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Quorum Sensing: Fact, Fiction, and Everything in Between

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I. PREFACE

Prior to 1994, quorum sensing was commonly referred to as “autoinduction” (Fuqua *et al.*, 1994; Nealson *et al.*, 1970). Autoinduction was originally described for the Gram-negative marine organism *Vibrio fischeri* in early 1970s. The series of experiments conducted by Kempner and Hanson (1968) revealed induction of bioluminescence in freshly inoculated *V. fischeri*. The culture luminesced in response to media that was previously conditioned by the same bacterial strain. Nealson *et al.* (1970) were the first to propose that the autoinduction of luminescence in *V. fischeri* occurs on the transcriptional level and that the process is regulated by extracellularly secreted components (Eberhard, 1972).

The term “quorum sensing” was introduced by Dr. Steven Winans in 1994, who was putting together one of the first review articles on autoinduction in bacteria. Somehow, the word “autoinducer,” a term used to describe the small diffusible molecules involved in the process, just did not seem right to the young professor. Part of that dislike was due to common confusion of the term “autoinduction” with “autoregulation” (Fuqua *et al.*, 1994). Also, the cross-species induction of the bioluminescence had been reported by Greenberg *et al.* (1979) so by 1994, the term “autoinduction” became somewhat inaccurate. Winans was determined to come up with a new name that was innovative, descriptive, and most importantly, catchy. Assisted by his literary-minded brother-in-law, Dr. Winans generated dozens of possible terms including “gridlockins,” “communiolins,” and “quoromones.” None of the terms themselves became popular, but the notion of a quorum was accepted by Winan's colleagues and eventually made it into the title of the chapter. The term “quorum sensing” spread like wildfire, making its way into virtually every paper involving autoinduction written afterward.

II. INTRODUCTION

During our investigation of the quorum-sensing processes in *Listeria monocytogenes*, we stumbled on one assay that simply refused to offer meaningful results. This generally accepted assay was designed for the detection of the “universal” cell-to-cell signaling molecule, AI-2. A meticulous search through the literature led us to a long-forgotten study that was published in the early 1970s and overlooked in a number of recent publications. Locating this study was crucial for our research project (Turovskiy and Chikindas, 2006), but more importantly, this find propelled us to investigate other ambiguous aspects related to current quorum-sensing theories.

Although defined as a cell density-dependent process, quorum sensing is commonly considered to be “more than just a numbers game” (Xavier and Bassler, 2003) and is seen as being synonymous with bacterial communication. Words such as language (Taga *et al.*, 2001) and behavior are frequently used to depict this process in literature. Clever and witty quorum-sensing manuscript titles such as “Mob psychology” and “Bacterial social engagements” shift the process even further into a social realm.

The appeal of all these allegories is understandable, as is the enthusiasm of the researchers who make these comparisons; however, scientific theories cannot survive solely due to their appeal. When asking questions of current theories, it is best to go back to the roots of their inception. Hence, in the following chapter, we review quorum-sensing data that led not only to the formation of commonly accepted theories but also to conflicting theories which, for one reason or another, never became popular, and finally those that are currently falling apart from the lack of supporting evidence. All in all, the purpose of this chapter is the search for a better understanding of the phenomenon known as quorum sensing.

III. THE BASICS OF MICROBIAL LINGUISTICS

A. Autoinducers: The language of prokaryotic communication

Quorum sensing (QS) was originally described in the Gram-negative marine organism, *V. fischeri*. This bacterium can inhabit light organs of some marine animals, such as *Euprymna scolopes*, with the cell density often reaching 10^{10} – 10^{11} CFU/ml (Fuqua *et al.*, 1994). The microorganism bioluminates in these symbiotic associations (Lupp *et al.*, 2003), but not in a planktonic state where its cell density is below 10^2 CFU/ml (Fuqua *et al.*, 1994). The bioluminescence results from the enzymatic reaction driven by luciferase and apparently expression of the *V. fischeri*'s luciferase gene is regulated through QS (Winans and Bassler, 2002). Only two proteins, LuxI and LuxR, are involved in this regulatory pathway. LuxI catalyzes synthesis of 3-oxo-C6-HSL, which diffuses freely from each cell and can accumulate in the environment if the bacterial population density is high enough. This acylated homoserine lactone (AHL) can be detected by a sensor kinase protein, LuxR, which also has a response regulator domain. LuxR binds the promoter of *lux* operon, thus inducing the expression of *V. fischeri*'s luciferase as well as LuxI and LuxR (Fig. 7.1).

LuxR/I-type systems were identified in more than 70 Gram-negative species (Henke and Bassler, 2004a). Each LuxR-type sensor protein is very specific at detecting a particular AHL signaling molecule. The AHL-type pheromones differ in their acyl chain length, degree of saturation, and the substituent located on the third carbon (Fig. 7.2). Although it was originally believed that AHLs diffuse passively through cellular membranes, the long-chain molecules are actively transported via efflux and influx pumps (Smith *et al.*, 2004). The LuxR/I-type QS systems are known to regulate elastase and rhamnolipid (virulence factors) production in *Pseudomonas aeruginosa*, exoenzyme (another virulence factor) and antibiotic production in *Erwinia carotovora*, bioluminescence in *V. fischeri*, and also pigment/antibiotic production in *Serratia liquefaciens* (Henke and Bassler, 2004a).

Interestingly, *Salmonella enterica* and *Escherichia coli* have a LuxR homologue (SdiS), but lack any genes homologous to *luxI*. There is also no evidence that either of these two organisms produce AHLs (Smith *et al.*, 2004). It is thought that *S. enterica* is capable of responding to AHLs produced by other enteric bacteria through expression of *rck* operon and several other genes, presumably for protection against the host's defenses (Henke and Bassler, 2004a). Some systems unrelated but similar to LuxR/I QS were identified in a few Gram-negative organisms, namely AinS from *V. fischeri*, and HdtS from *P. fluorescens* (Bassler, 2002).

Gram-positive species predominantly utilize small posttranslationally modified peptides for cell-to-cell signaling. These peptide autoinducers are exported via ATP-binding cassette (ABC)-type transporters (Fig. 7.3). The genes encoding the precursor peptide, membrane-bound sensor kinase protein, and the transporter machinery are usually located in a single gene cluster (Bassler, 2002). The regulation of gene expression by peptide pheromones is achieved through a two-component signal transduction system. This system will be illustrated in Section III.B using the example of the *nis* operon. The peptide-type autoinducers are known to regulate

competence and sporulation processes in *Bacillus subtilis*, virulence and biofilm formation in *Staphylococcus aureus*, and nisin production in *Lactococcus lactis*.

B. Autoinducers with antimicrobial activity

Kaufmann *et al.* (2005) observed that *N*-(3-oxododecanoyl) homoserine lactone, one of the autoinducers used by *P. aeruginosa*, is also an effective bactericidal agent. The autoinducer itself and the corresponding product derived from a spontaneous Clasen-like condensation (Kaufmann *et al.*, 2005), 3-(1-hydroxydecylidene)-5-(2-hydroxyethyl) pyrrolidine-2,4-dione, were effective against all tested Gram-positive bacteria. On the other hand, *P. aeruginosa* as well as other Gram-negative bacteria were not affected by either of these two compounds. The bactericidal property of *N*-(3-oxododecanoyl) homoserine lactone was detected at concentration ranges which are typical for this compound in *P. aeruginosa* biofilms (Kaufmann *et al.*, 2005). The authors speculate that other known autoinducers may also perform additional biological functions. For instance, the well-known group of autoinducers with potent antimicrobial activity is the class I bacteriocins, the so-called lantibiotics (Kleerebezem, 2004).

Bacteriocins are small antimicrobial molecules of proteinaceous nature, which are produced ribosomally by virtually all bacterial species to control other microorganisms competing for the same ecological niche (Klaenhammer, 1993). Generally, these molecules have a narrow range of activity that is usually restricted to Gram-positive species, closely related to the producer strain.

The observation that some microorganisms produce bacteriocins in a cell density-dependent manner led to the discovery of QS involvement in the synthesis of these peptides. Originally, it was noticed that, when diluted in fresh media, some strains would stop producing bacteriocins. However, the synthesis would resume if filter-sterilized spent medium from the same strain was added (Eijsink *et al.*, 2002).

Class I bacteriocins (lantibiotics) undergo extensive posttranslational modification prior to being secreted. This class of antimicrobial peptides (AMPs) is produced by lactic acid bacteria (LAB). Lantibiotics have unusual amino acids, such as dehydroalanine and dehydrobutyrine as well as thioether bridges called (β -methyl) lanthionines (Kleerebezem, 2004), and are generally hydrophobic. These molecules are known for their broad range of activity, stability to heat, and inherent safety, which makes them excellent candidates as food preservatives (Kleerebezem, 2004).

Biosynthesis of at least some of the lantibiotics is quorum-sensing dependent. Nisin from *L. lactis* and subtilin from *B. subtilis* are structurally very similar. The regulatory machineries for the synthesis of these two molecules have a lot of similarities as well (Kleerebezem, 2004).

All genes necessary for nisin production are arranged in a single gene cluster (Kleerebezem, 2004). The expression of *nisABTCIP* is regulated by the P_{nisA} promoter. This operon includes the structural gene for nisin precursor peptide (*nisA*) and genes necessary for maturation (*nisB*, *nisC*, *nisP*), export of (*nisT*) and immunity to nisin (*nisI*). The regulatory genes (*nisRK*) and the rest of the immunity genes (*nisFEG*) are under the control of P_{nisR} and P_{nisF} , respectively. NisK and NisR constitute a two-component signal transduction system. When a mature nisin molecule binds to NisK, the signal is transduced to a response regulator NisR, which subsequently is able to bind to P_{nisA} and P_{nisF} (Fig. 7.4). The promoter for *nisRK* is not responsive to nisin (Kleerebezem, 2004). Subtilin has almost identical regulatory system with minor differences. Both nisin and subtilin are true pheromones with the antimicrobial properties.

Class IIa bacteriocins are small, heat-stable molecules with a highly conserved YGNGV consensus motif in the N-terminus (Hechard and Sahl, 2002). They are also known as anti-listeria bacteriocins (due to their high activity against foodborne pathogen *L. monocytogenes*) or pediocin-like bacteriocins (named after the first well-studied class IIa bacteriocin). These molecules are synthesized as prepeptides and are processed during translocation across the membrane. The synthesis of many class IIa bacteriocins, such as plantaricin from *Lactobacillus plantarum*, is regulated by peptide pheromones. It was reported that some of these pheromones may have antimicrobial activity themselves (Eijsink *et al.*, 2002), but it is insignificant in comparison with the activity of the actual bacteriocin. The signal transduction is conveyed via a standard two-component system (in some publications, this system is addressed as a three-component system because of the structural gene for pheromone itself) (Eijsink *et al.*, 2002).

The QS regulation of sakacin K was studied in detail (Brurberg *et al.*, 1997). The bacteriocin-like pheromone binds to a sensor kinase protein that activates the appropriate response regulator. The response regulator interacts with promoters upstream of the regulatory and transport operons as well as with the promoter of the structural gene itself. The bacteriocin's structural gene is under stringent control of this system, while the regulatory and transport genes appear to be less responsive to the regulation because the pheromone is exported by the very same ABC transporter (Eijsink *et al.*, 2002).

C. Multiple quorum-sensing systems: Integrating the sensory information

A number of prokaryotes utilize multiple QS systems. The “sensory” information collected through these systems has to be integrated for targeted gene expression. Multiple QS systems can share a single genetic regulon; they can target sets of overlapping genes or regulate seemingly unrelated genetic clusters.

P. aeruginosa has two LuxI/R-type QS systems, LasI/R and RhlI/R. These systems work in parallel, but some of the genes targeted by LasR overlap with the genes targeted by RhlR. One of these overlapping genes is *rhlI*. As a consequence, RhlR/I system is turned on not only by accumulation of RhlR's cognate C4-HSL but also by the activation of LasR/I system (Henke and Bassler, 2004a). The result of interaction between *rhl* and *las* signaling pathways is a sequential gene expression. The genes targeted by RhlR are expressed after the genes targeted by LasR. According to Henke and Bassler (2004a) the sequential gene expression is needed for proper maturation of biofilms or successful infection process.

Either of the two peptide pheromones from *B. subtilis*, ComX and CSF, can stimulate the expression of its target genes while repressing the genes targeted by the second peptide (Henke and Bassler, 2004a). This mode of regulation assures that the two sets of genes will not be expressed at the same time. ComX controls the genes necessary for genetic competence, while CSF targets genetic apparatus necessary for sporulation. According to Henke and Bassler (2004a), such stringent regulation is employed since genetic competence and sporulation are two mutually exclusive physiological states for a bacterium.

The QS regulon of *V. harveyi* is controlled by three separate QS systems. Each of these three systems has a distinct autoinducer synthase and a specific hybrid sensor histidine kinase protein. HAI-1 (harveyi AI-1) and AI-2 were discovered more than a decade ago (Bassler *et al.*, 1994). *V. harveyi*'s AI-1 is *N*-(3-hydroxybutanoyl) homoserine lactone (HSL), which is synthesized by LuxLM. This protein is not related to *V. fischeri*'s LuxI but it performs a similar function (Federle and Bassler, 2003). *N*-(3-hydroxybutanoyl) homoserine lactone binds its specific sensor protein LuxN.

LuxS is required for the biosynthesis of AI-2, which is 3A-methyl-5,6-dihydro-furo(2,3-*b*)(1,3,2)dioxaborole-2,2,6,6A-tetraol. AI-2 is detected by a soluble periplasmic protein LuxP consequently leading to activation of a hybrid two-component sensor kinase response regulator protein, LuxQ.

The third QS system in *V. harveyi* was discovered more recently. The still uncharacterized autoinducer CAI-1 is synthesized by CqsA and detected by its cognate sensor CqsS. The Cqs system was first characterized in *V. cholerae* (Henke and Bassler, 2004a), which is where the name for this system comes from (*cholerae* quorum sensing).

The rest of the signaling cascade in *V. harveyi* is shared by all the systems (Fig. 7.5). LuxN, LuxQ, and CqsS dephosphorylate the shared phosphotransferase LuxU, which indirectly activates the response regulator LuxR.

In *V. harveyi*, QS is known to regulate bioluminescence, type III secretion, and metalloprotease production (Henke and Bassler, 2004a). The expression of all identified genes in *V. harveyi* QS regulon is regulated exclusively via the Lux circuit. The activation of each system seems to have an additive effect on the regulation of gene expression (Mok *et al.*, 2003).

In the foodborne pathogen *V. cholerae*, AI-2 and CAI-1 downregulate the expression of virulence factors like cholera toxin and toxin-coregulated pilus, as well as the expression of 70 other virulence-related genes. Most of these genes are required for the attachment of the pathogen to intestinal epithelial cells (Federle and Bassler, 2003). AI-2 and CAI-1 also downregulate the expression of genes responsible for biofilm formation, while upregulating the expression of Hap protease, the enzyme facilitating the detachment of *V. cholerae* cells from the intestinal walls (Federle and Bassler, 2003). The analysis of the *V. cholerae* QS regulon suggests that at high cell densities, this parasite tends to abandon its host and reenter the environment, possibly due to nutrient depletion.

D. The “Environment Sensing” theory: So much for social engagements of bacteria!

Autoinducer-mediated regulation of gene expression is well established in many species of bacteria, that is *V. harveyi*, *V. fischeri*, *P. aeruginosa* (Bassler, 2002; Bassler *et al.*, 1993; Lupp *et al.*, 2003). The benefits of this process are commonly explained in terms of a concerted response on a population level (Henke and Bassler, 2004a; Winans and Bassler, 2002). In fact, the very term “quorum sensing” implies a population density-dependent process (Fuqua *et al.*, 1994). Although this cooperative explanation is appealing to many, it may not be the most accurate description of the phenomenon.

The extracellular concentration of autoinducers may reflect population density of a microorganism *in vitro*, due to mixing of the producer cells within the constraints of a vessel. However, the situation is likely to be different *in vivo*, where the concentration of a secreted autoinducer may also depend on the diffusion and flow properties of the environment. In many natural habitats of bacteria, these properties are fluctuating and therefore quite unpredictable, that is soils before and after the rain or tooth enamel before and after consumption of a beverage. Consequently, laboratory conditions cannot simply be extrapolated *in vivo*.

Alternatively, autoinducers may be used by individual bacterial cells to sense the flow dynamics of their immediate environment, as opposed to the population density. This “Environment Sensing” theory was proposed by Redfield (2002) but has been overlooked by most researchers thus far. Redfield (2002) theorized that the environment or the diffusion sensing may allow bacteria to prevent wasteful synthesis of extracellularly secreted substances such as bacteriocins, siderophores, exoenzymes, and other effector molecules. These molecules increase nutrient availability for their producers, provided that they remain close to the cell.

For example, bacteria break down extracellular macromolecules through the use of the secreted enzymes such as proteases, cellulases, pectinases, collagenases, and chitinases (Redfield, 2002). The success of this process largely depends on the properties of the surroundings (Fig. 7.6). High flow rates can wash the exoenzymes and the products of their digestive reactions away from the producer cell, rendering the extracellular digestion process ineffective. Relatively small metabolic burden is associated with autoinducer synthesis (Keller and Surette, 2006; Redfield, 2002). The restricted diffusion and mixing in the immediate environment of the single cell, a property that is essential for the effectiveness of the secreted products, can be sensed by the cell through the extracellular accumulation of autoinducers. The fact that QS-regulated genes most commonly encode extracellular products and the proteins necessary for their posttranslational modification and secretion (Kleerebezem, 2004; Redfield, 2002) supports the “Environment Sensing” theory behind the autoinduction (QS) phenomenon.

The direct benefits obtained by the individual cells through the flow dynamics “awareness” may favorably account for at least the initial steps of the evolution of autoinduction pathways. According to Redfield (2002), the synchronized population-wide response (i.e., QS, as it is commonly defined) may simply be a side effect of the Environment Sensing. The variety of the autoinducer-regulated processes is vast; however, the fundamental function of these processes and the driving force behind their evolution may still be elusive. We speculate that in the near future, perception of QS in the scientific community will shift more toward the Environment Sensing theory.

IV. LOST IN TRANSLATION

A. AI-2: The most talked about molecule in the field

In the past two decades, enormous scientific resources have been invested into the search to better understand the elusive molecule known as AI-2. We are aware of at least six independent microarray studies aimed at investigating global transcriptional response of various microorganisms to AI-2-mediated QS (DeLisa *et al.*, 2001; Joyce *et al.*, 2004; Merritt *et al.*, 2005; Ren *et al.*, 2004; Yuan *et al.*, 2005; Zhou *et al.*, 2003). Knockout mutations of *luxS* (AI-2 synthase) have been constructed and characterized in about two dozen bacterial species. In most of these cases, the *luxS* orthologue has been cloned and its functionality has been confirmed. The structures of at least five LuxS orthologues have been determined through resolution of the protein's x-ray diffraction patterns (Das *et al.*, 2001; Hilgers and Ludwig, 2001; Lewis *et al.*, 2001; Rajan *et al.*, 2005; Ruzheinikov *et al.*, 2001).

The molecular structure of AI-2 has been revealed through X-ray crystallography when the molecule is cocrystallized with its two known cognate sensor proteins (LuxP and LsrB). Originally discovered in *V. harveyi*, AI-2 is now known to be produced by more than 70 bacterial species and more than 50 *luxS* (AI-2 synthase) homologues were identified in sequenced bacterial genomes (Sun *et al.*, 2004). Although still a matter of debate, AI-2-mediated QS is thought to regulate the expression of numerous phenotypes in various bacterial species. Among many other traits, AI-2 is thought to regulate motility in *Campylobacter jejuni*, biosynthesis of the antibiotic carbapenem in *Photorhabdus luminescens*, and the expression of virulence factors in *Streptococcus pyogenes* (Xavier and Bassler, 2003). AI-2 has been proposed to be and is widely accepted in the scientific community as the universal cell-to-cell signal in prokaryotic microorganisms.

This famous, or rather “infamous,” molecule and its parental gene (*luxS*) were given a dedicated section in at least 12 review articles. At least three review articles have been solely committed to the discussion of AI-2-mediated QS (De Keersmaecker *et al.*, 2006; Vendeville *et al.*, 2005; Xavier and Bassler, 2003). Finally, being a subject of controversy, *luxS*/AI-2 inevitably has its own devoted section in this chapter as well.

The publications dedicated to arguably the most talked about molecule in the field are purposely reviewed more or less in a chronological order in the following sections. This arrangement gives the reader a chance to follow the events that led to the formation and subsequent decline of the cross-species communication paradigm.

B. The early years of research: AI-2 goes interspecies

It all began with a publication by Greenberg *et al.* (1979), which reported bioluminescence in *V. harveyi* in response to application of culture fluids from several nonluminous bacterial species. A decade later, one of the *V. harveyi* autoinducers (AI-1) had been identified as *N*-3-hydroxybutanoyl homoserine lactone, which belonged to the same class of molecules as the previously identified *N*-3-oxo-hexanoyl-HSL from *V. fischeri*. Surprisingly, the enzymes responsible for synthesis and detection of the AI-1 (LuxM and LuxN, respectively) do not belong to the LuxI/R family of proteins (Bassler *et al.*, 1993). The identification of the system components required for the AI-1-mediated QS in *V. harveyi* led researchers to believe that an additional, still unidentified autoinducer was utilized by the microorganism in the cell-to-cell signaling processes (Bassler *et al.*, 1993).

Even before the key components of the AI-2-mediated QS system were characterized, the autoinducer could be detected using a constructed *V. harveyi* mutant, BB170 ($\Delta luxN$), as a reporter strain. This *Vibrio* strain was used by Bassler *et al.* (1997) to illustrate that cross-species induction of luminescence in *V. harveyi* is triggered by AI-2. As a result, AI-2 received the esteemed title of “interspecies communication signal” (Bassler *et al.*, 1997).

The actual structure of AI-2 was determined when the molecule was serendipitously crystallized in a complex with its cognate receptor protein, LuxP (Chen *et al.*, 2002; Ringe, 2002). As the structure of *V. harveyi*'s LuxP was being resolved through x-ray crystallography, a ligand, identified as AI-2, has been noticed in the cleft between the two LuxP domains. Based on the electron density analysis, Chen *et al.* (2002) proposed the structure of AI-2 as a furanosyl borate diester. The involvement of the Boron atom was also confirmed by NMR spectroscopy and electrospray ionization mass spectrometry (ESI-MS) (Chen *et al.*, 2002).

As the sequence of the AI-2 synthase (LuxS) became available, homologues of the gene coding for this enzyme were identified across various bacterial species (Bassler, 2002; Schauder *et al.*, 2001). Armed with the *luxS* sequence and an easy AI-2 detection assay, researchers were compelled to investigate AI-2-mediated QS in non-*Vibrio* species.

The simplest of these early studies (see references in Table 7.1) included the construction of *luxS* deletion mutants in the studied microorganisms and subsequent confirmation of the LuxS functionality as an AI-2 synthase. The functionality of the *luxS* homologue was typically confirmed by cloning the gene into *E. coli* DH5 (AI-2 strain). The phenotypical changes of *luxS*-null mutations were typically attributed to AI-2/*luxS*-mediated QS. In fact, that is how most of the contemporary evidence for the function of AI-2 as a QS signal has been generated. Table 7.1 lists a few studies that were conducted using the methods just described.

Phenotype rescue was often attempted by reintroduction of *luxS* back into a knockout mutant. As a rule of thumb, reintroduction of *luxS* under the influence of its original promoter would rescue the mutant phenotype (Table 7.2).

Occasionally cell-free culture fluids from known AI-2 producers were used to rescue the mutant phenotypes (Table 7.3). It is important to note that the rescue of the phenotype with the cell-free culture media is an extremely simple procedure. However, on many occasions it has not been reported at all (Tables 7.1 and 7.2). The procedure was successful in the rescue of some

phenotypes (Table 7.3; Sperandio *et al.*, 1999); none-the-less, these results were questioned after the case of EHEC as described in Section IV.C.

C. The pivotal case of EHEC

Without a doubt, the most extensive study of *luxS*-dependent QS in non-*Vibrio* species has been conducted in *E. coli*. Both commensal and pathogenic strains have been investigated with regard to the presence of this QS system (Challan *et al.*, 2006; DeLisa *et al.*, 2001; Sperandio *et al.*, 2001). In fact, *E. coli* DH5 α , which is a *luxS*⁻ strain, is commonly used for cloning and characterization of *luxS* orthologues from various bacterial species (Elvers and Park, 2002; Fong *et al.*, 2001; Winzer *et al.*, 2002b).

At least three research groups have independently conducted studies aimed at investigating the role of AI-2/LuxS in global transcriptional regulation of the *E. coli* genome. The regulation of *lsr* operon in *E. coli* K12 will be described in Section IV.E. This section is mainly concerned with the “case of” enterohemorrhagic *E. coli* (EHEC), as the results of these studies had a significant impact on what was to become the accepted methodology in this area of research.

Enterohemorrhagic *E. coli* (EHEC) O157:H7 is a foodborne pathogen notorious for causing outbreaks of bloody diarrhea and hemolytic-uremic syndrome (Sperandio *et al.*, 1999, 2003). The virulence factors of O157:H7 are localized within the chromosomal pathogenicity island known as locus of enterocyte effacement (LEE). The majority of these genes are arranged in five consecutive polycistronic operons, *LEE1*–*LEE4* and *tir*. *LEE2*, *LEE3* and *tir* are regulated in a cascade manner by Ler, the transcriptional activator encoded in *LEE1* (Sperandio *et al.*, 1999, 2003). The involvement of AI-2-mediated QS in regulation of LEE genes in EHEC has been meticulously investigated in the past years (Sperandio *et al.*, 1999, 2001, 2003). In the study conducted by Sperandio *et al.* (1999), various strains of *E. coli* were transformed with *lacZ* reporter fusions constructed under the control of the predicted *LEE1*–*LEE4* and *tir* promoters so that their QS activation patterns could be investigated in different backgrounds. Media conditioned by strains of *E. coli* that contained functional *luxS* (including the *luxS*-complemented DH5 α) directly activated *LEE1* and *LEE2* promoters while inducing *LEE3* and *tir* through Ler. Conversely, the medium conditioned by DH5 α (*luxS*⁻) did not have any effect on the transcription of LEE genes. These results led the authors to the conclusion that AI-2 was the compound responsible for induction of these virulence genes in EHEC.

In a later study, gene array approach was used by Sperandio *et al.* (2001) to determine the actual extent of transcriptional regulation in EHEC 86–24 that is mediated through the AI-2-dependent QS. Two sets of radioactively labeled cDNA that were derived from EHEC 86–24 and its isogenic *luxS* mutant, respectively, were hybridized to an *E. coli* K-12 gene array, which was subsequently scanned and analyzed for differences in pixel intensity. The data analysis revealed that roughly 10% the genome is differentially transcribed in the *luxS* mutant in comparison to its parental strain, thus indicating that QS may play a role in global regulation of EHEC gene expression (Sperandio *et al.*, 2001).

An independent microarray study conducted by DeLisa *et al.* (2001) and published the very same year as Sperandio *et al.* (2001) seemed to confirm the tremendous impact of AI-2 on global transcriptional regulation in *E. coli*. The study investigated transcriptional response of the *E. coli* W3110 *luxS*-null mutant to extracellularly added AI-2. Medium conditioned by the *E. coli* AI-2 producer strain, W3110, was used as the source of AI-2, and the medium conditioned by the W3110 *luxS*-null mutant was used as a corresponding negative control. RNA isolated from the cells exposed to these conditioned media was used for hybridization with the DNA microarray and for subsequent transcriptome analysis. DeLisa *et al.* (2001) reported that roughly 6% of the *E. coli* genome was transcriptionally regulated through the

AI-2-dependent QS, even though criteria for significance in this study was less stringent than the criteria used by Sperandio *et al.* (2001).

The results of these two microarray studies further elevated the standing of AI-2 to the status of global regulator, at least for *E. coli* strains (Sperandio *et al.*, 2001). Then, a short article written by Winzer *et al.* (2002a) completely changed everything. Winzer *et al.* (2002a) dug through the literature that was published almost four decades ago and stumbled on the fact that LuxS had been described previously as a “RH cleavage enzyme” that is involved in important physiological processes of some bacteria (Duerre and Miller, 1966; Miller and Duerre, 1968). The implication of this discovery is that *luxS*-null mutant phenotypes described for a number of prokaryotic species (Tables 7.1, 7.2, 7.3) may be caused by the altered physiology of the cells and not by QS. As a result, the phenotype rescue procedures using conditioned media preparations of AI-2 should be interpreted with great caution as well, at least in the case of *E. coli* strains. For example, the media conditioned by the *E. coli* O157:H7 and the corresponding *luxS* mutant are likely to differ tremendously in composition (Winzer *et al.*, 2002a) because the two strains have 10% of the genome experiencing altered expression (Sperandio *et al.*, 2001).

Synthetic AI-2 was not available at the time the studies conducted by DeLisa *et al.* (2001) and Sperandio *et al.* (2001) took place, and that is why cell-free culture fluids (conditioned media) had been utilized by the researchers as a supply of AI-2 (Sperandio *et al.* 2003). The later studies conducted with the purified and the synthetic AI-2, however, clearly indicated that AI-2 cannot induce virulence (LEE) genes in EHEC (Sperandio *et al.*, 2003). Apparently, the induction of LEE genes by the culture fluids of *luxS*⁺ *E. coli* strains that had been reported by Sperandio *et al.* (1999) was due to an unrelated and as-yet unidentified compound, termed AI-3. The *luxS*-knockout mutation affects biosynthesis of AI-3 through the shift of cellular metabolic processes which are related to the physiological role of *luxS* (Walters *et al.*, 2006). AI-3, in turn, has been proposed to be the “true” interspecies and possibly even an interkingdom communication signal (Sperandio *et al.*, 2003). The subject of AI-3 will not be critically reviewed in this chapter, as the research in the area is still in its infancy (Clarke *et al.*, 2006; Reading and Sperandio, 2006; Walters *et al.*, 2006). It is worth pointing out, however, that the notion of interkingdom communication is rather difficult to comprehend from the evolutionary perspective (Winzer and Williams, 2003). Most significantly, the study by Sperandio *et al.* (2003) illustrated that the conditioned media approach that has been used to study AI-2-mediated QS in bacteria may result in conclusions that are inaccurate. Additionally, in the case of EHEC, it had clearly been demonstrated that many phenotypes of *luxS*-null mutants result from the alteration in the metabolism of the cells (Walters *et al.*, 2006). The reported metabolic function of *luxS*, and the data collected from the EHEC studies that completely supported this new finding, changed the course of AI-2-related research.

D. The role of *luxS* in cell physiology: Activated methyl cycle

The activated methyl group is required for a number of essential cellular processes in both prokaryotes and eukaryotes. *S*-adenosyl methionine (SAM) is the major methyl donor of the cell (Winzer *et al.*, 2002a). SAM-dependent methylation of DNA, RNA, proteins, and certain metabolites is carried out by dedicated transmethyases with the formation of *S*-adenosylhomocysteine (SAH) that serves as a feedback inhibitor for SAM-dependent methyltransferases (Winzer *et al.*, 2002a). The molecule is highly toxic and it is being recycled by cells via two major pathways.

Some phyla of the Bacteria kingdom and all of the Archaea and Eukarya kingdoms utilize a one-step detoxification pathway (Sun *et al.*, 2004) that involves SahH. This enzyme converts SAH into homocysteine and adenosine (Fig. 7.7). Phylogenetic distribution of this pathway implies its ancient origins (Sun *et al.*, 2004).

The alternative, two-step pathway for detoxification of SAH is employed by some species of γ -, β -, and ϵ -proteobacteria and by all Firmicutes. The first step of the pathway is the conversion of SAH into adenine and *S*-ribosyl homocysteine (SRH), a reaction catalyzed by Pfs. In the second step, SRH is converted into homocysteine and DPD (the precursor for AI-2) by LuxS. The cycle is completed as homocysteine is converted into methionine and subsequently activated back into SAM (Fig. 7.7). DPD formed in the reaction catalyzed by LuxS is rather unstable. The molecule exists in equilibrium with numerous furanones that are formed from spontaneous cyclization (Fig. 7.8). One of this furanones can react with borate to cause the formation of AI-2. Since AI-2 synthesis is tightly linked to important metabolic processes of a cell, theoretically, this molecule could be used to gauge not only the density of a population but also its metabolic state (Xavier and Bassler, 2003). Xavier and Bassler (2003) have argued that AI-2 production has indeed been the driving force behind the evolution of the two-step SAH recycling pathway.

The fact that perfectly viable *luxS*-null mutants have been constructed for numerous bacterial species allows for speculation that the reaction catalyzed by Pfs is sufficient for the recycling of toxic SAH (Xavier and Bassler, 2003). Recent studies indicate that this is not exactly the case. Elevated levels of SAH were detected in the culture fluids of the *luxS*-null mutant constructed from *L. monocytogenes* EGD-e (Challan *et al.*, 2006). Nevertheless, this mutant strain has not been compromised in its ability to grow in the planktonic state.

Theoretically, a *luxS*-null mutant accumulates SRH, while homocysteine is depleted from its cytoplasm. The mutant cells are thought to compensate for the homocysteine deficiency through synthesis of the molecule from oxaloacetate (Kaper and Sperandio, 2005; Reading and Sperandio, 2006). Homocysteine is used for the *de novo* synthesis of methionine, while oxaloacetate along with l-glutamate is used for aspartate synthesis (Reading and Sperandio, 2006; Walters *et al.*, 2006). The global effects of these metabolic shifts are rather difficult to predict as the phenotypes exhibited by *luxS*-null mutants may be caused by a combination of the disturbances in both, QS and metabolic processes of a cell. Generally, discrimination between the possible role of *luxS* in QS and the role of this gene in the central metabolism of a cell proved to be rather challenging (De Keersmaecker *et al.*, 2006).

The effect of the activated methyl cycle (AMC) disruption in some microorganisms can be assessed through evaluation of indirect evidence. For example, despite numerous studies that were aimed at investigation of AI-2-mediated QS in *E. coli*, to date, the genes harbored by the *lsr* operon are the only genes of the microorganism's genome that were shown to be directly regulated by AI-2 (Walters *et al.*, 2006; Xavier and Bassler, 2005). In view of this fact, it is likely that most of the genes influenced by the *luxS*-knockout in *E. coli* are affected by metabolic shifts associated with the disruption of AMC (Sperandio *et al.*, 2001; Walters *et al.*, 2006). Transcriptome analysis conducted by Sperandio *et al.* (2001) has revealed that the *luxS*-null mutant has altered expression of genes involved in biosynthesis, metabolism, and transport of amino acids; genes involved in biosynthesis and metabolism of nucleotides; as well as genes involved in catabolism of carbon compounds (Sperandio *et al.*, 2001; Walters *et al.*, 2006). A few simple but effective experimental approaches have also been employed by the researchers to account for the pleiotropy of *luxS*. For example, the effects of the growth medium supplemented with AMC intermediates or aspartate on a *luxS*-null mutant phenotypes have been investigated for *L. monocytogenes*, *S. enterica* serovar typhimurium, and *E. coli* (Challan *et al.*, 2006; Miller *et al.*, 2004; Walters *et al.*, 2006). Also, attempts were made to complement the phenotype of the EHEC *luxS* mutant by transforming the cells with the plasmid containing functional *sahH*. Presumably, the recombinant cells would metabolically “bypass” the dysfunctional components of the AMC (Walters *et al.*, 2006); the procedure was successful at restoring the mutant's transcription of the *LEE1* promoter to the wild-type levels. Finally, the phenotype rescue attempts were conducted by growing the mutants in coculture with their

parental strains so that both populations are exposed to the same signaling molecules (Challan *et al.*, 2006; Doherty *et al.*, 2006).

Some evidence for the significance of LuxS in the central metabolism of a cell has been obtained through the comparative genomic analysis. The study conducted by Sun *et al.* (2004) involved analysis of 138 fully sequenced genomes. The reciprocal best hit strategy was utilized to search for genes that are orthologous to the key players in the AMC as the function of orthologues is likely to be conserved (Sun *et al.*, 2004). Results of this study indicate that roughly 20% of the investigated organisms lack the set of genes necessary for conversion of SAH into homocysteine (*sahH* or *pfs/luxS*). Most of these organisms, however, are either symbionts or parasites that may rely on their host for metabolic processes such as the “handling” of SAH (Sun *et al.*, 2004).

With only a few exceptions, bacteria that have the ability to convert SAH into homocysteine also have the necessary enzymes to regenerate this intermediate back into SAM, thus completing the cycle. In contrast, the cognate sensor protein for AI-2 (LuxP) and the key components of the signal transduction circuitry triggered by binding of AI-2 to LuxP (LuxQ and LuxU) seem to be restricted to the *Vibrio* species (Sun *et al.*, 2004). In fact, the lack of AI-2 receptors in non-*Vibrios* has been for years the major missing link in the theory regarding AI-2 being the “the universal” signaling molecule.

***E. Isr* operon: The missing link... is still missing**

It is unclear whether DPD or any of its derivatives (Fig. 7.8) passes across bacterial cytoplasmic membrane through passive diffusion, as these molecules are highly polar (Bassler, personal communication). AI-2 can exert its “signaling effect” by binding to membrane-associated receptors which are unrelated to *Vibrio*'s LuxP. AI-2 can also enter the cell via active transport and the signal transduction then can be initiated from within the cell. Prior to 2001, however, LuxP was the only protein reported to bind AI-2. There is a possibility that a knockout mutation of an as-yet-unidentified AI-2 receptor can be lethal for the microorganism, making it difficult to identify the protein via genetic screening (Bassler, personal communication). Nevertheless, the vigorous search for the AI-2-regulated genes led researchers to the discovery of LsrB, a protein which was later shown to directly interact with AI-2.

The study was triggered by the observation that *S. typhimurium*, along with *E. coli* and a few other microorganisms, seem to degrade their extracellularly excreted AI-2 at the onset of the stationary growth phase. The LuxS of *S. typhimurium* is capable of producing AI-2 and the molecule can be detected in this organism's culture fluids all throughout the exponential growth phase. However, AI-2 levels seem to diminish as the population transcends into stationary growth phase (Surette and Bassler, 1998; Taga *et al.*, 2001, 2003).

Taga *et al.* (2001) screened 11000 random insertion mutants and identified eight genes which were differentially transcribed in *S. typhimurium* 14028 and in its isogenic *luxS*-null mutant SS007. One of the identified genes, *metE*, is induced by homocysteine and, consequently, it was ruled out as a true target of AI-2 regulation (Taga *et al.*, 2001, 2003). The remaining seven genes were located in a single operon *lsrACDBFGE* (*lsr* for *luxS*-regulated) that was shown to be activated by extracellularly added synthetic AI-2 (Taga *et al.*, 2001, 2003). The *lsr* operon has also been characterized in *E. coli* (Xavier and Bassler, 2005), and has identical gene arrangement to the *lsr* operon in *S. typhimurium* except for *lsrE*.

The first four genes of the *lsr* operon code for the ABC-type transporter highly homologous to the Rbs transport apparatus. The *rbs* operon that has mostly been studied in *E. coli* (Taga *et al.*, 2001) harbors genes involved in transport and phosphorylation of ribose and an identical set of genes has been identified in *S. typhimurium*. Similar to ribose, AI-2 is phosphorylated

as it enters the cell through its transporter. The transcription of *lsrACDBFGE* is induced by interaction of the phosphorylated AI-2 (AI-2-P) with the repressor protein, LsrR. Genes coding for the transcriptional regulator and cytoplasmic kinase, *lsrR* and *lsrK*, respectively, transcribed divergently of *lsrACDBFGE*.

The ultimate fate of the phosphorylated AI-2 is still unclear, although it has been suggested to be similar to the fate of pentose sugars; AI-2-P is possibly converted to DHAP and then channeled in to a glycolytic pathway (Xavier and Bassler, 2005). It is known that the enzymes coded by *lsrG* and *lsrF* are involved in the reactions that lead to degradation of AI-2-P. However, the products of these enzymatic reactions are yet to be identified (Taga *et al.*, 2003).

Analogous to some operons coding for sugar transporters (i.e., *mal* in *E. coli*), the activation of *lsr* is dependent on cAMP-CAP (Xavier and Bassler, 2005). The accumulation of glycerol-3-phosphate in the cytoplasm of mutant *E. coli* cells led to the repression of *lsr* through the cAMP-CAP-dependent mechanism. The *lsr* operon is also repressed by dihydroxy-acetone phosphate (DHAP). The repression of *lsr* by this molecule is independent of cAMP-CAP, and it may be facilitated through the direct interaction of DHAP with *lsr* operon repressor, LsrR.

The structure of the AI-2 molecule in complex with its periplasmic binding protein, LsrB, has been determined through x-ray crystallography (Miller *et al.*, 2004). Surprisingly, AI-2 bound to LsrB appeared to be (2R,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (*R*-THMF) instead of the familiar furanosyl diester borate. The discrepancy in the “appearance” of AI-2 (s) has been explained in terms of boron availability. Boron is abundant in marine environment where *Vibrios* reside, but it is scarce in the intestines where *E. coli* and *S. typhimurium* typically dwell (Waters and Bassler, 2005). It is worth noticing that the chemical structure of *R*-THMF is very similar to the structure of D-ribose (Fig. 7.9). Additionally, LsrB shares a significant structural homology with the ribose-binding protein (RBP) from *E. coli* and galactose-binding protein from *S. typhimurium* (Miller *et al.*, 2004). There is also evidence that AI-2 in *E. coli* is being reinternalized through an alternative low-affinity transporter, possibly through Rbs (Taga *et al.*, 2003; Vendeville *et al.*, 2005).

One theory is that analogous to acetate, DPD is not a preferable source of carbon; however, it can be utilized during a period of starvation (Winans, 2002). In the absence of glucose, the molecule is reinternalized via the Lsr transporter (Surette and Bassler, 1998; Taga *et al.*, 2001) and presumably metabolized as a last resort. Alternatively, the function of *lsr* is sometimes interpreted in terms of QS. Faithful advocates of the “microbial Esperanto” theory currently view AI-2 as a group of molecules derived from DPD (Rickard *et al.*, 2006). Accordingly, microorganisms such as *E. coli* and *S. typhimurium* sequester AI-2 to interfere with the “social activities” of the competing bacteria. While this interpretation may be reasonable for those who accept the proposed role of AI-2 as the universal autoinducer, it may not be as obvious for those who reject it or just feel it is unsubstantiated.

F. Multilingual bacteria: Another look at the role of interspecies communication in *V. harveyi*

The role of “interspecies communication” in *Vibrio* species is far from being clear. It is commonly believed that AI-1 is used by *V. harveyi* to count “thy self,” while AI-2 is used to count potential competitors (Bassler, 2002; Bassler *et al.*, 1997; Waters and Bassler, 2006). Utilization of a two-autoinducer QS system can create four distinct input states for a cell: no autoinducers, AI-1 only, AI-2 only, and both AI-1 and AI-2 (Bassler *et al.*, 1997; Mok *et al.*, 2003). Each of these states theoretically could trigger a unique mode of gene expression. Bioluminescence data supported this hypothesis and the later discovery of the third signal used by *V. harveyi*, CAI-1, implied a possibility of the eight input states (Henke and Bassler,

2004b). However, the study conducted by Mok *et al.* (2003) has indicated that the common notion regarding the function of AI-2 in *V. harveyi* is likely to be mistaken.

The purpose of the study conducted by Mok *et al.* (2003) was the identification of novel AI-2-controlled genes and characterization of their control by the two autoinducers that were known at that time, AI-1 and AI-2. *V. harveyi* MM30 (*luxS*-null mutant, cannot produce AI-2) was subjected to random insertion mutagenesis using Mini-MulacZ transposon. Ten AI-2-controlled genes were identified through the screen of 6500 of such insertion mutants. LuxLM (AI-1 synthase) was then disabled in each fusion strain by in-frame deletion of *luxLM* on the chromosome. As a result, the 10 engineered strains would not synthesize endogenous AI-1 and AI-2. Transcription level of each fusion in response to the externally added autoinducer(s) was monitored through β -galactosidase assay. Strikingly, all the quorum-sensing-controlled target genes identified by that time in *V. harveyi* appeared to be regulated by both AI-1 and AI-2. This regulation takes place exclusively through the Lux circuit (Fig. 7.5) (Mok *et al.*, 2003). Most importantly, “*V. harveyi* quorum-sensing circuit ... discriminates between conditions in which both autoinducers are present and all other conditions” (Mok *et al.*, 2003). This binary mode of regulation can easily be achieved with a single autoinducer. The multiple QS systems of *V. harveyi* may allow this microorganism to distinguish between the environments and to express genes accordingly. The ability of the *V. harveyi*'s QS circuitry to only distinguish the coincidence of the autoinducers from other possible input states is rather difficult to explain in terms of “intraspecies and interspecies communication”. On the other hand, multiple autoinducers with different diffusive properties (as in the case of AI-1 and AI-2) can provide the cell with the information about its immediate environment (Redfield, 2002). Mok *et al.* (2003) speculates that some natural habitats of *V. harveyi* may be prone to accumulate both autoinducers. The light organ of a host that harbors *V. harveyi* is likely to be an environment that favors accumulation of all the autoinducers.

G. The recent years: Research involving synthetic AI-2

Synthetic AI-2 was not available for the earlier studies described in Sections IV.B and IV.C (Sperandio *et al.*, 2003). Researchers had to rely on culture fluids from AI-2 producers for the supply of this substance. The culture fluids, however, may contain numerous metabolic by-products and/or unidentified autoinducers (Sperandio *et al.*, 2003; Winzer *et al.*, 2002a). Inaccurate results can easily be obtained by this spent media-based approach, as has been illustrated by the case of EHEC. Several procedures (De Keersmaecker *et al.*, 2005; Meijler *et al.*, 2004; Semmelhack *et al.*, 2005) are now available for the *in vitro* synthesis of DPD (precursor of AI-2). This section goes over some of the recent studies that involved synthetic or purified AI-2.

Challan *et al.* (2006) have investigated the role of AI-2-mediated QS in attachment of *L. monocytogenes* EGD-e cells during biofilm formation. The *luxS*-knockout mutant that was constructed from the EGD-e strain produced denser biofilms than its parental strain. Interestingly, extracellularly added AI-2 did not have any effect on the number of the attached cells, while SRH affected the biofilm density for both the wild type and the mutant strain. Additionally, SRH (substrate for LuxS) was shown to accumulate in the culture fluids of the constructed knockout mutant. As a result, Challan *et al.* (2006) came to the conclusion that the mutant phenotype was due to the accumulation of SRH and not due to the disrupted quorum sensing.

Biofilms formed by the *Lactobacillus reuteri* 100–23 were similarly affected by *luxS*-knockout mutation. The mutant cells produced thicker biofilms on plastic surfaces *in vitro* and on epithelial surfaces in an animal model (Tannock *et al.*, 2005). Additionally, the intracellular ATP content of the planktonic mutant cells was 35% lower than the ATP content of the parental strain. *In vivo* competition experiments were used to test the ecological performance of the

luxS mutant. Although inoculated at the same level, the *luxS* mutant strain was outcompeted by other strains of the same species (Tannock *et al.*, 2005). According to the authors, it is unclear whether QS has anything to do with this observation. Additionally, *in vitro* biofilm phenotype of the mutant strain could not be rescued by addition of the concentrated AI-2 preparation.

Competition experiments with rather elegant design were also conducted by Doherty *et al.* (2006) in the study of the *luxS* function in *S. aureus*. The constructed *luxS*-null mutant did not have any obvious defects when grown in a rich growth medium (LB medium). In particular, the traits associated with the virulence of *S. aureus*, such as synthesis of hemolysins and extracellular proteases as well as biofilm formation, were not affected by the *luxS* knockout. Conversely, the growth of the *luxS* mutant was compromised under the sulfur-limited conditions. The mutant strain did not grow well in chemically defined medium that contained 5- μ M cysteine as a sulfur source. Doherty *et al.* (2006) have reasoned that cells with disrupted AMC (*luxS*-null mutants) increasingly rely on methionine uptake from the surroundings and this shift in metabolism, as opposed to QS defect, is responsible for the growth phenotype of the *S. aureus luxS*-null mutant. The hypothesis was tested through a competition experiment, which involved growth of the *luxS* mutant and its parental strain in a coculture under the sulfur-limiting conditions. The relative population sizes of the two strains following 24 hour of growth have indicated that the mutants were significantly outcompeted by the wild-type cells. Doherty *et al.* (2006) have argued that cells grown in a coculture have same pool of autoinducers; the fact that the growth defect of the *luxS*-null mutants was not relieved under these conditions indicates that the phenotype is not due to QS.

Rather unexpected results were obtained through two independent studies of AI-2-mediated QS in *Neisseria meningitidis*. The *luxS* mutant constructed from *N. meningitidis* MC58 did not exhibit abnormalities in growth kinetics (Schauder *et al.*, 2005). Proteomics analysis conducted by Schauder *et al.* (2005) revealed a lack of any major cellular response by the *luxS*-null mutant to synthetic AI-2. Essentially the same conclusion had been reached through a microarray study of the *luxS* mutant constructed from *N. meningitidis* Z2491. Dove *et al.* (2003) reported that the mutant did not exhibit any concerted transcriptional response to the added AI-2 in the form of culture fluid from the wild-type strain. Doherty *et al.* (2006) suggested that AMC may contribute differently to methionine biosynthesis in various species of bacteria. This variation may explain the vast differences between the transcriptional responses of the *luxS* mutants of *E. coli* and *N. meningitidis* to the media conditioned by their parental strains.

Functional *luxS* driven by its native promoter has been shown to be required for the formation of mature biofilms by *S. typhimurium* SL1344 (De Keersmaecker *et al.*, 2005). The biofilm formation defect of the *luxS*-null mutant could not be rescued by synthetic AI-2. Furthermore, the phenotype was not restored through supplementation of the biofilm medium with AMC intermediates: methionine, cysteine, or SAM. Quite the opposite, SAM actually amplified the effects of the original knockout. Surprisingly, *luxS* fused with the strong constitutive *nptII* promoter failed to rescue the biofilm phenotype when the gene was introduced into the mutant. However, when the mutant was complemented with *luxS* driven by its original promoter, the biofilm characteristics were restored to the wild-type level. The results of this study emphasize the significance of the *luxS* regulation and the possible effects of this regulation on the smooth running of AMC in *S. typhimurium*.

We are aware of one intriguing study in which a *luxS* mutant phenotype was successfully rescued by supplementing the culture media with the synthetic AI-2 (Rickard *et al.*, 2006). The focus of the study was the formation of mixed biofilms by the two commensal species of oral microflora, *Streptococcus oralis* 34 and *Actinomyces naeslundii* T14V. The biofilms investigated in this study were grown in the reusable flow cells with the saliva being pumped

through them. Rickard *et al.* (2006) reported that the dual-species biofilm cannot be formed by *A. naeslundii* T14V and the *luxS* mutant constructed from *S. oralis* 34. The biofilm growth was reestablished as the saliva passed through the flow cells was supplemented with the synthetic AI-2 at concentrations 0.08–0.8 nM (Rickard *et al.*, 2006).

The results of the phenotype rescue studies reviewed in this section are summarized in Table 7.4. Most of the rescue attempts involving purified or synthetic AI-2 were unsuccessful. At the same time, some rather atypical results were obtained in the study conducted by Duan *et al.* (2003). Duan *et al.* (2003) demonstrated that synthetic AI-2 can modulate gene expression in *P. aeruginosa*. The study involved transcriptional analysis of the promoters for 21 virulence factors in the pathogen's genome. Six of these genes were induced by supplementing the *P. aeruginosa* culture with synthetic AI-2 (Duan *et al.*, 2003). This study is unique in that *P. aeruginosa* does not have a *luxS* homologue in its genome, and therefore is unable to produce its own AI-2. One hypothesis is that this pathogen responds to AI-2 produced by commensal microflora in the lungs of a cystic fibrosis patient and activates its virulence based on their presence (Duan *et al.*, 2003).

DPD (the precursor to AI-2) is a common by-product of bacterial metabolism (Sun *et al.*, 2004; Winzer *et al.*, 2002a; Xavier and Bassler, 2003), so it is quite feasible that the microorganisms may have evolved pathways that utilize this molecule and its derivatives as a “cue” for the expression of certain genes.

The widespread occurrence of AI-2 is the main reason why this suspected “universal autoinducer” historically received a great deal of attention in the scientific community (Winzer *et al.*, 2002a). Most studies involving purified or the synthetic AI-2, however, indicate that QS mediated by this autoinducer may not be as widespread as was originally thought (Doherty *et al.*, 2006; Vendeville *et al.*, 2005). The actual signal transduction pathways linking the interaction of the AI-2 molecule and its cognate sensor protein(s) to the expression of the target genes have only been established in *V. harveyi*, *V. cholerae*, *S. typhimurium*, and *E. coli* (Xavier and Bassler, 2005). With that in mind, the biological role of AI-2-mediated gene regulation in *S. typhimurium* and *E. coli* is still a matter of debate since the only known functions of the target genes (*lsrACDBFGE*) are the uptake, phosphorylation, and degradation of AI-2 (Doherty *et al.*, 2006; Xavier and Bassler, 2005). The fact that the transcription of *lsr* is repressed by the common metabolic intermediates G3P and DHAP (Xavier and Bassler, 2005) supports the hypothesis that these two microorganisms reinternalize AI-2 simply to metabolize it in the absence of a preferred carbon source. Much of the early evidence for the AI-2-mediated QS in non-*Vibrio* strains has to be reevaluated using the purified compound; as of now, the majority of this evidence may be considered indirect or incomplete due to the complications associated with the involvement of *luxS* in the AMC (Doherty *et al.*, 2006). It is quite possible that outside the *Vibrio* genus, few organisms (if any) utilize AI-2 for genuine QS (Sun *et al.*, 2004).

H. AI-2 in foods: A few words about the currently accepted AI-2 detection assay

“The currently accepted AI-2 detection assay” (Rickard *et al.*, 2006) was first described by Bassler *et al.* (1993). The assay is based on the ability of *V. harveyi* cells at certain cell densities to bioluminate in response to externally added AI-2. Interestingly enough, the intensity of the bioluminescent response of the wild-type *V. harveyi* to AI-1 is much higher than its response intensity to AI-2 (Bassler *et al.*, 1997). In fact, AI-2 could not be detected in the culture fluids of some (now known) AI-2 producers using the wild-type *V. harveyi* as a reporter strain. Experiments were also conducted with $\Delta luxS \Delta luxM$ double mutant strain, MM77, which is unable to synthesize its own AI-1 and AI-2 but is able to respond to externally added autoinducers. These experiments revealed a 100-fold greater intensity of bioluminescence in response to AI-1 as compared to AI-2 (Mok *et al.*, 2003).

The currently accepted and widely used AI-2 detection assay utilizes the *luxN*-null mutant, BB170, as a reporter strain. This strain is not deficient in autoinducer synthesis, but presumably it cannot respond to AI-1 due to the lack of functional AI-1 receptor, LuxN.

The assay is initiated by mixing the reporter cells with the sample in question (10% v/v). At lower cell densities (10^4 – 10^6 CFU/ml) the reporter cells respond to externally added AI-2 because the level of endogenously produced autoinducers has not reached the critical point for the bioluminescent response to take place. The mixture is usually incubated for 3–6 hours, with bioluminescence readings and cell counts taken approximately every 30 min. Noninoculated growth medium is usually used as a negative control, while the culture fluids from wild-type *V. harveyi* strain BB120 are used as a positive control. AI-2-like activity is expressed in relative bioluminescence units (light intensity is normalized to either positive or negative controls) per cell of the reporter strain.

However, there are hidden obstacles to using the AI-2 assay. Back in the early 1970s, it was discovered that even a small quantity of glucose inhibits bioluminescence in *V. harveyi* species (Nealson *et al.*, 1972). It was proposed that this inhibition happens on the transcriptional level of the *Vibrio*'s luciferase biosynthesis, through a catabolite repression mechanism. The “glucose factor” was once again brought to the attention of the scientific community by De Keersmaecker and Vanderleyden (2003). Nonetheless, in many reported studies this fact was not taken into account (Lu *et al.*, 2004, 2005; Zhao *et al.*, 2006). Finally, it has been reported that in concentrations below inhibitory levels for bioluminescence of *V. harveyi* BB170, glucose effectively induces the bioluminescent response and therefore may cause false positive results. Glucose and perhaps other sugars tend to support the growth of *V. harveyi* to a much better extent than glycerol, the carbon source in autoinducer bioassay (AB) medium (Turovskiy and Chikindas, 2006). Therefore, during the incubation time (3–6 hours), the culture of the indicator cells with added glucose may reach the critical cell density for QS to take place through endogenously produced AI-2 (*V. harveyi* BB170 has functional *luxS*, and therefore can produce AI-2).

The three AI-2-related studies (Cloak *et al.*, 2002; Lu *et al.*, 2004; Zhao *et al.*, 2006) are of particular interest, as the data in these food-related applications are likely to be affected by glucose. The study by Cloak *et al.* (2002) was aimed at characterization of the AI-2 production by microorganisms in foods. *Campylobacter coli*, *S. typhimurium*, and *E. coli* O157:H7 were inoculated into chicken broth, milk, and apple juice. These microorganisms were grown at various temperatures and each spent medium was assayed for AI-2 presence. The highest AI-2-like activity was observed after 24 hour of growth for microorganisms grown at either 25°C or 37°C in milk and chicken broth. After 48 hour of growth, AI-2-like activity virtually disappeared. Not much AI-2 was produced during the growth at 4°C and “no notable AI-2 activity was evident in apple juice with any of the organisms examined under any of the conditions tested” (Cloak *et al.*, 2002). Apple juice is known for high glucose content and this is what probably inhibits bioluminescence in *V. harveyi* BB170, and consequently, the detection of AI-2.

Lu *et al.* (2004), on the other hand, have analyzed a number of foods for the presence of AI-2-like activity and also for the ability to “interfere” with the actual AI-2 from *E. coli*. Frozen fish, tomatoes, cantaloupes, tofu, and milk induced high AI-2-like activity in *V. harveyi* BB170. On the other hand, rinses from turkey patties, chicken breast, and homemade cheeses interfered with *V. harveyi*'s response to AI-2. Additionally, the food preservatives sodium acetate at 0.1%, sodium propionate at 0.16%, and sodium benzoate at 0.1% (final concentrations) inhibited *V. harveyi*'s response to AI-2 (Cloak *et al.*, 2002; Lu *et al.*, 2004). The study was conducted in the following manner: The whole fruits and vegetables were swabbed with cotton swabs and then these swabs were soaked in fresh AB medium. Beef and chicken patties were washed and

rinsed in AB medium. The medium was then assayed for AI-2 presence. The liquids from frozen fish and tofu packaging were analyzed directly as well as the whole milk samples. Blank AB medium was used as a negative control, and bioluminescence as low as five times higher than a negative control was considered significant. The AI-2-like activity associated with some foods such as frozen fish, tomatoes, and tofu could easily be caused by the presence of very low concentration of glucose in these samples. The bioluminescence assay was conducted in a 96-well plate reader, and final cell counts of *V. harveyi* BB170 were never determined.

The reported ability of propionates, acetates, and benzoates, at conceivable concentrations and neutral pH, to inhibit AI-2-like activity of AI-2 collected from the known AI-2 producer is intriguing. However, the data obtained by this group suggest that the listed compounds may interact with the intracellular signal transduction of *V. harveyi* BB170 and not with the molecule itself. What it means is that these compounds are unlikely to be effective against non-*Vibrio* species, since the signal transduction cascade for AI-2 seem to be restricted to *Vibrio* species only (Sun *et al.*, 2004; Fig. 7.10).

In the study by Zhao *et al.* (2006), BHI medium was used to conduct the AI-2 detection assay instead of the traditional, “sugar-free” AB medium. The BHI medium is rich in glucose and other carbohydrates which are funneled into glycolysis and possibly can inhibit luminescence in the *V. harveyi* reporter strain.

The growth of the reporter strain may also be hindered by high acidity of the added sample (De Keersmaecker and Vanderleyden, 2003). Also, there is 30–40% standard deviation associated with the method (Turovskiy and Chikindas, 2006). All the above factors must be considered if reliable data are to be collected using this method. The method may be improved through analysis of the bioluminescence kinetics as opposed to the analysis of the single reading (Y.T. and M.C., unpublished data).

V. QUORUM QUENCHING: ALL QUIET ON THE MICROBIAL FRONT

A. Halogenated furanones: The defense system of algae

The process of interference with bacterial quorum sensing is known as quorum quenching and this phenomenon was observed in both prokaryotes and eukaryotes. The Australian macroalga *Delisea pulchra* is known to produce a variety of halogenated furanones. These molecules interfere with AHL-dependent quorum-sensing systems through competitive inhibition at the LuxR-type receptor site. Halogenated furanones also accelerate the turnover time of LuxR family of proteins. This interference is thought to control bacterial biofilm formation on the algae's surface (McLean *et al.*, 2004).

B. AHL lactonases and acylases: Too early to judge

A number of bacteria produce AHL-degrading enzymes known as AHL lactonases and AHL acylases. Lactonases hydrolyze the lactone ring of AI-1-type autoinducers and are found in numerous *Bacillus* species (Zhang, 2003). It was shown that all the tested strains of *B. thuringiensis*, *B. cereus*, and *B. mycoides* were capable of degrading AHLs (Dong *et al.*, 2002). AHL acylases break the amide linkages of AHLs and were originally discovered in a soil isolate of *Variovorax paradoxus* and later in *Ralstonia* species (Zhang, 2003).

Some enzymes of eukaryotic origin were recognized to have similar activities against AHLs, that is paraoxonases and porcine kidney acylase (I) can degrade AHLs if the conditions are appropriate (Dong and Zhang, 2005). Porcine kidney acylase (I) seems to be widely conserved in eukaryotic organisms; however, the AHL-degrading activity exhibited by this enzyme is largely restricted to alkaline conditions, casting a doubt on the enzyme's *in vivo* role as a quorum quencher (Dong and Zhang, 2005; Xu *et al.*, 2003). Also, paraoxonases encoded by PON genes

are known to have important physiological functions. Both PON1 and PON2 have antioxidant properties, while PON1 is also involved in degradation of the toxic organophosphate (Billecke *et al.*, 2000; Dong and Zhang, 2005; Draganov *et al.*, 2000).

The *in vivo* function of bacterial AHL-degrading enzymes has also been debated (Roche *et al.*, 2004). Although these enzymes are commonly thought to function as QS disruptors, their primary physiological role may merely be an aspect of the cell's central metabolism. AHLs are abundant in the environment, making it likely for microorganisms to evolve ways to utilize these compounds as metabolites (Roche *et al.*, 2004). It is known that *Variovorax paradoxus* and *Arthrobacter* species are capable of using AHLs as a sole source of energy (Leadbetter and Greenberg, 2000; Park *et al.*, 2003; Roche *et al.*, 2004).

In mixed bacterial communities, enzymatic activities of different microbial species may complement one another (Roche *et al.*, 2004). Thus, species incapable of fully metabolizing AHLs may initiate degradation of these molecules through a lactonase- or acylase-catalyzed reaction, while the coinhabitants of the same niche may use the products of these reactions to complete the breakdown. This argument is supported by the fact that some soil bacteria can use homoserine lactones (products of acylase-catalyzed reaction) as a sole source of carbon (Yang *et al.*, 2006). Roche *et al.* (2004) have also argued that most enzymes capable of deactivating AHLs were identified through specific screens for this deactivation activity. However, catalytic activity of these enzymes *in vivo* may primarily be directed against substrates other than AHLs. At least one identified AHL acylase, PvdQ from *P. aeruginosa*, is known to be implicated in a biochemical pathway unrelated to quorum sensing. The acylase activity exhibited by PvdQ is thought to be directed toward biosynthesis of pyoverdine, a protein involved in iron acquisition (Lamont and Martin, 2003; Ochsner *et al.*, 2002; Roche *et al.*, 2004).

Furthermore, arrangement of the genes coding for some AHL-degrading enzymes often implies their role in central metabolism of a cell. For instance, *Agrobacterium tumefaciens*' *attM* is harbored by the operon that also contains homologues of succinate semialdehyde dehydrogenase and alcohol dehydrogenase (Roche *et al.*, 2004). Altogether, due to the lack of conclusive evidence it is too early to designate these enzymes as quorum quenchers.

C. Quorum quenching: Practical applications

Regardless of the function that AHL-degrading enzymes may play *in vivo*, a number of promising applications that involve these enzymes have been reported in the literature. Among the most prominent ones are the two *in vivo* studies of virulence attenuation in *E. carotovora* through interference with the pathogen's quorum-sensing pathways. *E. carotovora* is a plant pathogen and the expression of its virulence factors is known to be controlled through AHL-dependent quorum sensing. *B. thuringiensis*, on the other hand, displays strong AHL lactonase activity (Dong *et al.*, 2004). In one of the experiments, potato slices were dipped into *B. thuringiensis*' liquid culture and afterward inoculated with *E. carotovora*. Negative controls were dipped in sterile water and then inoculated with *E. carotovora* as well. *B. thuringiensis* did not inhibit the growth of the pathogen; however, lesions caused by the pathogen were significantly reduced in size (Dong *et al.*, 2004). The same procedure was repeated with a strain of *B. thuringiensis* which could not produce the lactonase (*aiiA*-null mutant) and resulted in the formation lesions at the sites where *E. carotovora* was inoculated, although these lesions were still less severe than the ones in the negative control. Dong *et al.* (2004) hypothesized that *B. thuringiensis* interfered with *E. carotovora*'s quorum sensing and thus made this pathogen more vulnerable toward the defenses of the potato plant (*Solanum tuberosum*). In another set of experiments, a transgenic *S. tuberosum* was constructed through an *A. tumefaciens*-mediated transformation of *aiiA*. Tubers from these genetically manipulated plants were immune to infection by *E. carotovora* (Dong *et al.*, 2001).

The importance of quorum sensing in the proliferation of pathogens and spoilage organisms in processed foods has not yet been confirmed (Smith *et al.*, 2004). It is difficult to make a definitive conclusion on whether the intervention with QS will make foods safer for consumption and/or if it will extend the products' shelf life. However, it is quite conceivable that quorum-sensing inhibitors (QSIs) will be identified in foods. For example, crude garlic extract contains at least three different compounds capable of interfering with the LuxR/I quorum-sensing system (Persson *et al.*, 2005; Rasmussen *et al.*, 2005). The toluene fraction of this extract was able to interfere with the formation of characteristic mushroom-like structures of *P. aeruginosa* PAO1 biofilms. The biofilms grown in the presence of 2% garlic extract were susceptible to treatment with antibiotics and detergents (Rasmussen *et al.*, 2005).

In another study, Wu *et al.* (2004) have demonstrated that a synthetic QSI, similar in structure to V-30 (produced by *D. pulchra*), can be effective in clearing *P. aeruginosa* lung infections in mice. Supposedly, this compound interferes with the proper formation of the *P. aeruginosa* biofilms, ultimately making the infection more susceptible to the animals' immune response. The assumption has been supported by an *in vitro* study (Wu *et al.*, 2004), which demonstrated that biofilms formed by *P. aeruginosa* in the presence of this QSI have increased susceptibility to tobramycin and SDS.

D. The available screening procedures for quorum-sensing inhibitors

Several biological screening systems for identification of QSIs have been described in the literature. The simplest, and probably the most effective, assay for detection of QSIs is based on the abilities of two naturally occurring microorganisms, *P. aureofaciens* 30–84 and *Chromobacterium violaceum* ATCC 12472, to regulate the expression of their pigmented molecules through QS. The screening procedure is conducted on the agar plates by overlaying samples with soft agar containing either of these two indicator organisms (McLean *et al.*, 2004). Purple-colored violacein is produced by *C. violaceum* and orange-colored phenazine is produced by *P. aureofaciens*. QSIs interfere with production of these pigments and can be easily identified through discoloration of the growing indicator strain. This assay is easy to utilize and it does not require the use of external AHLs.

The recombinant QSI selectors generally have *lux*-type promoter fused with a reporter gene. This fusion is inserted into a vector and then cloned into a model organism. In these recombinant systems, the expression of the reporter gene is under the control of AHL-mediated quorum-sensing processes. AHL antagonists inhibit the expression of the reporter gene and thus can be identified. Rasmussen *et al.* (2005) described the construction of three such recombinant screening systems called QSI1, QSI2, and QSI3. QSI1, the most successful of the three systems, is established in *E. coli* harboring the pJAB140 plasmid, which contains a *lux* promoter fused with *phlA*. PhlA is toxic for the cells, and in QSI1, the expression of this protein is dependent on the presence of external AHLs. AHL antagonists allow the indicator cells to grow in the presence of various AHLs. The limitation of this system is that it requires the use of external acyl homoserine lactones.

QSI2 contains a *sacB* marker fused with a *lasB* promoter and presumably responds to QSIs with a more narrow range of action due to specificity associated with *P. aeruginosa*'s LasR/I system (Rasmussen *et al.*, 2005). This system can be used for selection of antagonists specific to *P. aeruginosa* quorum-sensing system. The disadvantage of QSI2 is that it requires external AHLs and gives false positive results in response to glucose (Rasmussen *et al.*, 2005).

The most advanced microtiter plate assay for the selection of QSIs was described by Jafra and van der Wolf (2004). This assay is based on GFP-marked *E. coli*, which fluoresces in response to various external AHLs. QSIs inhibit this fluorescence without affecting the growth of the indicator *E. coli* cells. The advantage of the microtiter plate assay is that it allows for analysis

of multiple samples at once, and the fluorescence of *E. coli* cells can be monitored quantitatively. Similar microtiter plate assays which involve mutant *C. violaceum* or *P. aureofaciens* rely on the researcher himself for making a judgment about change of color in the culture of indicator cells (Jafra and van der Wolf, 2004).

VI. THE UPDATE

Recently, Waters and Bassler (2006) identified and characterized many novel QS-regulated genes in *V. harveyi* that were not reported previously by Mok *et al.* (2003). The previously reported genetic screen recognition of the QS regulon in *V. harveyi* (Mok *et al.* 2003) could have been biased towards the genes modulated by multiple autoinducers, since the procedure was conducted in an AI-2⁻ *Vibrio* strain that was still capable of producing AI-1 and CAI-1.

In contrast, the screen reported by Waters and Bassler (2006) conducted in an AI-1⁻/AI-2⁻ background, thus increasing the chance of identifying the genes that are solely controlled by a specific autoinducer. Although most of these newly-identified promoters were only responsive to the simultaneous presence of all three autoinducers (coincidence behavior), some displayed a graded response with detectable alteration in expression triggered individually by AI-1 or AI-2 (Waters and Bassler, 2006).

Still, all the known QS-controlled genes in *V. harveyi* share common regulatory components, a cascade leading to the phosphorylation/diphosphorylation of LuxR. The variation in binding affinities of the LuxR to the QS-regulated promoters is thought to account for the differences in gene expression response.

Although Waters and Bassler (2006) propose that *V. harveyi* QS signals AI-1, CAI-1 and AI-2 are used for intraspecies, intragenera and interspecies communication, respectively, we suggest that multiple QS systems are used by this microorganism to simply assess its immediate environment (IV.F).

VII. CONCLUDING REMARKS

There is no doubt that the practical applications of quorum-sensing research carry enormous potential (Dong and Zhang, 2005; Zhang and Dong, 2004); however, it is crucial to realize that the direction of scientific exploration has always been influenced by the opinions of the scientific community. There are numerous examples in the history of science of appealing yet inaccurate functional theories that directed research onto a path of ambiguity. Our attempt to utilize the relatively simple AI-2 detection assay initiated numerous questions (Turovskiy and Chikindas, 2006), which led us to uncover conflicting theories in every major aspect of quorum-sensing research.

The very function of quorum sensing is not completely clear. The evolution of this process in bacteria could possibly have been driven by the need to sense the flow dynamics of their immediate environment, as opposed to the need for a concerted response. Since multiple factors could influence the direction of development in evolution, both theories could be correct and therefore have a right for coexistence. Conversely, the theory of AI-2-mediated interspecies communication seems likely to remain weak or even fall apart from insufficient supportive results. Data collected over the past decade indicate that in most species of bacteria, the molecule may simply be a metabolic by-product and nothing more. Finally, the function of the AHL-degradative enzymes is still ambiguous. These enzymes may function as quorum quenchers; however, they may also be involved in the central metabolism of a cell.

We end this investigative, possibly controversial review with Einstein's guiding principle behind science itself, "Never stop asking questions."

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FIGURE 7.1.

The LuxI/R families of proteins are commonly involved in synthesis and detection of the QS signaling molecules in Gram-negative bacteria.

FIGURE 7.2.
AHL-type autoinducers differ in the structure of their acyl carbon chain.

FIGURE 7.3.

Quorum sensing in Gram-positive bacteria is typically mediated by oligopeptides, which are modified from the precursors prior to being effluxed from the cell.

FIGURE 7.4.

Transcription of the nisin gene cluster is regulated through QS. Genes coding for NisR and NisK are constitutively transcribed. Although regulated by P_{nisaR} , *nisaRK* can also be transcribed together with *nisaABTCIP*; polycistronic mRNA containing *nisaABTCIPRK* has been reported (Kleerebezem, 2004). In contrast to P_{nisaR} , P_{nisaA} and P_{nisaF} are induced by extracellular nisin molecules. Nisin binds to the membrane-associated kinase, NisK, inducing the phosphorylation of the response regulator protein, NisR. The activated NisR induces the transcription of the transcriptional units driven by P_{nisaA} and P_{nisaF} (*nisaA*, *nisaABTCIP*, *nisaABTCIPRK*, and *nisaFEG*).

FIGURE 7.5.

Multiple QS systems of *V. harveyi* regulate the expression of their target genes via a common response regulator protein, LuxR. The three signal transduction pathways that link the QS sensor proteins to the target genes converge via a shared phosphotransferase, LuxU.

FIGURE 7.6.

Secreted exoenzymes are commonly employed by bacterial cells to break down macromolecules. In contrast to the simplistic view (left), the success of the extracellular digestion largely depends on diffusion and flow in the vicinity of the cell (right). This figure was reproduced with minor changes from Redfield (2002) with the perm author and the publisher.

FIGURE 7.7.

The two major schemes of the AMC. The white and gray arrows depict alternative biochemical pathways for the recovery of homocysteine from the toxic SAH. This figure was reproduced with minor changes from Sun *et al.* (2004) with the permission of the author and the publisher.

FIGURE 7.8.

A series of compounds formed from the spontaneous cyclization of DPD is currently referred to as “AI-2.” *S*-THMF-borate, the original AI-2, was recognized as a ligand for LuxP in *V. harvei*. As the LsrB ligand, *R*-THMF, was identified, the term “AI-2” had to be broadened to include other derivatives of DPD shown in the figure.

FIGURE 7.9.

The *lsr* operon of *E. coli* and *S. typhimurium* is activated by extracellular AI-2. The genes harbored by *lsr* encode proteins necessary for reinternalization, processing, and possibly degradation of AI-2 (Vendeville *et al.*, 2005).

FIGURE 7.10.
Quorum sensing: fact, fiction, and everything in between.

TABLE 7.1

The simplest studies of the AI-2-mediated QS in non-*Vibrio* species involved characterization of *luxS*-null mutants (based on the review by Vendeville *et al.* (2005))

Species name	Knockout mutant phenotype	References
<i>Streptococcus pyogenes</i>	Elevated SLS hemolytic activity Reduced proteolytic activity Media specific growth defect	Lyon <i>et al.</i> , 2001
<i>Helicobacter pylori</i>	Thick biofilms	Cole <i>et al.</i> , 2004
<i>Serratia</i> 39006	Decreased carbapenem production	Coulthurst <i>et al.</i> , 2004
<i>Campylobacter jejuni</i>	Reduced: Motility Autoagglutination <i>flaA</i> transcription	Jeon <i>et al.</i> , 2003
<i>Porphyromonas gingivalis</i>	Deficiency in: Exoproteases Rgp/Kgp Haemagglutinin activity	Burgess <i>et al.</i> , 2002

TABLE 7.2

Generally the *luxS* mutant phenotype could be successfully rescued through gene complementation (based on the review by Vendeville *et al.* (2005))

Species name	Knockout mutant phenotype	Phenotype rescue by gene complementation	References
<i>Streptococcus gordonii</i>	Downregulated <i>gtfG</i> , <i>fruA</i> , <i>lacD</i> Defect in formation of mixed species biofilms	ND Successful	McNab <i>et al.</i> , 2003
<i>Neisseria meningitides</i>	Attenuated <i>in vivo</i>	Successful	Winzer <i>et al.</i> , 2002b

TABLE 7.3

Some studies reported successful phenotype rescue by the AI-2-containing conditioned media (based on the review by Vendeville *et al.* (2005))

Species name	<i>luxS</i> -null mutant phenotype	Phenotype rescue by		References
		Gene complementation	Conditioned media	
<i>Shigella flexneri</i>	Deficiency in <i>virB</i> expression	ND	Partial	Day and Maurelli, 2001
<i>Clostridium perfringens</i>	Deficiency in: Toxin production	ND	Successful	Ohtani <i>et al.</i> , 2002
<i>Serratia marcescens</i> 274	<i>pfoA</i> mRNA	Partial	Successful	Coulthurst <i>et al.</i> , 2004
	Impaired virulence	ND	ND	
	Deficiency in: Prodigiosin production Hemolytic activity	Successful Successful	Successful ND	

TABLE 7.4

Synthetic AI-2 has been utilized in recent AI-2-related studies

Species name	<i>luxS</i> -null mutant phenotype	Phenotype rescue	
		Gene complementation	Synthetic AI-2
<i>Staphylococcus aureus</i>	Reduced ability to compete under sulfur-limiting conditions	Successful	Not successful
<i>Listeria monocytogenes</i>	Denser biofilms	Successful	Not successful, phenotype is due SRH
<i>Salmonella typhimurium</i>	Cannot form mature biofilms	Successful only with the <i>luxS</i> driven by pCMPG5664	Not successful
<i>Neisseria meningitidis</i>	No major effect (proteomics and microarray studies)	Not applicable	No effect
<i>Lactobacillus reuteri</i>	Thicker biofilms, lower ATP content	Successful	Not successful
<i>Streptococcus oralis</i>	Defect in mixed biofilm formation	Successful	Successful