive and no longer supresses LuxR, which, as a result, upregulates the transcription of the luciferase operon. This image was adapted from Bandara et al (2012).

2. Evolutionary foundations of quorum sensing diversity

As with all complex systems in nature, quorum sensing is preceded by a long evolutionary history. For the purpose of this literature study, this history will be divided into evolution from a phylogenetic perspective and an ecological perspective. The phylogenetic approach will focus on the genetic basis underlying quorum sensing gene distribution, whilst the ecological perspective attempts to elucidate on bacterial group behaviour. Indeed, as is the case in higher organisms, bacteria are subject to behavioural processes such as division of labour, altruism, cooperation and competition.

2.1. Phylogenetic perspective

In eukaryotes, evolution is effectuated by the transfer of genetic traits to the next generation either by sexual or asexual reproduction. This allows researchers to pursue the evolutionary history of certain genes or traits by comparing genetic information of successive species. Horizontal gene transfer complicates this process (Madigan et al., 2012). Horizontally refers to the transfer of genes between individuals of the same generation, where one cell donates genetic information to a recipient cell. In contrast, vertically refers to the transfer of genes from the previous generation to the next, by cell division. Horizontal gene transfer can occur by transformation, transduction and conjugation (Figure 11). In transformation, free DNA from a lysed cell is taken up by the recipient cell. In transduction, viruses mediate the transfer of DNA by disrupting and taking up donor cell DNA. In conjugation, prokaryotes require cell-to-cell contact. Cytoplasmic or chromosome-integrated plasmids in the donor cell are transferred to the recipient cell. In contrast to transformation and transduction, the bacterial cell is not lysed in conjugation.

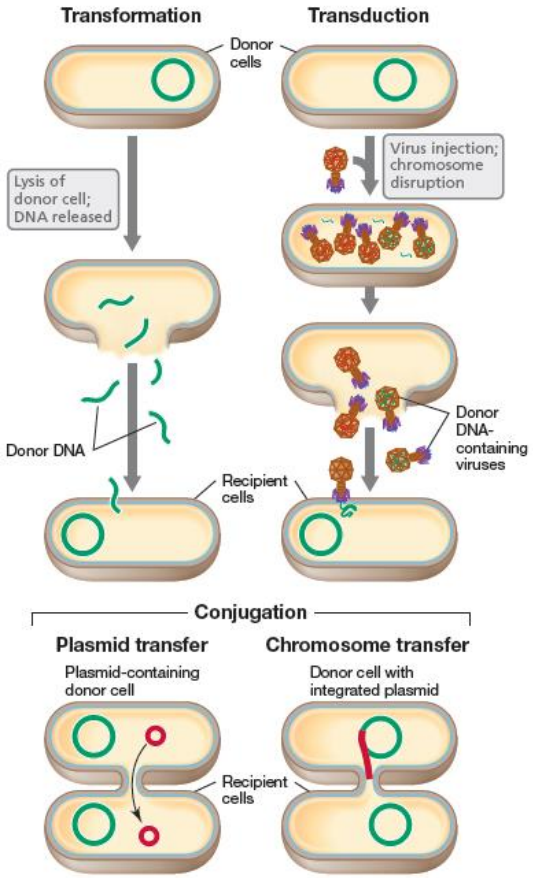

Figure 11. Diagram depicting the three major modes of horizontal gene transfer. This image was adapted from Madigan et al (2012).

How is it possible to draw conclusions from evolutionary analyses of quorum sensing genes? The answer lies in the genetic variation of the bacterial genome. Several clues are found to indicate horizontal gene transfer took place. First, when proteins are found that only have homologues in distantly related species, it is possible that the protein was obtained by horizontal gene transfer. Secondly, genes that were gained by horizontal gene transfer often show deviant guanosine and cytosine content (GC content) or codon usage bias from the rest of the genome (Madigan et al., 2012).

Horizontal gene transfer has been shown to be important when analysing the evolutionary history of quorum sensing (Gray & Garey, 2001; Lerat & Moran, 2004). Homologous genes of *luxI* and *luxR* have been found not only on bacterial chromosomes but also on more readily transferred extrachromosomal elements and plasmids. In their phylogenetic analysis of 76 individual LuxI and LuxR homologues, Gray and Garey (2001) also show the importance of horizontal gene transfer. Furthermore, they propose the potential for

acquired regulatory systems to be incorporated into already existing ones. This could be part of explaining the complexity of some quorum sensing pathways that are known to date.

Only 4 phyla make up over 90 % of all characterised bacterial genera: Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes (Madigan et al., 2012). As for the LuxI/LuxR system, it is proposed this system arose early in the evolution of Proteobacteria and subsequently spread to each group within this phylum (Gray & Garey, 2001). Correspondingly, the Proteobacteria phylum distinguishes itself by all its members being gram-negative. As was shown later, the approach used by Gray and Garey (2001) did contradict with a new finding. Lerat and Moran (2004) found that the LuxI/LuxR family actually consisted out of two separate families. Separation of the two families, called A and B, was based on their differences in sequence and subsequently in their function. Family A LuxR homologues exist as monomers in their inactive form. When activated by binding AHL, these monomers form a homodimer that binds the promoter sequence of their target genes. In contrast, family B LuxR homologues appear to form dimers and bind to the promoter sequence in the absence of AHL binding. It was also shown that family A grouped into α-, β- and γ-Proteobacteria, whilst family B exists only in γ-Proteobacteria (Lerat & Moran, 2004). This difference in function could indicate an early divergence of the two families. More research is needed, however, as the difference in function has not yet been accounted for in all family members.

Another interesting find is that the origins of the mechanisms underlying quorum sensing most likely date from ancient times, in the very early evolution of bacteria (Lerat & Moran, 2004). Furthermore, through the course of this evolutionary journey, rarely did receptor and signal synthase genes acquire a new partner. This indicates that most gene partners are likely to have shared evolutionary histories.

2.2. Ecological perspective

The social behaviour of microorganisms can be compared to macroorganisms in many ways. For example, the cooperative assembly of biofilms is socially related to domicile creation of higher organisms, such as burrows, nests, hives and galls of many social organisms (Crespi, 2001). Cooperative hunting, comparable to wolf packs can be compared to quorum sensing induced virulence of *Staphylococcus* species. In both cases, teamwork is required to take down larger 'prey'. Quorum sensing also resembles the way social animals communicate using pheromones (Crespi, 2001; Keller & Surette, 2006).

From an ecology-evolutionary perspective, quorum sensing is an odd process that seems to favour altruistic behaviour. Bacteria produce costly signals and respond to those signals to benefit other individuals at a personal cost (Keller & Surette, 2006). One might ask how this favours natural selection for that species. To favour natural selection, quorum sensing has to translate into a direct benefit for the individual and an increase in its fitness. In order to study the social interactions of microorganisms from an ecological point of view, one must differentiate between different chemical interactions (Keller & Surette, 2006). When referring to an AIs or a specific QSM, the term 'signal' is used in the current literature study. However, from an ecological perspective, a signal is any act, structure or chemical emission that alters the behaviour and gene expression of other organisms, evolved because of that effect and is effective because the receiver's response has evolved accordingly (Keller & Surette, 2006). In this case, a signal is deliberately sent from the emitter and deliberately received by the receiver and increases the fitness of both parties. In contrast, when a signal did not evolve specifically for that effect but induces it by chance, it is called a cue. Lastly, when a chemical emission lowers the fitness of the receiver by altering its behaviour and gene expression, it is called chemical manipulation.

Benefits of intraspecies communication

Formation of microcolonies in pathogenic bacteria is one such example of how altruistic group behaviour increases the overall fitness of the species. A single cell that starts excreting a costly virulence-associated substance will have little to no effect and decreases its fitness. In contrast, microcolonies facilitating the coordinated excretion of virulence associated substances are more advantageous and are favoured by natural selection (Keller & Surette, 2006).

Benefits of interspecies communication

Syntrophic relationships, the process by which multiple microorganisms cooperate to degrade a substrate neither can degrade alone, provides a clue towards the evolutionary benefit of interspecies communication (Keller & Surette, 2006). In one such example, degradation of aromatic compounds is energetically favourable only if bacteria in the first biochemical steps are close to methanogens or sulphur-reducing bacteria. Especially characteristic in anoxic catabolism, syntrophy often occurs to account for the lack of electron acceptors other than CO_2 (Madigan et al., 2012). However, it is still unclear whether these organisms coevolved or if syntrophy is merely a result of bacteria preferentially associating with species that harbour complementary metabolic machinery (Keller & Surette, 2006).

Benefits of interkingdom communication

Certain squids harbour a symbiotic relationship with *V. fisheri* bacteria. For bacteria, this relationship allows the development of biofilms, motility and colonisation alongside light production (Keller & Surette, 2006). For the squid, colonisation by these bioluminescent bacteria is required for the light organ to develop normally. It was even shown that mutants defective in producing light are less competitive in long-term survival of the light organ than are light producing bacteria (Visick, Foster, Doino, McFall-Ngai, & Ruby, 2000). This indicates that the host has a way of selecting against cheaters. In this case, cheating is the behaviour of exploiting cooperation between organisms by imposing fitness costs on them, while providing fitness benefits to themselves (Crespi, 2001).

2.3. Mixed disciplines

The evolutionary basis of quorum sensing has now been discussed on the level of phylogenetic exchange and the ecological approach of fitness and natural selection. Both bear a close resemblance to each other as the vertical transfer of genes, discussed in §2.1., is directly linked to natural selection and fitness. The second approach to quorum sensing evolution showed that social behaviour between bacteria and hosts can closely resemble social behaviour in higher organisms. However, according to a newly emerging mix of disciplines within cognitive biology and microbiology, more approaches can be taken to explain the development of bacterial group behaviour.

In a review by P. Lyon (2015), she argues that bacteria show evidence for capacities encompassed by cognition. Besides perception and communication (quorum sensing), she argues that bacteria possess the capacity to exhibit valence, memory, learning, anticipation and signal integration (decision making). To measure the cognitive intelligence of bacteria, a formula for ‘bacterial IQ’ was introduced based on the genome size and proportion of DNA segments encoding signal transduction proteins (P. Lyon, 2015). Following her review of the current state of ‘cognitive microbiology’, P. Lyon (2015) raises awareness towards investigating into microbial awareness and learning, object sensing and coupling prokaryotic oscillatory activity to eukaryotic endogenous brain activity. Although not discussed further in the current literature study, P. Lyon (2015) sets a curious basis for further research into microbial behaviour and its connection to higher organisms. After all, we might have a shared ancestry.

3. Gram-negative quorum sensing

As a model system for gram-negative quorum sensing, the basics of the LuxI/LuxR system have already been discussed, along with a general overview of AHL AI molecules. However, gram-negative bacteria exhibit more complex forms of communication, as will be reviewed in this paragraph. Perhaps not surprising, most gram-negative quorum sensing systems are described in the Proteobacteria phylum. Here, the more complex quorum sensing systems of *P. aeruginosa*, *Agrobacterium tumefaciens* and *V. harveyi* will be discussed along with several QSMs other than AHL.

3.1. The *P. aeruginosa* RhlI/RhlR system

Homologous quorum sensing systems to LuxI/LuxR have been found in around 25 species (Bassler & Miller, 2013). Some are as simple as the LuxI/LuxR system; others exhibit more complex ways to communicate. In species of *P. aeruginosa*, quorum sensing is governed by two separate systems named the LasI/LasR system and the RhlI/RhlR system. The former one was named for its effect on elastase production, the latter for its effect on rhamnolipid production (Bandara et al., 2012). Both homologous to the LuxI/LuxR system, these contribute to *P. aeruginosa* virulence and are well documented because of that.

In this system, LasI and RhlI function as the signal synthases, producing *N*-(3oxododecanoyl)-homoserine lactone (PAI-1) and *N*-(butyryl)-homoserine lactone (PAI-2), respectively (Bassler & Miller, 2013). LasR and RhlR function as signal receptors and activate multiple genes associated with virulence, as will be discussed further on. As with the LuxI/LuxR system, *P. aeruginosa* quorum sensing has a self-activating function in which LasR activates *lasI*. Novell to this system; activated LasR works in tandem to activate the *rhlR* gene and thus RhlI/RhlR quorum sensing is dependent on the LasI/LasR system.

As cell numbers grow, LasI-dependent AI accumulates in the environment and eventually diffuses into the cell. At the same time, RhlI-dependent AI also accumulates. When a threshold is reached, AIs bind their respective receptor proteins LasR/RhlR and activate transcription of their target genes (Figure 12). In this case, activated LasR activates both virulence genes and *rhlR*, making the RhlR-activated genes dependent on LasR activation. Meanwhile, LasI-dependent AI negatively regulates the activity of RhlR by competitively binding it. This process makes sure that RhlR-activated genes are only transcribed once the LasI/LasR regulated genes have been transcribed and implicates *P. aeruginosa* species' ability to precisely time its gene expression (Bassler & Miller, 2013).

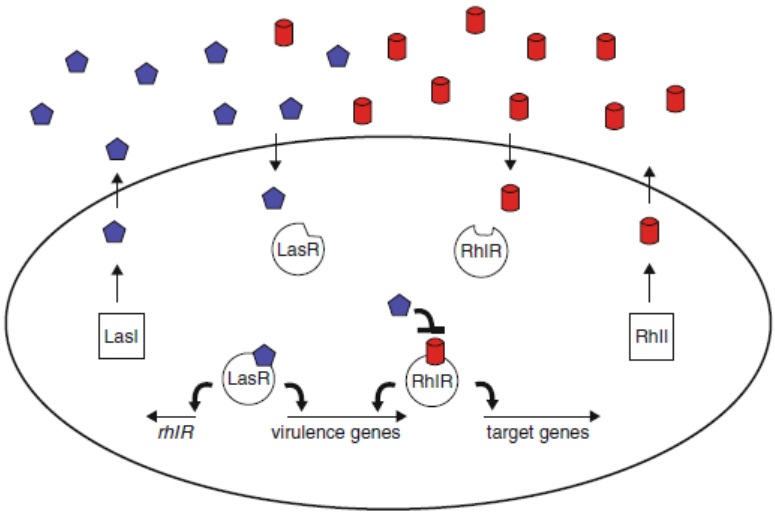

Figure 12.Diagram of the P. aeruginosa LasI/LasR and RhlI/RhlR system. Both systems control important genes in P. aeruginosa virulence. RhlR functioning is dependent on LasR activation, as can be seen in the diagram. At the same time, LasI-dependent autoinducer negatively controls RhlR activity by competitive inhibition. This diagram was adapted from Bassler and Miller (2015).

Virulence in *P. aeruginosa* is controlled by these two quorum sensing systems. First, the LasI/LasR system controls genes that are involved in biofilm formation (e.g. elastase production), production of extracellular enzymes (e.g. exoproteases and toxin *toxA*) and motility. Secondly, the RhlI/RhlR system also controls elastase, *toxA* and exoprotease production, rhamnolipid, pyocyanin and cyanide production along with several other compounds (Bandara et al., 2012). In total, *P. aeruginosa* is believed to regulate some 300 genes based on quorum sensing systems.

The *P. aeruginosa* quorum sensing system appears to be even more complex when a third AI was discovered. The *Pseudomonas* quinolone signal (PQS) regulates *lasB*, one of the virulence genes that encodes for elastase (Bassler & Miller, 2013). Interesting about PQS is that the signal is a quinolone. Halogenated quinolones are often used in clinical practice as potent antibiotics to both gram-negative and gram-positive bacteria. This might suggest that *P. aeruginosa* uses the signal for communication and as a way to compete with other species.

3.2. The *A. tumefaciens* TraI/TraR system

Another species within the Proteobacteria phylum, *A. tumefaciens*, exhibits a quorum sensing system that is partially dependent on a signal from its host plant (Bassler & Miller, 2013). These bacteria are in part responsible for crown gall tumours by transferring a plasmid, called Ti for tumour-inducing plasmid, to the nuclei of its plant host. Conjugation of the plasmid between individuals of *A. tumefaciens* requires a signal (opine) from its host and an AHL signal produced by the AI synthase TraI. The LuxI/LuxR-like quorum sensing system TraI/TraR is located on this Ti plasmid itself (White & Winans, 2007). Remarkably, the enzymes required to synthesise opines are also located on the Ti plasmid. In *A. tumefaciens*, two types of Ti plasmids can be observed (White & Winans, 2007). The nopaline-type Ti plasmid and octopine-type Ti plasmid. The two systems differ slightly in the arrangement of their operons and the genes they encode. Here, octopine-type Ti plasmids are discussed and the way this quorum sensing regulates plasmid conjugation in *A. tumefaciens*.

Octopine enters the cell through ABC transporters and interacts with the transcriptional regulator OccR (Figure 13; White & Winans, 2007). This interaction results in the activation of the *occ* operon, *traR* and *occR*. The *occ* operon contains genes for the uptake and catabolism of octopine, with the *traR* gene located at the distal end of the operon. The encoded TraR receptor protein then interacts with TraI-dependent AI, which is constitutively present at low concentrations (Bassler & Miller, 2013). Following this event, TraR-AI complexes activate several genes. One of these genes is *traI*, resulting in the characteristic positive feedback

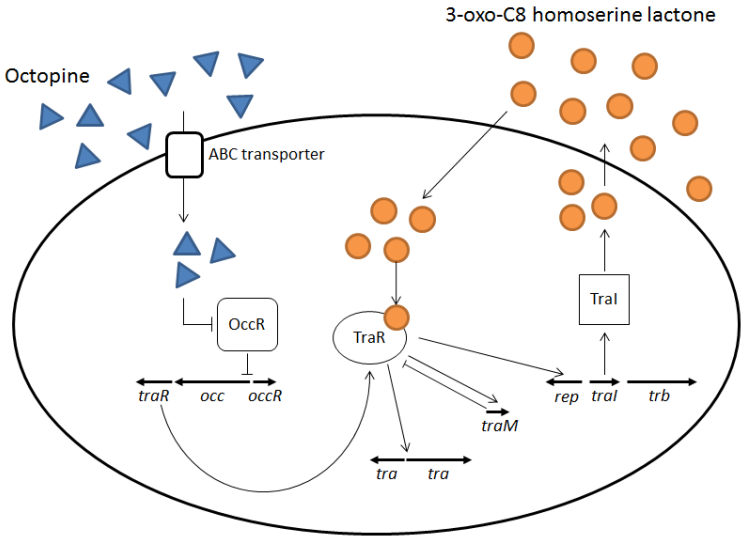

Figure 13. Simplified diagram of A. tumefaciens quorum sensing on an octopine-type Ti plasmid. The depicted genes are located in operon structures o the octopine-type Ti plasmid. The large oval pictures an A. tumefaciens cell. Here, TraI/TraR quorum sensing is dependent on activation by host cells secreting octopine, which is taken up via ABC transporter systems. This diagram was compiled by the author, with information from both White and Winans (2007) and Bassler and Miller (2013).

loop. Other operons that are activated by TraR-AI complexes are *tra,* responsible for mobilisation, *trb*, responsible for mating pores and *rep*, responsible for vegetative replication (Bassler & Miller, 2013; White & Winans, 2007). Notably, TraR-AI complex also activates *traM*, which negatively regulates the quorum sensing system by competitively binding TraR (Bassler & Miller, 2013). In addition to TraM, the TraI/TraR system is also regulated by TrlR and possibly by the products of *attKLM* (White & Winans, 2007). However, these will not be discussed in this study.

3.3. The *V. harveyi* multisystem network

Recall the LuxS system in species of *Vibrio*. In contrast to the RhlI/RhlR system in *P. aeruginosa*, that works in tandem or the TraI/TraR system in *A. tumefaciens*, which is dependent on an outside cue, the already briefly discussed LuxS system in *V. harveyi* actually works in parallel with another quorum sensing system. A very surprising fact to the *V. harveyi* multisystem is that no functional protein is homologous to the LuxI/LuxR system found in *V. fisheri* (Bassler & Miller, 2013). This is because even though the functions of LuxLM and LuxS are similar to LuxI and *V. harveyi* LuxR is functionally similar to *V. fisheri* LuxR, no structural similarities are found between the proteins. The multisystem can be divided into two systems (Figure 14). System 1 utilises the autoinducer 1 (AI-1, an AHL derivative) molecule, which is synthesised by LuxLM and received by LuxN. System 2 utilises the AI-2 molecule (a furanosyl borate diester), which is synthesised by LuxS and received by LuxQ. In both systems, low concentrations of the AI molecule lead to a phosphorylation cascade by either LuxN or LuxQ. Successive phosphorylation of LuxU, LuxO and LuxR inhibits translation of the *luxCDABE* operon. At high AI concentrations, LuxN and LuxQ switch from kinase activity to phosphatase activity and initiate a dephosphorylation cascade of LuxU, LuxO and LuxR. Once this has happened, the *luxCDABE* operon can be transcribed. Additional AI-1 and AI-2 specific targets have been identified in *V. harveyi*, but these results remain unpublished (Bassler & Miller, 2013)

AI-1-like molecules were only shown to exist in one other species of bacteria so far. In contrast, AI-2-like molecules are shown to exist in multiple species of bacteria (Bandara et al., 2012; Bassler & Miller, 2013). This led researchers to believe that AI-1 is used for intraspecies communication whilst AI-2 is used for interspecies communication. Another hypothesis is that these two systems allow *V. harveyi* to sense both own-species as well as different-species cell densities in its surrounding area (Bassler & Miller, 2013). Bassler and Miller (2013) propose that this gives *V. harveyi* a selective advantage by allowing it to sense the amount of competition for scarce nutrients. In hindsight, this could be a form of 'decision making', as P. Lyon (2015) described it to be part of the microbial cognitive toolkit. The ability to synthesise AI-2 has been

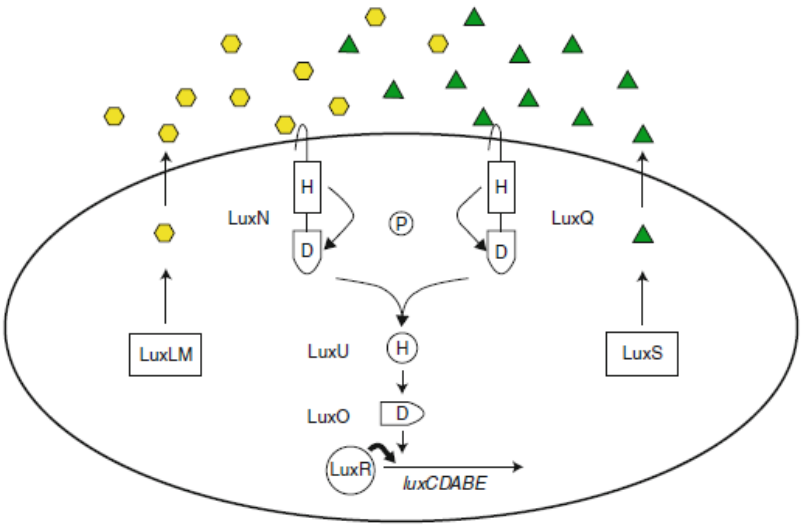

Figure 14. Diagram of the V. harveyi multisystem quorum sensing pathway. AI-1 (yellow), produced by LuxLM and AI-2 (green), produced by LuxS are received by their respective sensor kinases LuxN and LuxQ. This leads to a dephosphorylation cascade which ends in transcription of the luxCDABE operon. This diagram was adapted from Bassler and Miller (2013)

found in over 55 species of both gram-negative and gram-positive bacteria (Bandara et al., 2012). As such, this molecule as well as more bacteria that utilise this system will be discussed further in paragraph 5 of this chapter: interspecies quorum sensing.

3.4. Other quorum sensing molecules

So far only systems that use AHLs as AIs have been elaborated on. Fortunately, AHLs do not have a complete monopoly on gram-negative quorum sensing as will be discussed next.

The "A-signal"

The gram-negative soil bacterium *Myxococcus xanthus* harbours a complex and not yet fully understood quorum sensing system (Bassler & Miller, 2013). These bacteria colonise dead plant material and do this in swarms. This allows *M. xanthus* individual cells to profit from neighbour excreted hydrolytic enzymes. Another feature of *M. xanthus* is that it forms fruiting bodies. The formation of these structures is induced by high cell density under limiting nutrient conditions, making it an ideal set-up for quorum sensing.

Quorum sensing by *M. xanthus* requires a different signal than AHL, called the A-signal. In contrast to an acylated homoserine lactone, the A-signal is composed of a mixture of amino acids that are produced as a consequence of the enzymatic activity of extracellular proteases (Bassler & Miller, 2013). To generate the A-signal, three genes are required. First, a 2CR sensor kinase *asgA* and transcriptional regulator *asgB* and secondly a housekeeping σ-factor encoded by *asgC*. So far, two proteins are known to be involved in receiving the A-signal: the 2CR sensor kinase SasS and response regulator SasR. Phosphorylated SasR interacts with alternative σ^{54} to activate genes involved in spore differentiation. Another protein, called SasN, is known to negatively regulate this system. However, the function of SasN is not yet known. More recently than the research used by Bassler and Miller (2013), another signal called the "C-signal", was coupled to the fruiting body formation of *M. xanthus* (Konovalova, Wegener-Feldbrügge, & Søgaard-Andersen, 2012). This research also suggested that the A- and C-signal act sequentially and are linked by a shared timing mechanism. Nonetheless, much is left to be elucidated on this system.

Diffusible signal factor

Another QSM that has been identified is the diffusible signal factor (DSF). First discovered in species of *Xanthomonas campestris* pv *campestris*, the DSF represents a whole new family of QSMs that appear to be widespread amongst gram-negative bacteria (Deng, Wu, Tao, & Zhang, 2011). The signal itself is proposed to be an unsaturated fatty acid, giving even more variety to the set of quorum sensing systems found in gram-negative bacteria. Even though the regulatory pathways of DSF remain to be elucidated, the molecule was shown to affect biofilm development by suppressing genes for extracellular matrix formation (Bandara et al., 2012). Several other functions of DSF have been found in species of *Stenotrophomonas maltophilia*, where it might control motility, exoprotease synthesis, antibiotic and heavy metals resistance, lipopolysaccharide synthesis, cell aggregation and virulence. DSF-like molecules also appear to regulate many functions in other species, such as biofilm dispersal in *Escherichia coli.* Interestingly, biofilm dispersal and subsequent inhibition of new biofilm formation in *E. coli* might be affected by a DSF-like molecule synthesised by *P. aeruginosa*. More so, DSF has also been shown to inhibit the morphological transition of the yeast *Candida albicans* (Deng et al., 2011).

So far, the bacteria that were found utilising DSF belong to the *β*-Proteobacteria and *γ*-Proteobacteria (Deng et al., 2011). The DSF-based quorum sensing systems that are used by these bacteria can be divided into two groups. The *γ*-Proteobacteria, except *P. aeruginosa*, use a system that is distinguished by colocalization of genes (clustering of coregulated genes on the genome) encoding key components of this system, like

RpfF, RpfC and RpfG. In contrast, other bacteria do not share these genetic regions and homologs to these genes are often located in different places on the genome (Deng et al., 2011).

3.5. Concluding remarks on gram-negative quorum sensing

So far, several quorum sensing systems in gram-negative bacteria have been discussed with homologs to these systems found in many other species of bacteria (Bandara et al., 2012; Bassler & Miller, 2013). In general, the AHL-based system LuxI/LuxR works as a paradigm to describe the mechanisms behind gram-negative quorum sensing. However, as has been discussed, many other signalling molecules are utilised by gram-negative bacteria to effectuate communication on both intra- and interspecies level. Besides the homoserine lactones of the LuxI/LuxR system and its many homologs, gram-negative bacteria can also communicate using amino acids, furnosyl compounds, fatty acids and several other chemical compounds that have not yet been reviewed. As was also discussed, gram-negative bacteria utilise a wide range of proteins to receive signals, by either directly interacting with the signalling molecule from within the cell (LuxR-like), or by receiving the signal and transducing it via 2CR systems (LuxN/LuxQ-like). Even so, this is only the tip of the iceberg if this study were to discuss all systems known to date. It is also worth noticing that some of these systems are also used in interspecies and even interkingdom signalling, the function of which will be reviewed more profoundly later in this study.

4. Gram-positive quorum sensing

In the following paragraph, several gram-positive quorum sensing systems will be elaborated. In general, gram-positive quorum sensing systems involve the post-translational modification of its peptide signal molecules and recognition by sensor components of 2CR signal-transduction systems (Kleerebezem et al., 1997). Also common to gram-positive cell-cell signalling is that signalling molecules are exported through ABC-exporter proteins. In absolute contrast to gram-negative systems, is the absence of any AHL-like molecules. The exception is *γ*-butyrolactone in *Streptomyces spp.,* which is structurally homologous to AHL. Lastly, as with many gram-negative communication systems, the genes involved in the signal production and reception are transcriptionally linked.

Several gram-positive quorum sensing systems are discussed, starting with two systems that control competence in *Streptococcus pneumoniae* and *Bacillus subtilis*, followed by *γ*-butyrolactone signalling in species of *Streptomycin*.

4.1. Regulation of competence in *Streptococcus pneumoniae*

Bacteria have the potential to alter their genetic make-up by transformation (Figure 11). First described in *S. pneumoniae*, this process requires bacteria to take up DNA from the surrounding environment. The ability to do so is known as (genetic) competence (Bassler & Miller, 2013). The development of the competent state in *S. pneumoniae*, as most likely in most other naturally competent bacteria, is controlled by quorum sensing. Somewhat unique in comparison to other bacteria, however, *S. pneumoniae* is able to acquire DNA regardless of its sequence, whilst other bacteria . This feature allows it to acquire DNA regardless of the species of origin. Noteworthy is that this system shares homology to the previously described Agr system in *S. aureus* (Kleerebezem et al., 1997).

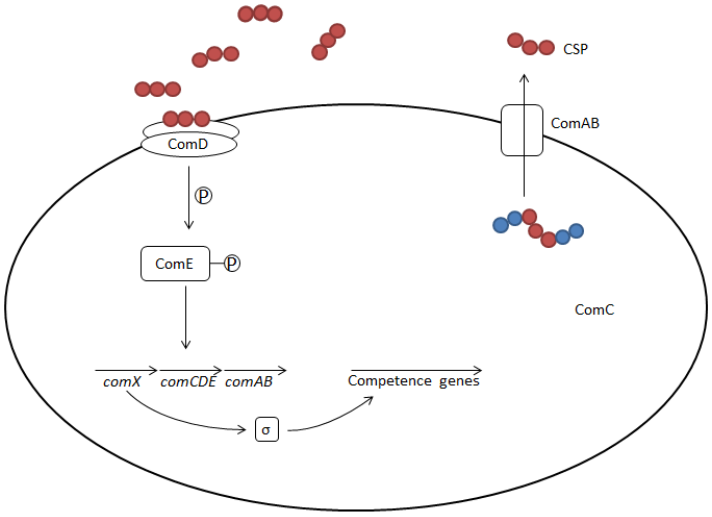

Figure 15. Diagram of the competence stimulating quorum sensing system in S. pneumoniae. The oligopeptides signal CSP regulates the competent state by means of activating ComD and ComE and comX indirectly. The actual cleavage position of ComC might not be accurate in this depiction. This diagram was compiled with information from Reading and Sperandio (2006) and Bassler and Miller (2013).

In *S. pneumoniae*, the signalling molecule is called the competence stimulating peptide (CSP). This molecule is cleaved from a 17 amino acid precursor called ComC (Figure 15) and is excreted by the ABC transporter ComAB (Bassler & Miller, 2013). When high enough cell numbers are reached, CSP is sensed by a 2CR sensor kinase ComD which is followed by a phosphorylation cascade. Eventually, phosphorylation of the response regulator ComE results in activation of the *comX* gene, which encodes an alternative σ-factor. Besides *comX*, ComE also positively regulates the *comCDE* and *comAB* operons (Reading & Sperandio, 2006). The alternative σ-factor, in turn, regulates the genes required for competent state development (Bassler & Miller, 2013).

4.2. Regulation of competence in *Bacillus subtilis*

Another well-described system that regulates competence is found in *B. subtilis*. Besides the system being more complex than in species of *S. pneumoniae*, the *B. subtilis* system also differs in the temporal onset of competence. In *S. pneumoniae*, CSP induced competence is developed in early log-phase (Bassler & Miller, 2013). Supposedly, the reason for this is to acquire heterologous DNA. In contrast, *B. subtilis* competence is developed in the transition from log-phase to stationary phase, in which cell numbers are high and cell lyses occur. In other words, *S. pneumoniae* uses competence to take up DNA from other species as well as its own, whilst *B. subtilis* controls competence to make sure it inherits exclusively own-species DNA.

In *B. subtilis*, two QSMs called ComX and the competence and sporulation factor (CSF) control the development of a competent state (Bassler & Miller, 2013). The ComX-system directly affects gene expression whilst the CSF-system serves a regulatory function by inhibiting ComX-system repressors (Figure 16). Even though the CSF-system utilises a different mechanism than most other systems, it is still considered to be part of quorum sensing because of its reliance on cell-density.

ComX is synthesised from a 55 amino acid precursor and exported, both by the ABC transporter ComQ (Aggarwal & Federle, 2014). When threshold cell densities are reached, ComX is sensed by the ComP 2CR sensor kinase (Bassler & Miller, 2013). As a result, ComP initiates a phosphorylation cascade that leads to the activation of response regulator ComA and subsequent transcription of *comS*. In turn, the protein encoded by *comS* functions to protect a protein called ComK from proteolytic degradation. ComK serves as the transcription factor activating competence stimulating genes. This system is negatively controlled by RapC, which is part of a family of response regulator aspartate phosphatases (Rap proteins). RapC counteracts the ComX-system by cleaving the phosphate group of P-ComA (Aggarwal & Federle, 2014). The CSF-system relieves ComA inhibition by inhibiting RapC activity.

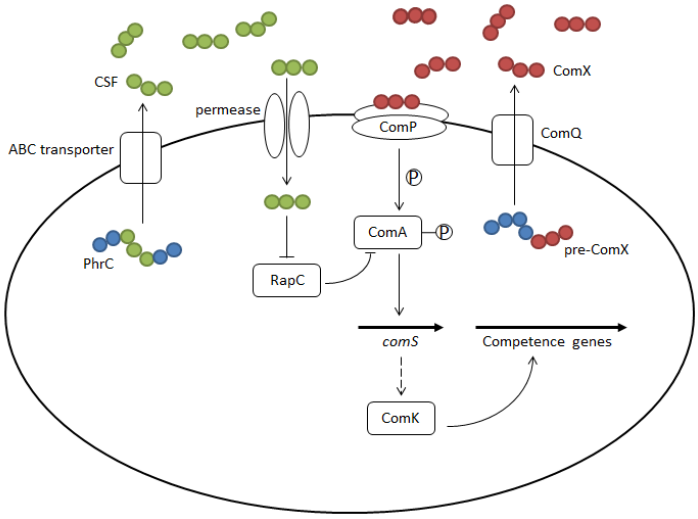

Figure 16. Diagram of the competence stimulating quorum sensing system in B. subtilis. Here, the oligopeptide QSM ComX works in tandem with CSF to activate the expression of competence stimulating genes. The system is additionally regulated by RapC, which counteracts activity of ComA. The actual cleavage position of PhrC might not be accurate in this depiction. This diagram was compiled with information from Bassler and Miller (2013) and Aggarwal and Federle (2014).

CSF is synthesised from a precursor peptide, called PhrC, which got its name as a phosphatase regulator (Aggarwal & Federle, 2014). When CSF is processed from PhrC and subsequently exported from the cell, it is internalised again by an oligopeptide permease when the threshold concentration is reached (Bassler & Miller, 2013). Internalised CSF binds to RapC and inhibits its function, thus relieving ComA inhibition and stimulating the development of competence.

4.3. *Streptomyces* γ-butyrolactone signalling

The gram-positive systems that have been elaborated thus far utilise AIPs as signalling molecules to control gene expression in a density-dependent manner. For some time, the gram-negative genus *Streptomyces* are known to exhibit AHL-like molecules called γ-butyrolactones (GBLs) to regulate morphological development and secondary metabolism, such as antibiotics (Du, Shen, Yu, Bai, & Li, 2011). Several systems have been described, such as the A-factor/ArpA system in *Streptomyces griseus*, SCB-1/ScbR in *Streptomyces coelicolor*, IM-2/FarA in *Streptomyces lavendulae* and VB/BarA in *Streptomyces virginiae*. In general, this system works analogously to the manner in which AHL-signalling is achieved (Biarnes-Carrera, Breitling, & Takano, 2015). A GBL (A-factor, SCB-1, IM-2 or VB), is synthesised by a synthase protein and regulates a homodimer receptor protein. In most cases, the receptor represses transcriptional activators when not bound to a signal.

The A-factor/ArpA system in *S. griseus* is the most profoundly described GBL-based system in species of *Streptomyces,* to date (Biarnes-Carrera et al., 2015). In this system, the GBL signal A-factor is synthesised by AfsA, which is transcribed from the *afsA* gene (Du et al., 2011). Hypothetically, the A-factor signal diffuses to and from the environment in an AHL-like manner. Once internalised again, the signal acts on ArpA, a transcriptional repressor of the gene *adpA* when no signal is present (Ohnishi, Yamazaki, Kato, Tomono, & Horinouchi, 2005). The A-factor-ArpA complex is released from the DNA and transcription of *adpA* can take place. The gene *adpA* encodes a pleiotropic transcriptional activator AdpA, which means it activates a large subset of genes. Figure 16 displays a simplified depiction of the A-factor/ArpA system, as drawn by the author of the current study. This depiction is by no means complete and contains hypothetical connections (orange).

Whilst the GBL systems of multiple species of *Streptomyces* are structurally highly similar, very different effects on secondary metabolism are observed (Du et al., 2011). In *S. griseus*, GBL quorum sensing facilitates the regulation of morphological differentiation (serine proteases, metalloproteases, aerial mycelium formation, septum formation and sporulation) and streptomycin production, amongst other antibiotics

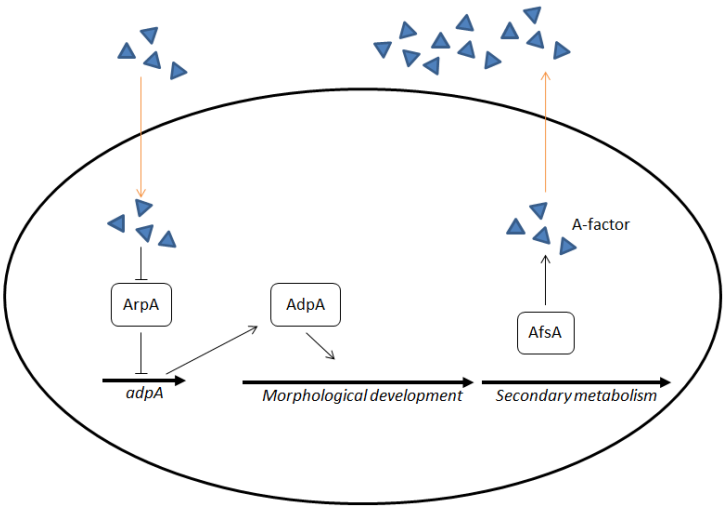

Figure 17. Simplified diagram of the S. griseus A-factor/ArpA system. This gram-negative system is highly analogous to AHL-based quorum sensing. As such, the figure shows known (black) as well as hypothetical pathways (orange). This depiction was compiled by the author with information from Biarnes-Carrera et al. (2015), Du et al. (2011) and Ohnishi et al. (2005).

(Biarnes-Carrera et al., 2015; Ohnishi et al., 2005). In *S. coelicolor*, a homologous protein to AdpA is also found and serves a self-regulatory function (Biarnes-Carrera et al., 2015). In contrast to *S. griseus*, however, antibiotic production in *S. coelicolor* is not controlled by A-factor. Instead, three signalling molecules are used called SCB1, SCB2 and SCB3. Still, a lot is left to be discovered about the biochemical pathways that make up these different GBL systems.

4.3. Concluding remarks on gram-positive quorum sensing

In total, four gram-positive quorum sensing systems have been discussed in this study. First, the *S. aureus* Agr system, which is used as a model to describe most forms of gram-positive quorum sensing. Secondly, this study elaborated the *S. pneumonia*e CSP and *B. subtilis* CSF systems as a simple and more complex model of competence regulation in gram-positive bacteria, respectively. Lastly, the GBL system in *S. griseus* was displayed which showed great analogy to gram-negative AHL-based quorum sensing.

At first sight, the gram-positive peptide language might seem less complicated or diverse than the systems described in the gram-negative discussion. However, it should be taken into account that AHLs and related chemical structures have less potential for diversification. In contrast, a single peptide QSM, which typically consists out of 5 to 17 amino acids, allows for many different conformational options in primary, secondary, tertiary and quaternary structure. Be that as it may, the gram-positive paradigm still consists out of a modified peptide signal which is secreted by a dedicated ABC transporter and a 2CR transduction path wich acts on the signal (Bassler & Miller, 2013). The GBL system in *Streptomyces* species is a clear exception to that paradigm and *B. subtilis* CSF molecules also utilise different means of internalisation.

5. Interspecies signalling

Individual species of bacteria are shown to hold tight species-specific relationships to accurately determine the need for morphological and physiological changes. However, *in vivo*, bacteria rarely live in uniform communities of a single species. Complex biological structures such as sludge and flocs found in wastewater treatment plants, plant rhizospheres or biofilms in most other bacterially occupied surfaces require specific and unique signalling methods for interspecies communication and cooperation for establishment, sustainability and survival (Bandara et al., 2012). These conversations are not all charitable, however, as quorum sensing can also be used by bacteria to obstruct the competition.

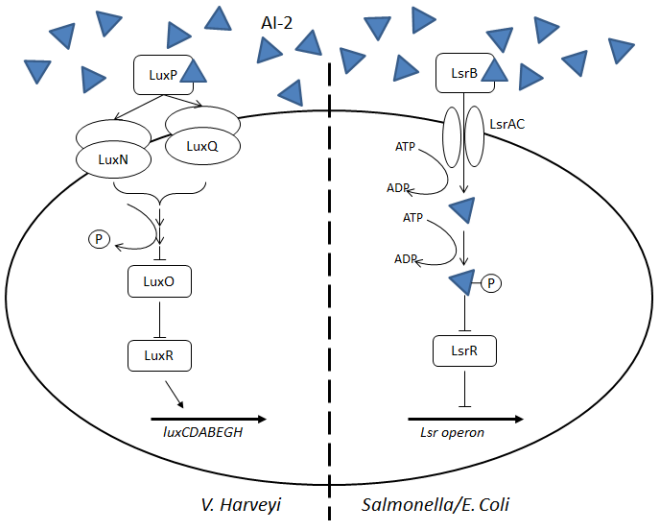

Figure 18. Diagram of different detection methods of AI-2 (blue triangles) in V. harveyi (left) and Salmonella/E. coli (right). These systems are based on 2CR signal transduction and import of AI-2 signalling molecules, respectively. This image was adapted from Bandara et al (2012) and edited for this study.

5.1. AI-2, the universal signalling molecule

Recall the *V. harveryi* LuxS system in §1.2 and §3.3. The *luxS* gene, which encodes the synthase protein for AI-2, has been found in a wide range of gram-positive and gram-negative bacteria (Bandara et al., 2012). Even more species seem to react to AI-2 signalling because the LuxS enzyme itself is not needed for signal detection. For example, in *P. aeruginosa*, AI-2 promotes virulence but cannot be produced by the bacterium itself (P. Williams & Cámara, 2009). Instead, *P. aeruginosa* utilises AI-2 produced by bacteria in its surrounding area to promote virulence. Another feature of AI-2 signalling, though synthesis is fairly similar in many species of bacteria, is the diversity at which the signal is detected (Bandara et al., 2012).

This study already depicted the basic means by which *V. harveyi* uses AI-2 as a signalling molecule. A completely different way of detecting the AI-2 signal, however, has been found in species of *Salmonella* and *E. coli* (Walters & Sperandio, 2006). In *Salmonella* and *E.* coli, AI-2 is recognised in the periplasm by its cognate receptor, LsrB (LuxS regulated; Figure 18). LsrB-AI-2 interacts with a membrane bound ABC transporter LsrAC causing AI-2 transport into the cell at the cost of ATP (Bandara et al., 2012). Once inside the cytoplasm, AI-2 is phosphorylated by LsrK. Phosphorylated AI-2 is bound by the transcriptional repressor LsrR, which subsequently is released from the *lsr* operon.

The diversity by which AI-2 controls expression of different genes in different organisms becomes apparent when reviewing the regulatory pathways it affects. Research shows the affiliation of AI-2 with biofilm formation, virulence, type III secretion systems, iron acquisition, heme acquisition, protease production, bioluminescence, colony morphology, siderophore production, pleiotropic protein expression, motility, cell division, metabolism, toxin production, bacteraemia infection, antibiotic production and ABC transporter expression (Bandara et al., 2012, and references therein). This makes it complicated to draw profound “interaction maps”, explaining the spatial and temporal biochemical relations between species. Bandara et al (2012) describe the oral cavity as one such well-studied theatre of such complex interactions, biofilm formation by *Porphyromonas gingivalis* and *Streptococcus gordonii*, for example. Mutant LuxS in *P. gingivalis* resulted in reduced virulence gene expression and failed to develop biofilms on pre-existing *S. gordonii* biofilms, *in vitro*.

5.2. Other interspecies communication

The profoundly discussed AHL molecules are also shown to be involved in interspecies quorum sensing. For example, AHLs secreted by *P. aeruginosa* in cystic fibrosis (CF) patients are recognised unidirectional by *B. cepacia* (Bandara et al., 2012). It is believed that *P. aeruginosa* acts on *B. cepacia* species in this manner to

induce virulence and host infections. *P. aeruginosa* competitiveness is also shown to be of superior nature. Some AHL-derivatives that are generated by spontaneous AHL degradation possess bactericidal activity. Gram-positive bacteria such as *S. aureus* are affected by this phenomenon whereas gram-negative bacteria such as *P. aeruginosa* are not.

Another form of competitive interspecies signalling is the way *E. coli* and *Salmonella enterica* serovar *typhimurium* are able to receive, but not synthesise AI-1 (Bandara et al., 2012). In these bacteria, AI-1 is received through the LuxR homolog SdiA. This protein positively regulates genes associated with virulence and host immune response evasion. In enterohemorrhagic *E. coli* (EHEC) and *S. typhimurium*, this allows for alteration of gene expression in response to AI-1 production in the gastrointestinal tract of their host. Another molecule that might play a role in intestinal interspecies quorum sensing is AI-3. However, much is left to be elucidated on this molecule.

To date, many QSMs have been identified to play a role in interspecies quorum sensing. However, as has been stated before, these complex social interactions are hard to map and research surrounding these conversations is in its infancy. Several other known molecules are indoles, DSF, CSP (Bandara et al., 2012), peptidoglycan and antibiotics (Shank & Kolter, 2009). Together, these molecules serve both competitive (growth inhibition, virulence inhibition, bactericidal, other forms of inhibition) and cooperative functions (growth attraction, growth stimulation, germination, secondary metabolite induction, biofilm stimulation and other forms of stimulation).

5.4. Interkingdom signalling

In nature, microbes and eukaryotic organisms hold a tight relationship to one another. In fact, humanity would not survive under entirely sterile conditions. The same applies when looking at the plant kingdom. In rhizospheres, plants hold tight relationships with microbes and cooperatively provide each other with nutrients. Indeed, microorganisms share many relationships with their bigger, multi-celled counterparts, as will be briefly reviewed. One such system will be profoundly discussed, making use of AI-3, epinephrine and norepinephrine as signalling molecules in the interaction between humans and *E. coli*.

Most bacteria in the human gastrointestinal (GI) tract microbiome, around 10^{14} against 10^{13} mammalian cells, serve to shape mammalian innate immunity and aid in nutrient processing (Walters & Sperandio, 2006). The human body, as well as bacterial cells, employ several hormones and hormone-like molecules to communicate in this tight-knit community. The aforementioned EHEC pathogen is able to hijack these signals and use them to its own advantage.

EHEC is a notorious bacterium, most commonly associated with hospital outbreaks and causes bloody diarrhoea and haemolytic uremic syndrome (HUS, red blood cell destruction and kidney failure) (Walters & Sperandio, 2006). The genes that encode virulence in EHEC are located on an island of several operons, called the *LEE* pathogenicity island. Several genes called *LEE1, LEE2, LEE3, tir (LEE5)* and *LEE4* encode several virulence factors, including type III secretion systems (TTSS), intimin (an adhesin) and Tir (the intimin receptor). TTSS is responsible for the translocation of Tir to mammalian epithelial cells. The *LEE* island is activated by Ler and further positively regulated by GrlA. Negative regulation occurs by means of GlrR. Two very potent toxins, Shiga toxin 1 and 2, are released upon EHEC cell lyses. These toxins are encoded by a λ-like bacteriophage that enters the lytic cycle when EHEC expresses a cell stress response.

In humans, epinephrine and norepinephrine affect intestinal smooth muscle contraction, submucosal blood flow, chloride secretion and potassium secretion in the intestines (Walters & Sperandio, 2006). These

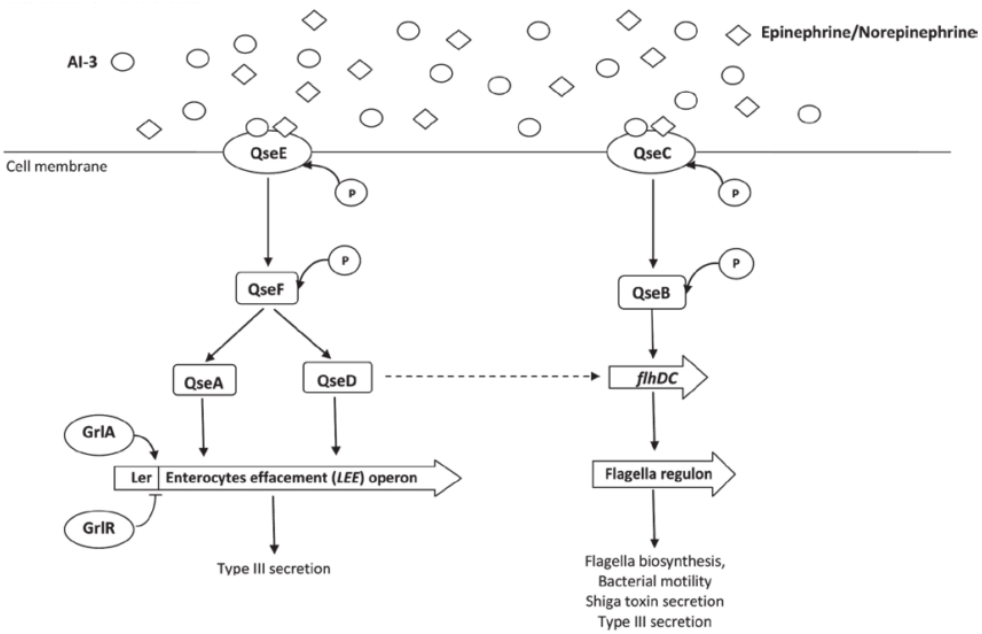

Figure 19. The AI-3/epinephrine/norepinephrine signalling system in EHEC bacteria. Microbially produced AI-3 works in conjunction with mammalian epinephrine/norepinephrine to promote virulence in EHEC and cause infection in the human GI tract. This diagram was adapted from Bandara et al. (2012).

molecules are expected to serve an important role in the biological management of the GI tract. In conjunction with GI microbially produced AI-3, the two molecules epinephrine and norepinephrine trigger a signalling cascade that leads to EHEC virulence (Figure 19). AI-3 is sensed by a 2CR system comprised of a sensor kinase QseC and a response regulator QseB. Activated QseB upregulates the master flagellar regulator genes (Bandara et al., 2012). After interacting with epinephrine and norepinephrine, AI-3 also upregulates the LEE pathogenicity island. The interaction between EHEC and humans is not one-sided (epinephrine/norepinephrine received by EHEC). In fact, AI-3 mediated motility of EHEC allows it to penetrate the submucosal epithelial cells of the GI tract and interact with mammalian. This interaction is made possible by the proteins encoded by the LEE pathogenicity island. EHEC attaches to these cells and further colonises the submucosal lining of the human GI tract, promoting infection (Bandara et al., 2012; Walters & Sperandio, 2006).

Discussed several times before in this study, *P. aeruginosa* makes a potent pathogen that is amply present in individuals suffering from CF. Research has shown that microbial AIs are able to regulate eukaryotic gene expression, as appears to be the case with *P. aeruginosa* AHL signals (S. C. Williams et al., 2004). The hypothesis in the study by S. C. Williams et al. (2004) was that autoinducing signals PAI-1 and PAI-2 interacted with outer membrane receptors on mammalian cells, considering the similar nature of QSMs to mammalian hormones. Interestingly, though, the research team found that PAI-1 and PAI-2 could actually enter and function in mammalian cells, as long as the cognate receptor proteins were available. In the discussed investigation, the authors used artificial LasR/RhlR-based proteins to act as receptors within the mammalian cell. The active LasR/RhlR-based proteins were transported to the nucleus of the mammalian cell, mediated by the nuclear localisation signal, and were able to interact with mammalian genes.

Chapter III – Quorum Quenching
Jamming bacterial communication

From understanding the basic principles in which bacteria interact with their environment to the evolutionary foundations and profound systems of bacterial communication, there are also ways to manipulate quorum sensing. Many potent pathogenic bacteria make use of these discussed systems to consentaneously infect their host. Some notorious organisms such as EHEC, *S. aureus* and *P. aeruginosa* have already been discussed and receive a great deal of attention in the research on this field as well. These organisms rely partly on their quorum sensing systems to form a coordinated attack on the host. Principally, quorum sensing allows pathogenic bacteria to avoid host immune systems until a threshold cell density is reached, by suppressing virulence factors (Galloway, Hodgkinson, Bowden, Welch, & Spring, 2012).

With the on-going development of antibiotic resistance and the affiliated outbreaks of multi-resistant bacteria, science turns to alternative ways of controlling bacterial infection. Indeed, quorum sensing is a potential target for antibiotic alternatives, as is outlined in this chapter. Besides, nature has already found its way to repel pathogenic quorum sensing bacteria, as was shown in species of the seaweed *Delisea pulchra* (Bassler & Miller, 2013). This plant species has the ability to produce halogenated furanones which are structurally related to AHLs. The *D. pulchra* furanones inhibit swarming motility in *Serratia liquefaciens* and several other bacterial species, reducing their ability to colonise surfaces and consequently reducing their pathogenic efficiency towards the plant.

The principle of manipulating quorum sensing to suppress virulence without affecting bacterial growth or viability has been coined “quorum quenching” (Bandara et al., 2012). The fact that quorum quenching does not affect bacterial growth or viability is especially alluring (Galloway et al., 2012). By specifically targeting quorum sensing pathways, no disruption of selective pressure in the natural environment would occur. Current antibiotic treatments often disrupt the natural microbial environment of patients, making them vulnerable to new infections and increasing the speed at which resistance against the treatment develops (Reuter, Steinbach, & Helms, 2016).

Quorum quenching is achieved on four levels: (1) signal production, (2) the AI signal, (3) antagonism of the regulator and (4) inhibition of regulator DNA binding (Reuter et al., 2016). This chapter will outline several basic strategies on artificial inhibition within these levels. The following paragraphs will also give more in-depth examples of quorum quenching in species of *V. fisheri*, *S. aureus* and *P. aeruginosa*, three model organisms in the present study. In general, however, approaches to quorum quenching rely on inhibition of histidine kinase sensors, antagonistic relations on receptor-ligand binding domains, sequestering of AHLs and AIPs by high-affinity compounds and inhibition of effectors (Galloway et al., 2012; G. J. Lyon & Muir, 2003; Reuter et al., 2016).

1. Quenching AHL-based systems

Recall that in AHL-based systems, three major components make up the pathways that many gram-negative bacteria utilise: a LuxI-type signal synthase, an AHL-type signalling molecule and a LuxR-type signal receptor. These three components serve as the most straightforward means by which AHL-based quorum sensing systems could be inhibited (Galloway et al., 2012). Indeed, many strategies revolving the inhibition of gram-negative quorum sensing involve manipulation of one or more of these components. Starting with signal production, a surprisingly low number of publications exist on the modulation of LuxI-type signal synthases, as research seems to focus more on the AHL signal itself (Galloway et al., 2012). Even so, research on the *P.*

aeruginosa PQS system led to the discovery of a potent class of inhibitors that target the production of PQS and its precursor (Maurer et al., 2015). The inhibitor was able to inhibit synthesis of PQS and its precursor, as well as biofilm formation in *P. aeruginosa* altogether.

At the level of AI inhibition, several more techniques appear effective in undermining gram-negative quorum sensing. One such technique focuses on the sequestration of the AHL signal itself (Reuter et al., 2016). Signal sequestering polymers (SSPs), designed with a high affinity towards specific AHL molecules in *V. harveyi* were able to suppress quorum sensing induced bioluminescence. Moreover, these polymers appeared to be non-toxic to mammalian cells, making them ideal for clinical practice. Other forms of AI inhibition act by attacking the signalling molecule directly. For example, acylase enzymes are able to degrade AHL signalling molecules and thwart virulence in *P. aeruginosa* (Reuter et al., 2016 and references therein).

Antagonising the receptor-AHL complex can be achieved by engineering modified AHL signals. In one such example, chemically modified AHLs showed binding activity to LuxR-like receptors but lacked the ability to cause the necessary conformational change (G. J. Lyon & Muir, 2003). This caused the receptor-AHL (modified) complex to be ineffective in changing gene expression. In general, functional modification of AHL is accomplished by modifying the acyl chain, replacing the amide function with sulfonamides, ureas and sulfonylureas or modifying the lactone ring. Maurer et al. (2015) display an in-depth overview of these modifications and their effects on their cognate receptor proteins.

The last level of inhibition is by preventing the signal-receptor complex from binding DNA. In a knock-out experiment, in which a selected gene is disabled (knocked out), on *P. aeruginosa*, a non-functional *qslA* gene caused an increased expression of virulence factors. This gene normally serves as an anti-activator gene and the gene product QslA normally interacts with LasR to the extent that LasR can no longer bind its target promoter (Reuter et al., 2016). This feature makes QslA a possible quorum sensing inhibitor because it is able to prevent the expression of virulence genes.

Although the principle means that disrupt AHL-based signalling is straightforward; disrupt the synthase, the signalling molecule or the receptor, a lot is left to be elucidated on the matter. For example, several factors have to be taken into account, such as the effect of a single molecule on multiple species of bacteria (ex. AI-2) and enzymatic degradation of signalling molecules in *in vivo* situations (Galloway et al., 2012). The former could still disrupt the natural habitat, as do antibiotics. The latter could potentially render a quorum quenching molecule ineffective, because of its degradation by the host or the bacterium itself.

2. Quenching AIP-based systems

Aside from the traditional levels of quorum sensing inhibition, used to describe quorum quenching methods in AHL-based systems, AIP-based systems have additional targets for inhibition. AIPs in gram-positive bacteria need to be externalised by exporters and internalised by importers, which can be inhibited. Few cases have been described on inhibition of AIP signal synthesis, as is the case in research on AHL-based inhibition. In the Fsr system of *Enterococcus faecalis*, ambuic acid works as a quorum sensing inhibitor by targeting biosynthesis of the signalling molecule GBAP (Maurer et al., 2015). Specifically, the biosynthesis of this molecule is disrupted by inhibiting the protease function of the FsrB exporter protein, which is to cyclise and process the GBAP propeptides FsrD (somewhat comparable to the function of ComAB in *S. pneumoniae*, Figure 15).

Once an AIP is secreted into the environment, it can be attacked directly by means of antibody binding (Reuter et al., 2016 and references therein). In this approach, antibodies were designed in mice to target AIP-IV of a respective *S. aureus* group IV strain. The anti-AIP-IV antibodies showed a high specificity in binding to the AIP-IV signalling molecules. Application of these antibodies on mice with *S. aureus* associated abscess formation yielded similar positive results.

Inhibition of the receptor-signal complex can also be achieved through several means. First, Maurer et al. (2015) describe two methods, one in which an engineered peptide competitively binds the receptor histidine kinase FsrC in *E. faecalis*, and one in which siamycin I inhibits phosphorylation of the response regulator FsrC. Similar interactions have been identified for other species. Two antagonists named solonamide A and B, for example, are able to inhibit quorum sensing in the highly virulent Methicillin-Resistant *S. aureus* (MRSA) strain (Reuter et al., 2016).

Also similar to AHL quorum sensing inhibition, in AIP signalling, the transcription factor can be inhibited from binding their cognate promoter sequence. In *S. aureus*, a compound named savirin (*S. aureus* virulence inhibitor) was shown to inhibit AgrA DNA binding, by binding to it (Reuter et al., 2016). The specificity of savarin for *S. aureus* becomes apparent when taking into consideration that this compound preferentially binds *S. aureus* over *Staphylococcus epidermis* AgrA, which share a high similarity. *S. epidermis* serves an important role in the defence against skin pathogens, which makes this specificity desirable.

3. Mixed-tactics quenching

In light of the onset development of antibiotic resistance in bacteria, more alternatives other than quorum sensing inhibition have been proposed. Another large area of investigation focuses on antimicrobial peptides (AMPs), for their ability to kill antibiotic-resistance pathogens (Maurer et al., 2015). However, AMPs do not get the specificity that is desired and are still likely prone to development of resistant bacterial strains. As such, a new class of antimicrobials are introduced, called pheromone-guided antimicrobial peptides (PG-AMPs). In essence, PG-AMPs are a combination of QSMs and AMPs, therefore combining the effective killing-ability of AMPs and specific targeting-ability of QSMs. Several PG-AMPs have already been synthesised and tested. In one such case, the PG-AMP C16G2 was used in mouth rinse on human volunteers to test its effect against *Streptococcus mutans*. This research found that C16G2 is a potent substance in inhibiting *S. mutans* and reducing overall dental biofilm formation.

Tests with IMB-2, another PG-AMP show that these molecules can be engineered to uphold in a wide variety of environments including high salt concentrations and low pH values (Maurer et al., 2015). These results suggest that perhaps the answer to antibiotic resistance is not an either/or situation. Rather, a combination and even collaboration of several new antimicrobial techniques could be considered to replace antibiotics. Although the likelihood of bacteria developing resistance to quorum sensing inhibitors is less probable than antibiotic resistance, cases of this are still observed (Kalia, Wood, & Kumar, 2014). Considering the major regulatory pathways that are controlled by quorum sensing, it is also important to acknowledge the strong evolutionary drift towards survival. Kalia et al. (2014) argue that systems might evolve that include inhibition of anti-QSMs and/or virulence mechanisms independent of AHL-based pathways. In-depth research in the development of resistance to these new anti-microbial agents is of the essence, especially when considering the past mistakes that led to antibiotic resistance.

Conclusions

The microbial social network

It seems apparent that bacteria can no longer be seen as individual cells, operating in isolation and solemnly for their own survival without interaction, altruism and other forms of social behaviour. Instead, complex communication systems have been uncovered in many species of bacteria that control many forms of coordinated, multicellular behaviour. In some cases, such as the highly developed biofilms that harbour compartments of differentiated bacteria, one might even consider these structures part of a multicellular organism. Even more fascinating, one might consider these interactions to be a part of the human organism (Bassler, 2009).

The present study set out with a goal to elucidate on how quorum sensing facilitates the communication between bacteria in a microbial population. To answer this question, the chapters of this investigation sought answers to the following questions: what differences do gram-positive and –negative bacteria show in quorum sensing, what distinguishes interspecies from intraspecies quorum sensing, how can quorum sensing be inhibited and what are the influences of microbial quorum sensing on higher organisms?

Within the microbial world of communication, a clear division between gram-positive and gram-negative communication is found. Here, gram-positive bacteria exert the language of peptide molecules and gram-negative bacteria utilise AHLs or otherwise small molecules to communicate. In both cases, communication gives bacteria the ability to act as a multicellular unit, the purpose of which serves towards an evolutionary advantageous position (Bassler & Miller, 2013). This division caused the emergence of three models in their respective model organisms, one model for AHL-signalling, one for AIP-signalling and one for interspecies signalling. All models have in common that they rely on the increasing concentration of AI as cell density grows and that the reaction they provoke serves a positive autoregulatory function.

The LuxI/LuxR system in *V. fisheri*, in which AHLs synthesised by LuxI diffuse towards the environment and are received by LuxR when internalised again, serves as a basic model for all related AHL-based signalling pathways (Bassler, 1999). Here, signalling molecules are not dependent on transporters to carry them across the membrane, with exceptions such as the TraI/TraR system in *A. tumefaciens* (White & Winans, 2007) and AI-1 signalling in the multisystem of *V. harveyi* (Bandara et al., 2012; Bassler & Miller, 2013). In contrast, gram-positive AIP signalling is largely dependent on export by ABC-transporters which modify the pro-peptides signal on export (Bandara et al., 2012). The model system that represents this class of quorum sensing is found in *S. aureus*, where it controls the *RNAII* and *RNAIII* operons, affiliated with *S. aureus* virulence. In the *S. aureus* Agr system, pro-peptides are cleaved and exported by AgrB and sensed by the 2CR histidine kinase AgrC. This interaction leads to a phosphorylation cascade, activating the response regulator AgrA and consequently to the activation of its cognate promoter sequences.

Both forms of quorum sensing show increasing complexity when bacteria use multiple quorum sensing systems to regulate gene expression, such as is the case in *B. subtilis* and *V. harveyi* (Bassler & Miller, 2013; Reading & Sperandio, 2006). Moreover, communication becomes even more elaborate when bacteria start to communicate amongst different species. To this extent, AI-2 has been assigned the position of a universal signalling molecule, with over 55 species of bacteria responding to its presence (Xavier & Bassler, 2003). The extremely virulent EHEC bacteria is able to hijack and utilise mammalian hormonal signalling molecules (Bandara et al., 2012). EHEC utilises these hormones in conjunction with interspecies AI-3 signalling to control the LEE pathogenicity island and dictate infection of the human GI tract. Luckily, however, as has

been reviewed in chapter III, science has come to understand ways to employ quorum sensing to our own benefit as well. By developing our understanding of quorum quenching, it is possible to manipulate these intimate relationships amongst bacteria and create potent alternatives to antibiotics (Maurer et al., 2015; Reuter et al., 2016). The way in which an actively communicating community of microbes can be disrupted is achieved on the levels of signal production, the signal itself, antagonism of the regulator and inhibition of the DNA binding capacity of the regulator (Reuter et al., 2016).

Quorum sensing facilitates the communication between bacteria in a microbial community by the secretion of messages into the environment. These signalling molecules convey information about cell density, the metabolic potential of the environment and community composition. Accordingly, bacteria sense and integrate these signals to adapt to their ever-changing environment. In this way, quorum sensing facilitates perception, communication and decision making and contributes to the possible existence of bacterial cognition.

Discussion and future perspectives

This literature study aimed to elucidate the means by which bacteria communicate amongst themselves and others in an active microbial community. The findings in this study demonstrate that bacteria use complex signalling systems that excrete and integrate signals, resulting in changes in gene expression (Bandara et al., 2012). The main difference that is observed between gram-positive and gram-negative bacteria is the use of AIPs and AIs, respectively. Intraspecies signalling makes use of species-specific molecules that are not recognised by other species. In contrast, interspecies signalling makes use of common QSMs such as AI-2, which are observed in many bacteria (Bassler & Miller, 2013). These different quorum sensing systems are potential targets for new antimicrobial agents (Maurer et al., 2015). Considering the widespread use of quorum sensing systems in bacteria, these antimicrobial agents might find use in many areas.

The findings of the current study are congruent with current literature on quorum sensing. These findings and those stated in reviews used in this investigation, characterise quorum sensing as a means of signalling and communication amongst bacteria (Bandara et al., 2012; Bassler & Losick, 2006). However, according to Keller and Surette (2006), a signal – and inherently communication – is an act, structure or chemical emission that alters the behaviour and gene expression of other organisms, evolved because of that effect and is effective because the receiver's response has also evolved. This complicates the terms 'signal' and 'microbial signalling'. In the literature, these terms are used to describe organised communication between bacteria (Suchetha et al., 2015). Although bacteria developed quorum sensing circuits early on in their evolution, it is not certain that it developed specifically for that effect (Lerat & Moran, 2004). Horizontal gene transfer most likely caused the spread of quorum sensing systems throughout different bacteria phyla, which resulted in the possibility of interspecies communication (Gray & Garey, 2001). Similarly, however, it is not clear whether these interactions developed because of that effect. If the interactions evolved for another reason than the effect they have on microbial communities, Keller and Surette (2006) describe, it is called a cue. This discrepancy could provide another area of investigation, especially when considering a convergence between microbiology and ethology.

Quorum sensing is a fascinating phenomenon that involves many aspects, most of which are yet to be discovered in coming research and unpublished work. According to Bassler and Miller (2013), the study of quorum sensing is still in its infancy. Accordingly so, several quorum sensing systems were pieced together in the present study, of which no model was described yet. This resulted in some diagrams of the present study being compiled by the author with information from separate studies (e.g. Figure 13 and 15). Experimental proceedings are needed to confirm these models. Furthermore, the current literature study only aimed to elucidate quorum sensing in Bacteria. This has the possibility of underexposing quorum sensing altogether, as the same process is also found in Archaea and Eukaryotes (Bandara et al., 2012).

Questionable aspects of quorum sensing research include the ease by which phenotypic change is coupled to quorum sensing regulation. For example, research into quorum sensing involvement in biofilm formation is often achieved by introducing mutants. A mutant strain of *S. aureus*, for example, which is defective in one or more components of the Agr signalling system, will not form adequate biofilms. Considering the results from the present study, this is no surprise. However, a common practice that has been observed from the studies (and references therein) used in this investigation is to introduce mutations in global regulatory networks and consecutively assessing the biofilm phenotype. In such a case, it would be surprising if the added mutation did *not* cause a change in biofilm phenotype. Similarly, Parsek and

Greenberg (2005) argue that generating a mutation in a global regulatory network and the subsequent change in motility, surface appendages or chemistry of the cell surface might always translate to a divergent biofilm phenotype.

The ability to target quorum sensing as an alternative to antibiotics is a promising field of further research. Considering the onset development of multiresistant bacteria such as MRSA, EHEC and other superbugs, this is especially important to clinical healthcare. The discussed polymers in chapter III serve as a possible research platform for the invention of antibacterial coatings on hospital equipment (Maurer et al., 2015). Similarly, other methods such as AMPs and crossover PG-AMPs serve a promising role in microbial infection control. This research should not only contain itself to clinical practices, however. Recall from the introduction that biofilms also play a major role in wastewater treatment. The example used there involved sulphur bacteria forming biofilms that produce sulphuric acid, a highly corrosive component. Another problem that is yet unsolved in wastewater treatment is the sudden formation of “bulking sludge” or “foaming”, in which filamentous bacteria exert an explosive proliferation. The formation of an excess of foam leaves the wastewater treatment plant with a problem, solid particles will no longer be able to settle and thus the plant is left with wastewater effluent (Martins, Pagilla, Heijnen, & Van Loosdrecht, 2004). It is possible that quorum sensing underlies this process and serves as a possible target for solving the problem.

Reflectie

In de afgelopen maanden heb ik mogen werken aan mijn vakwerkstuk over de communicatie tussen bacteriën. Deze onderneming gaf mij de kans om me compleet te verdiepen en specialiseren in een onderwerp naar keuze. Dit heb ik als erg prettig ervaren en gaf mij veel voldoening. Het resultaat zoals deze hierboven uiteengezet is, ben ik daarom ook erg tevreden mee. Gedurende het schrijven van dit werkstuk heb ik mij mogen verdiepen in de microbiologische aspecten van de biochemie en cellulaire communicatie. Daarbij heb ik veel kennis moeten toepassen uit de vakken die mij in het Windesheim curriculum aangeboden zijn, alsmede gedurende mijn pre-master op de Radboud universiteit. Ik heb met dit werkstuk ook geprobeerd om een bewijsstuk neer te zetten die getuigd van mijn potentie om door te stromen naar een universitaire master.

Het schrijven van dit vakwerkstuk vergde veel tijd, inzet en vaak ook inzicht. Het gebruik van diverse reviews en het omzetten van meerdere reviews naar een enkele, samenhangende tekst ging mij goed af. Wat ik echter nog erg lastig vind, is het binden van een algehele conclusie uit verschillende experimentele onderzoeksrapporten. Deze rapporten zijn vaak beschrijvend geschreven zonder harde conclusies te bieden. Het kost ook veel moeite om te verdiepen in de manier waarop het onderzoek uitgevoerd is om de validiteit te beoordelen. Ik verwacht echter dat dit makkelijker gaat, naarmate er zelf ook onderzoek naar dit onderwerp gedaan wordt. Je bent in dat geval immers al bekender met de populaire onderzoeksmethodieken in dat onderzoeksveld.

Wat ik als een erg mooie ervaring heb gezien gedurende dit laatste collegejaar, was het colloquium van de vakwerkstukken. Om in een grote collegezaal te staan en je onderzoek te presenteren aan een groep gelijkgestemden is een ervaring die ik graag nog vaker wil meemaken. Daarop heb ik nog wel aan te merken dat het gewicht en de formaliteit van het colloquium nog beter zouden zijn wanneer deze aan het einde van het jaar zou worden georganiseerd. Natuurlijk begrijp ik ook dat dit om meerdere redenen bemoeilijkt wordt.

Tot slot hoop ik dat u veel leesplezier heeft ondervonden aan het vakwerkstuk en dat ik mijn enthousiasme over de complexiteit van onze microben wellicht heb kunnen overdragen. Het is bij mijn begeleiders al enige tijd bekend dat ik sterke twijfels heb over mijn voortzetting in het middelbaar onderwijs. In plaats daarvan ambieer ik een vervolgcarrière in de research. Ongetwijfeld zal ook hier onderwijs een grote rol spelen, wees het universitair of middels een docerende functie op het hbo.

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Bijlage 1 – Onderzoeksplan

1 Inleiding

Tot voorkort werden bacteriën in definitie beschouwd als zelfstandige organismen die fundamentele processen als zelfregulatie en voortplanting individueel uitvoeren. Een ontdekking uit de jaren 60 van de vorige eeuw bracht deze definitie echter onder revisie. Door de ontdekking van wat we nu 'quorum sensing' (QS) noemen is aan het licht gekomen dat bacteriën niet zo zelfstandig leven als voorheen gedacht werd.

QS is in zijn fundament een geadvanceerd communicatie systeem waarbij chemische moleculen worden gebruikt als boodschappers (Bassler & Losick, 2006). Quorum staat in deze context voor een groep cellen, een quorum, en wijst erop dat QS over processen gaat die alleen in groepen worden uitgevoerd (Bassler, 2002). Inderdaad, QS blijkt het regelsysteem achter een groot aantal 'sociaal-interactieve' processen tussen bacteriën. Tot deze processen behoren onder meer biofilm vorming, antibioticaproductie en resistentie, sporenvorming en pathogeniciteit (Bandara et al., 2012; Bassler & Losick, 2006; Bassler, 2002). Onderzoek naar QS verheldert niet alleen systemen die ten grondslag liggen aan bacteriële cel-cel communicatie maar blijkt ook nut te hebben in de farmaceutische industrie. Zo kan gericht ingespeeld worden op processen die aan de basis van pathogene bacteriepopulaties staan (G. J. Lyon & Muir, 2003).

Sinds de ontdekking van QS in de jaren '60-'70 is er veel onderzoek gedaan naar de regelmechanismen die achter dit proces zitten (Bandara et al., 2012). Daarbij zijn talloze quorum sensing molecules (QSM's) gevonden die betrokken zijn bij deze mechanismen (Bassler, 1999). Deze QSM's vallen in de categorie *autoinducers* (Madigan et al., 2012). Deze categorizering houdt in dat QSM's inspelen op veranderingen in populatiedichtheid, welke indirect effect hebben op genexpressie. Het complete regelsysteem en de verschillende facetten die hierbij betrokken zijn worden in dit onderzoek verder beschreven.

2 Doelstellingen

Zoals hierboven beschreven zijn de talloze QSM's en de mechanismen die zij aansturen van enorm belang voor de medische, ecologische en moleculaire microbiologie. QS vormt een fundamenteel aspect van de leefwijze van bacteriën in communicatie met elkaar en hogere organismen (Shank & Kolter, 2009). De doelstelling van dit onderzoek is om het complexe systeem achter bacteriële communicatie te verhelderen. Daarbij worden de verschillen tussen Gram-positieve en –negatieve bacteriën, de verschillen tussen *interspecies* en *intraspecies* communicatie, remming van QS en communicatie met hogere organismen in acht genomen.

2.1 Onderzoeksvragen

Om de doelstellingen te behalen hanteert dit onderzoek een hoofdvraag, onderverdeeld in een viertal deelvragen. Deze luiden als volgt (Engels volgt Nederlands):

- Hoe maakt quorum sensing communicatie mogelijk tussen bacteriën binnen een microbiële populatie? *(How does quorum sensing facilitate communication between Bacteria in a microbial population?)*
    - Welke verschillen zijn er in quorum sensing tussen Gram-positieve en –negatieve bacteriën? *(What differences do Gram-positive and –negative Bacteria show in quorum sensing utilization?)*
    - Welke verschillen zijn er in quorum sensing binnen dezelfde soort en andere soorten? *(What distinguishes interspecies quorum sensing from intraspecies quorum sensing?)*
    - Hoe kan quorum sensing geremd worden? *(How can quorum sensing be inhibited?)*
        - Welke invloeden heeft quorum sensing door een microbiële populatie op de mens?*(What are the influences of microbial quorum sensing on higher organisms?)*

2.2 Eindproduct

Door antwoord te vinden op de doelstellingen en onderzoeksvragen uit §2.1 moet dit onderzoek een algemene beschouwing over de huidige kennis van QS vormen.

3 Onderzoeksopzet

Het verloop van dit onderzoek zal plaatsvinden in een aantal rondes. Daarbij staat telkens analyse vanuit de literatuur centraal. Gezien de doelstellingen van dit onderzoek (het verkrijgen van een brede, algemene beschouwing over QS) ligt literatuur hier ook ten grondslag aan. In de volgende deelparagrafen worden de fasen van het onderzoek uiteengezet.

3.1 Oriëntatiefase

Voor de opstart van dit onderzoek is reeds literatuur geselecteerd. Deze literatuur is gekozen op basis van algemene inhoud over het onderwerp. Daarbij is voornamelijk voor wetenschappelijke recensies gekozen. Deze recensies geven namelijk een goed samenvattend beeld met basale voorbeelden van quorum sensing. In deze fase wordt tevens georiënteerd op de doelstellingen, richtlijnen en onderwerp van het onderzoek.

3.2 Vraagstelling- en informatiefase

De probleemstelling en deelvragen zijn geformuleerd op basis van persoonlijke interesse naar het onderwerp en het verwachtte eindproduct. Daarnaast is het persoonlijke leerrendement ook meegenomen in de formulering van deze delen. Aan de hand van deze eerste twee fases is het huidige onderzoeksplan vormgegeven en op feedback bijgesteld. Bij goedkeuring van het onderzoeksplan wordt verder gezocht naar relevante en verdiepende literatuur. Het doel van deze literatuur is om een breder begrip te krijgen voor de omvang van het onderzoek.Aan de hand van de bredere begripsvorming kan een grove opzet gemaakt worden van hoe het uiteindelijke verslag vormgegeven wordt.

3.3 Onderzoeks- en schrijffase

Aan de hand van de opzet in de vraagstelling- en informatiefase vangt het onderzoek aan. In dit geval betreft dit een literatuurstudie. De gevonden literatuur wordt aan de hand van een conceptversie uiteengezet in een beschouwing.

3.4 Herschrijffase

De herschrijffase fase van het onderzoek is een wisselwerking tussen feedback en aanpassingen daarop. Deze fase wordt gekenmerkt door uitwisseling van het onderzoek met docent en de critical friend.

3.5 Afwerkingsfase

Gedurende de afwerkingsfase is de inhoud van het onderzoek bekend en geschreven. De inleiding, conclusies, voorwoord en lay-out worden definitief gesteld.

4 Motivatie en leerdoelen

4.1 Persoonlijke motivatie

De motivatie die ik als schrijver van dit onderzoek heb ligt al in een ver verleden. Vanaf de eerste klas, de eerste keer dat ik door een microscoop keek, is mijn interesse gevestigd bij dat wat niet zichtbaar is. Dit heeft zich door de jaren verder ontwikkeld tot een voorliefde voor bacteriën en virussen. Deze voorliefde hoop ik verder te ontwikkelen met een vervolgstudie aan de Radboud Universiteit binnen de master microbiologie. Mijn huidige opleiding tot biologiedocent heeft een erg grote bijdrage geleverd aan de voorkennis die hiervoor nodig is, samen met een pre-master. Het onderwerp dat ik in dit onderzoek wil behandelen is echter nog maar mager aan bod gekomen.

Mijn interesse binnen de microbiologie zou een brug slaan tussen de medische en ecologische microbiologie. QS is een onderzoeksveld waarin dit sterk terugkomt omdat het proces in beide velden onmisbaar is. Bovendien werken QS systemen meer dan eens voor bacteriën met een ecologische rol terwijl ze voor hogere organismen ziekte veroorzakend zijn (Madigan et al., 2012).

4.2 Leerdoelen

Met de master als voor ogen wil ik deze scriptie graag laten werken als extra voorbereiding en een uitbreiding op de pre-master. Daarvoor heb ik de volgende leerdoelen gesteld.

Ik wil:

- mijn kennis verbreden over quorum sensing en bacteriële communicatie en daarbij kennis opdoen van verschillende communicatiemodellen van bacteriën;
- langere tijd zelfstandig aan een (literatuur)onderzoek kunnen werken;
- een grote hoeveelheid informatie verhelderd onder kunnen brengen in een enkele beschouwing en daarbij:
    - kritisch literatuur kunnen uitzoeken en beoordelen;
    - op literatuur en gevonden informatie kunnen reflecteren met behulp van andere bronnen.