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New Vocabulary for Bacterial Communication

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Quorum sensing (QS) is widely accepted as a procedure that bacteria use to converse. However, prevailing thinking places acyl homoserine lactones (AHLs) at the forefront of this communication pathway in Gram-negative bacteria. With the advent of high-throughput genomics and the subsequent influx of bacterial genomes, bioinformatics analysis has determined that the genes encoding AHL biosynthesis, originally discovered to be indispensable for QS (LuxI-like proteins and homologues), are often absent in QS-capable bacteria. Instead, the sensing protein (LuxR-like proteins) is present with an apparent inability to produce any outgoing AHL signal. Recently, several signals for these LuxR solos have been identified. Herein, advances in the field of QS are discussed, with a particular focus on recent research in the field of bacterial cell–cell communication.

1. Introduction

Cell-density-dependent bacterial gene expression was first described in *Vibrio fischeri* and *Vibrio harveyi*.[1,2] These early studies described the observation that, once the bacterial cell number reached a certain threshold (also called a quorum), gene expression was switched on, resulting in light production. Because a positive feedback loop was observed for this distinctive phenotype and a substance called an autoinducer, which accumulated in the growth medium and was involved in inducing the bioluminescence phenotype, the authors referred to this process as autoinduction.[1] This phenomenon has now been extensively explored, with quorum sensing (QS) having documented roles in many cellular processes other than light production.[3–5]

Early definitions of QS described circumstances whereby bacteria only produced certain chemicals under conditions at which there was a sufficiently high concentration of bacteria in a local environment.[6] Although this may be true, subsequent explanations evolved to suggest that signalling was more complex; these chemical signals were not required to solely communicate that there was a threshold level of bacteria, but to co-ordinate the production of (several different) beneficial compounds,[7] defining the need for multiple systems in a single bacterium. Redfield argued that the concept of threshold levels of bacteria required for signalling came about due to the inherent bias of cell-rich cultures in the laboratory, as opposed to the natural environments that these bacteria occupied.[8]

The first system described consisted of a regulator protein (LuxR) that detected an acyl homoserine lactone (AHL) produced by a cognate synthase (LuxI). However, affordable, high-throughput genomics, together with efficient assembly and annotation pipelines, has since opened the door for a more thorough investigation of bacterial genomes and their signalling potential. In addition to classical LuxI/LuxR circuits, *Pseudomonas aeruginosa* contains a second QS mechanism, involving LysR-type transcriptional regulators and 4-hydroxy-2-alkylquinolones, the derivatives of which, 4-hydroxy-2-heptylquinoline (HHQ) or 2-heptyl-3,4-dihydroxyquinoline (PQS), are responsible for switching on virulence through MvfR,[9–11] whereas small peptides (such as an autoinducing peptide (AIP) from *Staphylococcus aureus*[9]) have been implicated in Gram-positive QS pathways. Furthermore, the diffusible signal factor (DSF) was found as another type of signal for QS that was used, for example, by the $RpfF_{Bc}$/RpfR system.[10]

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Traditionally, it was thought that proteobacterial LuxR homologues required an AHL signal producing LuxI-like homologue. However, bacteria lacking LuxI have been discovered and the LuxR homologues have been termed LuxR "orphans"[11,12] or "solos"[13] due to their lack of a known signal synthase homologue. Additionally, SdiA from *Escherichia coli*, *Klebsiella* and *Salmonella* all lacked a synthase protein and were shown to detect AHLs from other species.[14,15] More recently, evidence

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Darko Kresovic studied bioinformatics at the Goethe University Frankfurt, Germany. During his PhD (2012–2016) at Frankfurt in the lab of Prof. Dr. Helge Bode, he investigated mainly unusual ketosynthase and LuxR solos. By using bioinformatic and biotechnological tools, the interaction of these two solos in novel bacterial cell–cell communication systems were studied in detail. In 2017, he joined GSK Vaccines Marburg and is currently responsible for transfer, improvement and robustness projects using small-scale models. These models cover bacterial and recombinant protein production processes across the GSK network.

Sophie Brameyer received her M.S. in biology from the Technical University Munich in 2011. She earned her PhD at the Ludwig Maximilians University (LMU) in Munich in 2015 in the lab of Prof. Dr. Ralf Heermann, where she discovered novel signalling molecules used for QS by *Photorhabdus* species. She then pursued her interest in the decision-making of single bacterial cells as a postdoctoral fellow in Dr. Jonathan Chubb's laboratory at University College London. Currently, she is working as a postdoctoral fellow in the group of Prof. Dr. Kirsten Jung at LMU studying bacterial adaptation, with teaching and supervising roles in the bioanalytical core facility for biomolecular interactions.

Helge Bode studied chemistry and biology in Göttingen and obtained his PhD from there in 2000. After postdoctoral stays at the German Research Center for Biotechnology (Germany) and Stanford University (USA), in 2005, he became Assistant Professor at Saarland University (Germany). From 2006 to 2010, he led a DFG-funded Emmy Noether group and from 2008 to 2017 he was the Merck endowed chair for Molecular Biotechnology in Frankfurt, where he is currently a full professor. He has >170 publications on all aspects of natural products, focussing on entomopathogenic bacteria. He has received ERC Starting and Advanced grants for his research.

Ralf Heermann studied biology, with focus on Microbiology, at the University of Osnabrück, Germany, where he also received his PhD in 2001. Following a postdoctoral stay at the Technical University in Darmstadt, Germany, he moved to the Ludwig Maximilians University (LMU) in Munich, where he started his own research group and received the "Habilitation" in 2010. In Munich, he was Head of the Bioanalytics core facility of the LMU Biocenter, which focuses on the identification and quantification of biomolecular interactions. Since 2018, he has been Full Professor for Microbiology at the Johannes Gutenberg University in Mainz, Germany. His research group is interested in bacterial communication, interkingdom signalling and signal transduction.

has also linked a subfamily of LuxR solos in plant-associated bacteria to both agonistic and antagonistic activities by small molecules produced by the plants that they infect.[16,17] Furthermore, in entomopathogenic bacteria of the genus *Photorhabdus*, it is now known that some LuxR solos are also not activated by AHLs. *Photorhabdus* undergo a life cycle involving symbiosis with nematodes followed by a pathogenic stage in insects (see ref. [18] for a review of this life cycle). These bacteria produce various secondary metabolites during this process to kill their insect host, utilise nutrients from the insect carcass, promote nematode development and subsequently colonise the newly spawned nematode progeny.[19–23] Despite never being isolated from the environment without their cognate nematode host, there is little reason to believe that these bacteria are incapable of surviving independently. Indeed, there is recent evidence that the bacteria can also live independently of the nematode symbiont in the rhizosphere, and a different way of communication has been suggested that the bacteria use in their different habitats.[24,25] However, the complexity of their life cycle presumably requires a certain level of robustness with respect to nutrient metabolism and secondary metabolite production. As such, it would not be unreasonable to speculate that they possess the capability for inter-kingdom signalling. Nevertheless, the relatively large numbers of LuxR solos in *Photorhabdus* provide a rare opportunity to study this class of regulators.[26] Recently, two new signals for LuxR solos were discovered in *Photorhabdus*: photopyrones (PPYs) and dialkylresorcinols (DARs), which are important for cell–cell communication.[27,28] Whether these proteins can be still be classified as LuxR solos is disputable, since their cognate signals have now been identified. However, these and other activation mechanisms beyond traditional AHL signalling are discussed herein.

2. Quorum Sensing (QS)

2.1. QS by two-component systems

In contrast to AHL signalling by Proteobacteria, examples of peptides as signalling molecules are widespread among those that are Gram-positive, with reports of peptides having a role in competence in *Bacillus subtilis* (Figure 1 A),[29,30] antibiotic production[31] and transfer of conjugative plasmids in *Enterococcus*.[32] The receptors of these types of QS systems typically are a "two-component"-type HK, which is in contrast to a LuxR, which is a membrane-bound receptor for detecting the signalling molecule from the outside. Signalling is mediated by autophosphorylation of the respective HK upon signal perception and a subsequent phosphotransfer of the phosphoryl group to the cognate cytoplasmic RR. The typical signalling molecules of these cell–cell communication systems are small peptides, such as the ComX of *B. subtilis* or AIP-I from *Salmonella enterica* (Figure 1 A, see ref. [33] for a review). However, fatty acid derived signalling has also been observed through two-component signal transduction in Gram-negative bacteria, as found in *R. solanacearum* and *R. pseudosolanacearum* (Figure 1 A). DSFs are also fatty acid derived QS signals that control biofilm formation and virulence in *Cronobacter turicensis*.[10] In *Burkholderia cenocepacia*, the QS signal receptor RpfR degrades intracellular cyclic diguanylate (*c*-di-GMP) upon sensing *cis*-2-dodecenoic acid, also called *Burkholderia* diffusible signal factor (BDSF).[34] The authors identified 3-OH PAME as a signalling molecule responsible for QS in *R. solanacearum*,[35] whereas 3-OH MAME regulates QS in *R. pseudosolanacearum*.[36] In both organisms, the production of 3-OH PAME and 3-OH MAME is catalysed by an enzyme named PhcB. The HK PhcS is the corresponding sensor and phosphorylates PhcR; the resulting activation of PhcA then leads to upregulation of several virulence factors.[37] Other Gram-positive organisms, such as *Staphylococcus* and *Streptococcus*, use peptides for cell–cell communication, leading to virulence factor production. One classic example is the post-translationally modified autoinducing peptide of *S. aureus* (Figure 1 A) that leads to the production of two divergent transcripts: RNAII and RNAIII.[38,39] Production of RNAIII leads to activation of the *agr* locus, as well as a number of major virulence factors of *S. aureus*, including a range of haemolysins, toxins and PVL, in addition to playing a role in biofilm detachment.[40,41] Another example of peptide signalling leading to virulence is the small hydrophobic peptide (SHP)/Rgg system in *Streptococcus*. A genomic screening performed on short peptides from *Streptococcus thermophilus* led to the identification of several pheromones found to activate specific regulators of the Rgg family.[42,43] Following on from this, the RovS transcriptional regulator in *Streptococcus agalactiae*, an opportunistic pathogen in children, was subsequently found to activate during infection, and thus, promote the bacteria's ability to invade hepatic cells.[44] In *Streptococcus*, a human antimicrobial peptide produced by leukocytes designated LL-37 was recently found to upregulate the production of virulence factors in group A *Streptococcus*[45] by direct binding to CsrS, which is the sensor protein of a two-component system hypothesised to play a role in oropharyngeal colonisation and invasion.[46,47] This discovery raises the question of how often specific interspecies crosstalk occurs and, more generally, if the substrate specificities for bacteria in QS systems are deliberately relaxed to exploit the production of analogous compounds by their respective hosts, symbionts or local competitors in the niche that they occupy.

2.2. LuxI/LuxR QS systems

In *V. fischeri* cultures, the molecule responsible for the observed autoinduction was identified as *N*-(3-oxohexanoyl)-L-homoserine lactone,[48] which was produced by the *luxI* gene product.[49] This molecule is believed to diffuse easily across the bacterial membrane and, at a threshold cell density, activate LuxR (Figure 1 A), which, in turn, binds to a so-called "*lux* box" upstream of the *luxICDABEG* operon, thereby activating transcription in a positive feedback loop, leading to rapid light production.[50] The soil bacterium *R. palustris* uses a LuxI synthase, RpaI, to produce *p*-coumaroyl-L-homoserine lactone by using environmental *p*-coumaric acid—a major aromatic monomer of lignin polymers that comprises over 30% of all plant dry material—rather than fatty acids from cellular pools, which

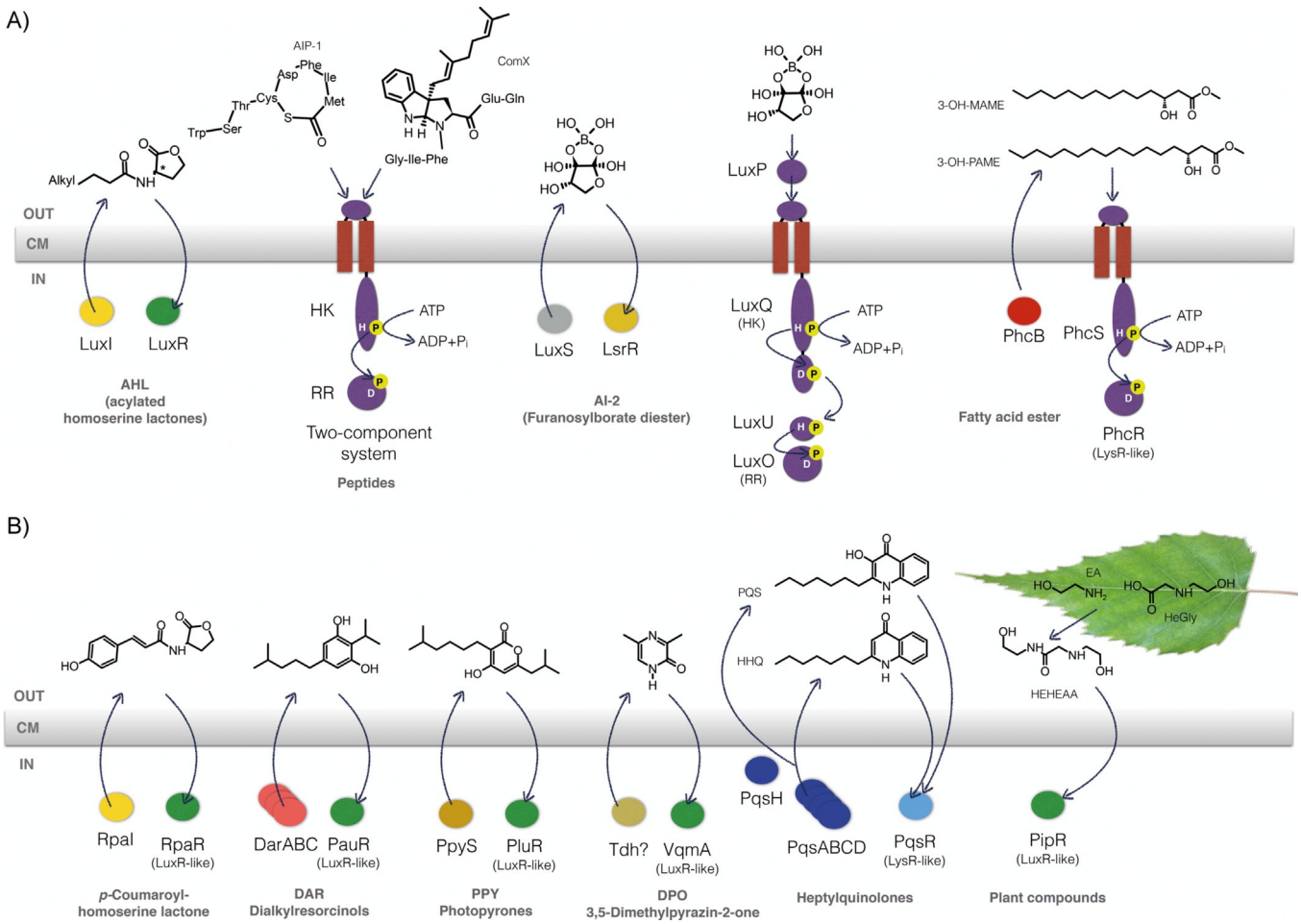

Figure 1. A) Representation of classical QS signalling pathways with AHL; AI-2; peptides from *S. aureus* and *B. subtilis*; and (*R*)-methyl 3-hydroxymyristic acid methyl ester (3-OH MAME)/(*R*)-methyl 3-hydroxypalmitic acid methyl ester (3-OH PAME) from *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum*. B) The nonclassical communication pathways of *Rhodopseudomonas palustris*, *Photorhabdus asymbiotica*, *Photorhabdus luminescens*, *Vibrio cholerae* and *P. aeruginosa*, as well as plant–bacteria inter-kingdom signalling in *Pseudomonas* GM79. The QS molecules are drawn at the site at which they are sensed by the cell (IN or OUT); transport over the cytoplasmic membrane (CM) is not depicted. Similar protein colour represents a similar protein family. LuxI homologues are depicted in yellow, LuxR homologues in green and LysR homologues in light blue. Typical domains of bacterial two-component systems are purple. HK = histidine kinase; RR = response regulator. LuxS/LsrR-derived AI-2 sensing is simplified and depicted without periplasmic capturing, transport and phosphorylation (see text for details). Notably, LsrR senses the phosphorylated form of AI-2, but LuxS produces non-phosphorylated AI-2. HEHEAA: *N*-(2-hydroxyethyl)-2-(2-hydroxyethylamino). HEHEAA is formed by the condensation of ethanolamine (EA) and *N*-(2-hydroxyethyl)glycine (HeGly).

is then sensed by the LuxR homologue, RpaR (Figure 1 B).[51] The concept of producing a QS molecule from a host-derived precursor intelligently combines QS with host or environment sensing.

It was proposed that LuxI and LuxR homologues were acquired, in many cases, by horizontal gene transfer, occasionally with one of the pair being lost, giving rise to the solos that have been found.[52] In particular, the authors noted the example of SdiA from *E. coli* as a prime candidate with a sequence closer to that of a pseudomonad sequence, rather than enterobacterial, with no apparent LuxI-like synthase present. This is supported by the inability of SdiA to be activated by signals produced by *E. coli* and its ability to detect AHLs from other bacteria.[15,53]

2.3. Autoinducer-2-related QS

In addition to AHLs, a new family of LuxI independent autoinducers, named AI-2, was subsequently described in *V. harveyi* and *V. fischeri*.[54,55] Furthermore, AI-2 was found among two other QS systems in the major human pathogen *V. cholerae*.[56] AI-2 is a furanosyl borate diester with a structure that differs significantly from that of AHL (Figure 1 A).[57] The synthase of AI-2, LuxS, is widespread throughout the bacterial kingdom because it is part of the activated methyl cycle, which is a metabolic pathway that utilises *S*-adenosylhomocysteine.[58,59] One by-product of this cycle is dihydroxypentanedione (DPD), which, after spontaneous cyclisation and coupling with borate, leads to AI-2 production. LuxP is a periplasmic protein that binds AI-2, leading to activation of LuxQ, which is a protein with a periplasmic sensor domain and a cytoplasmic RR domain. The phosphorylated LuxQ then phosphorylates LuxU,

which, in turn, activates the final RR, LuxO (Figure 1 A).[60,61] In *Salmonella* spp. and *E. coli*, there is another way AI-2 is sensed. The periplasmic LsrB protein detects AI-2, then AI-2 enters the cell through the Lsr ABC transporter, is phosphorylated by LsrK, and is subsequently presumed to interact with the transcriptional repressor, LsrR.[62] Other bacteria, such as *Helicobacter pylori*, developed a different strategy to sense AI-2 by using classical chemoreceptors to detect the QS molecule.[63] The widespread nature of LuxS led to the hypothesis that AI-2 might be used for universal interspecies communication, not dissimilar to the proposed function of LuxR solos.[64]

3. LuxR Solos

The prevailing theory for the purpose of LuxR solos is to sense exogenous signals, such as that mentioned above. However, we have now identified novel endogenous signals for two previously classified LuxR solos. A typical LuxR protein contains a C-terminal DNA-binding domain and an N-terminal signal-binding domain (Figure 3 A). A six-residue (WYDPWG) conserved motif is present in the signal-binding domain in AHL sensing protein homologues. However, in proteins activated by different signals, this motif is altered.[12,26] The motifs are now known for PPY (TYDQCS) and DAR (TYDQYI) sensing proteins.[27,28] However, most LuxR homologues with the more variable PAS4 signal-binding domain still lack a known activating molecule.[26] Interestingly, LuxR homologues lacking a cognate LuxI homologue appear to cluster in phylogenetic trees, relative to their specific niche. Plant associated bacteria form a clade that are lacking in diversity, while the two newly identified *Photorhabdus* signals also cluster together. Several clades on this tree currently lack any known signals. Although the majority of these branches represent protein homologues from *Photorhabdus* and *Xenorhabdus* species, a number of *Capnocytophaga*, *Sporocytophaga*, *Bacteroides*, *Myroides* and *Pseudoalteromonas* form the majority of the remaining leaves (Figure 3 A). The apparent clustering of protein homologues from different species existing in similar environmental niches may provide clues as to which signals activate those protein homologues with unknown folds. The high variation in the consensus sequence in PAS4 domain proteins suggests that either their activating substrate may not be as conserved as it is with AHLs, PPYs and DARs or that these domains represent a family of sensing proteins yet to be discovered with a range of activating compounds, such as those yet to be seen, which we have previously alluded to.[26] *P. aeruginosa* QS has been extensively studied with two LuxI/LuxR homologue pairs (named LasI/LasR and RhlI/RhlR) identified and one LuxR solo (QscR).[65–68] The LuxR solo in this strain demonstrated some interesting characteristics, with an apparently very promiscuous AHL-binding site. Varying levels of activity were observed when the protein bound several different AHL derivatives. This led to the regulation of genes that would normally be affected by LasR, leading to speculation of activation of two receptors with a single ligand.[69]

In addition to the two already known QS systems in *V. cholerae*, Papenfort et al. recently discovered 3,5-dimethylpyrazin-2-one (DPO) as a third autoinducer in *V. cholerae*.[70] DPO is sensed at a certain threshold by the LuxR solo VqmA through its PAS4 signal domain, which induces the expression of *vqmR* (Figure 1 B) to regulate the pathogenicity of *V. cholerae*.[71]

The first LuxR solo from *Photorhabdus* analysed in detail was PluR. Its target operon was identified from a proteome comparison between the parent strain and the Δ*pluR* mutant. It consists of six genes that might be responsible in the formation of a still unknown small molecule and is adjacent to *pluR*, with activation resulting in expression of the adjacent *pcfABCDEF* operon, which, in turn, causes cell clumping. Consequently, the operon was named *pcf* after "*Photorhabdus* clumping factor" with Pcf-derived cell clumping somehow contributing to the high pathogenicity of *Photorhabdus luminescens* towards insects. Knowledge of *pluR* and its target promoter enabled the construction of a mCherry-based reporter system. By using this system, analysis of the *P. luminescens* supernatant and pure compounds led to the identification of PPYs as the PluR ligands. From the PPY structure, it was postulated that a KS might be involved in the formation of the α-pyrone from two acyl moieties (Figure 2). Subsequent deletion of several genes encoding stand-alone KS finally led to the identification of the photopyrone synthase PpyS. Expression of *ppyS*, together with genes responsible for branched-chain FAB *bkdABC* from *P. luminescens,* resulted in PPY production in *E. coli*, and thus, confirmed the biosynthetic pathway.[27] PpyS belongs to a

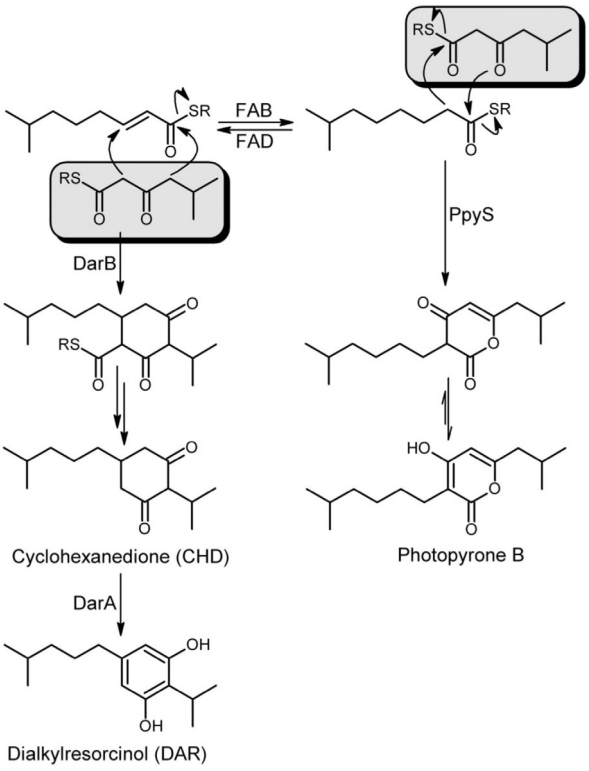

Figure 2. Biosynthetic pathways of PPY and DAR in *Photorhabdus.* Both pathways share one precursor (grey boxes). The different acyl moieties can be derived from fatty acid biosynthesis (FAB) or fatty acid degradation (FAD). PpyS and DarB represent the ketosynthases involved in biosynthesis and DarA is an aromatase that transforms CHD into DAR compounds.

new sub-class of KS, with similarity to FabH, involved in the initiation of FAB and, although its similarity to FabH makes it difficult to prove its specificity based only on protein similarity, very close homologues have also been identified in *Pseudomonas*, *Burkholderia* and *Anabaena* (Figure 3). Interestingly, α-pyrones (named pseudopyronins) are also known from *Pseudomonas* strains, but no correlation between pyrones and the PpyS-like KS has yet been made. However, the presence of such KSs in other bacteria might indicate a more widespread occurrence of these signalling molecules, in addition to their presence in *Photorhabdus* (Figure 3).

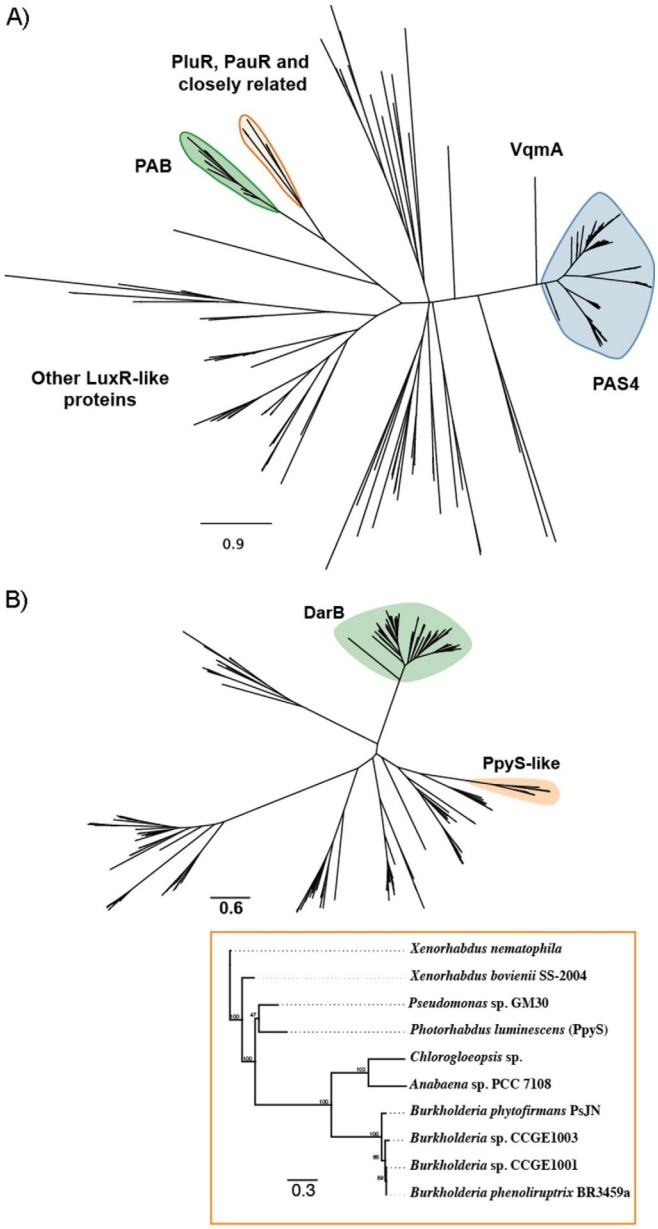

Figure 3. A) LuxR solos identified in bacteria. Highlighted are clades containing PluR/PauR responsible for sensing PPY and DAR, respectively (orange); plant-associated bacteria (green); those with an annotated unidentified fold in the sensor region (red); and protein homologues with a PAS4 domain (blue). B) KS phylogeny by using data from different subtypes of known ketosynthase collected through a BLASTp search against PpyS. Highlighted are clades containing DarB (green) and PpyS-like (orange) proteins; the inset shows the phylogeny of PpyS-like sequences and their strain of origin. Trees were formed by using a maximum-likelihood algorithm with the PHYML plugin of Geneious (v8.0.4). Values on branches represent bootstrapping ($n = 100$) percentages.

Following the discovery of PPY signalling in *P. luminescens*, a second LuxR solo (PauR) was examined in the insect and human pathogen *P. asymbiotica*. PauR is homologous to PluR and regulates expression of the *pcfABCDEF* operon.[28] Analysis of sequences in the signal-binding domain of PauR suggested that a molecule other than AHLs or PPYs was sensed by *P. asymbiotica*.[26] Different to *P. luminescens*, the *P. asymbiotica* genome lacks a *ppyS* homologue. In addition, this novel mechanism is not induced by either AHLs or PPYs.[28] The identified signal, 2,5-dialkylresorcinol, is produced by the action of three proteins in the *dar* locus. Interestingly, both PPY and DAR share the same acyl intermediate (Figure 2, grey boxes). Initially, the locus (consisting of three genes—*darA*, *darB* and *darC*) was investigated for its natural product producing capability.[72] Although it appears to be widespread within the proteobacteria (Figure 4), the genetic layout is not always intact. DarB catalyses the cyclisation of two acyl precursor molecules to form carboxy-CHD intermediates, which DarA subsequently converts into specific DARs, while DarC is an ACP for tethering the biosynthetic intermediates.[72] The majority of proteobacteria identified contained all three genes in the locus (94%); however, these are more often found in separate locations on the genome. Of those containing all three genes required for DAR synthesis, 47% also contained at least one LuxR solo, which suggested that they might respond to a signal similar to that identified in *P. asymbiotica*.[28] *Porphyromonas gingivalis*, on the other hand, is the only species identified that has a DarB homologue in the absence of either DarA or DarC (Figure 4). This raises the possibility that CHDs are responsible for signalling in this strain. CHDs and their derivatives were originally assigned roles in attracting pollinators to the *Chiloglottis* orchid.[73] Therefore, it is also plausible that CHDs are the signalling molecules in *P. gingivalis*.

4. LuxI Solos

Despite research focusing on novel activation mechanisms of LuxR solos, it is important to acknowledge the comparatively less common LuxI solo. What function do LuxI solos play in these bacteria? Perhaps these proteins are "junk DNA" that have yet to be lost through evolution. However, one could speculate that these play a critical role in bacteria that exist in mixed bacterial populations, such as biofilms. One explanation is that these LuxI solos produce molecules to mimic those of close competitors and interfere with cell processes. On the other hand, given the relative promiscuity of LuxR homologues, such as SdiA, and the ability of bacteria containing these SdiA-like proteins to detect signals emanating from other bacteria,[15] it would be prudent not to discount the possibility that these are present for the specific purpose of communicating with other bacteria containing LuxR homologues with a relaxed substrate specificity. It has been observed that the marine sponge symbiont *Ruegeria* sp. harbours, in addition

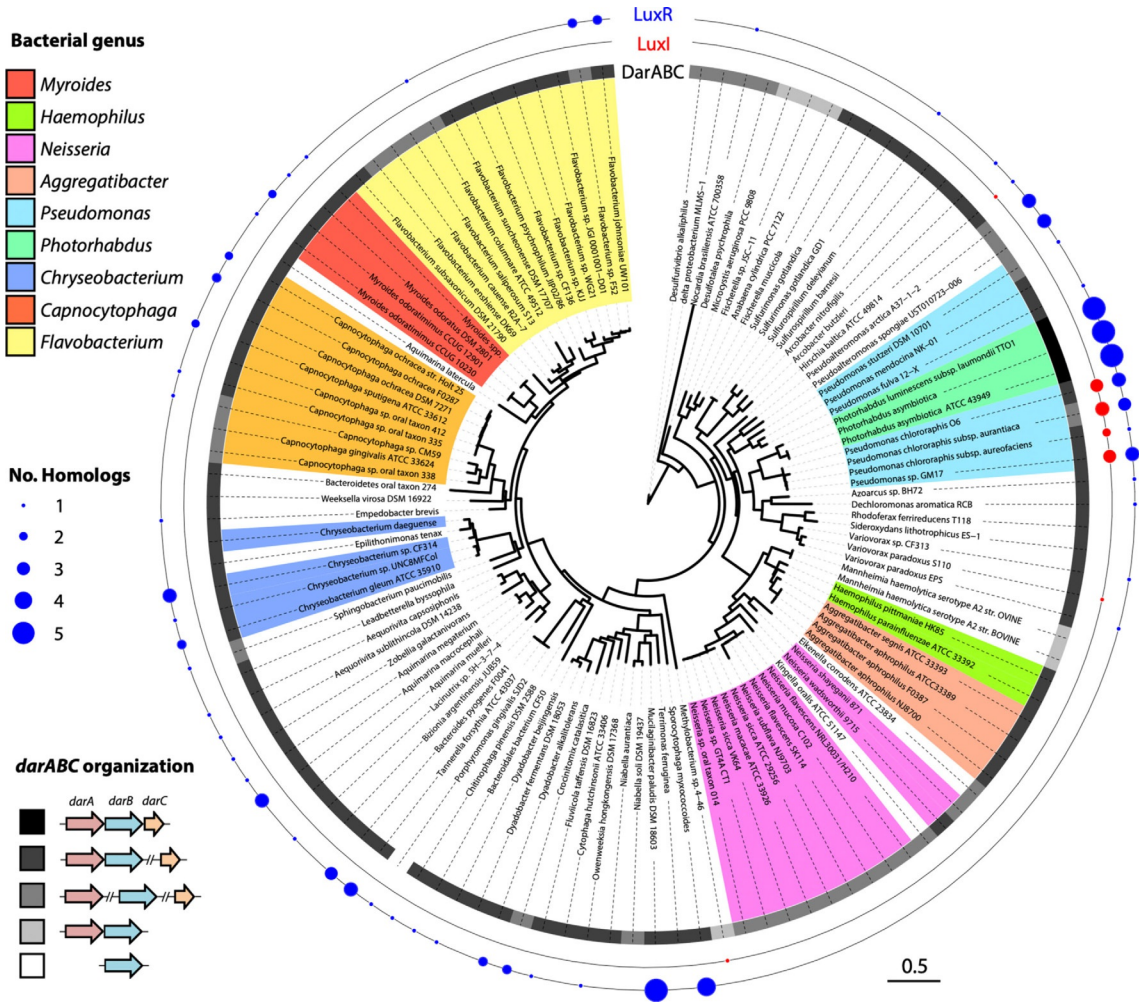

Figure 4. Neighbour-joining consensus tree of DarB homologues. DarB (WP_012794414.1) from *Chitinophaga pinensis* DSM 2588 was used to identify homologue-containing strains by using BLASTp. These strains were subsequently searched by using DarA (WP_012794415.1) and DarC (WP_012794409.1), also from *C. pinensis* DSM 2588, as query sequences. The phylogenetic tree was constructed in Geneious (v8.0.4) by using the PHYML plugin and bootstrapping to support branch formation ($n = 100$). The resulting tree was exported in Newick format and annotated by using the interactive tree of life (v2.1).[102,103] The innermost circle contains species and strain information highlighting dominant bacterial genera, with outer circles (from inside to outside) representing the genetic organisation of DarABC in the genome (greyscale), the number of LuxI homologues (red) and the number of LuxR homologues (blue).

to two pairs of *luxR/I* homologues, the gene *sscI*, which encodes a LuxI solo producing an AHL that contributes to motility.[74] One final explanation for the presence of LuxI solos in some bacteria is paracrine signalling. This phenomenon involves cell–cell communication over much shorter distances than that seen by QS. In *B. subtilis*, surfactin is activated through the action of the ComX peptide, which, in turn, leads to the production of an extracellular matrix (ECM).[29,75–77] However, only a subpopulation of the bacteria produces surfactin, with a different subpopulation that responds to the surfactin and produces the ECM, and those bacteria activated by surfactin then unable to respond to ComX.[78–80] The presence of LuxI solos could therefore be indicative of an undiscovered, analogous paracrine signalling molecule in QS bacteria that, for example, controls the production of virulence factors under specific environmental conditions.

5. Inter-Kingdom Signalling

Bacteria and their hosts communicate with each other by the use of hormonal signals through a process referred to as inter-kingdom signalling. Recent evidence shows that QS is not restricted to bacterial cell–cell communication, but also allows communication between microorganisms and their hosts.[78] This specific cell–cell signalling involves small molecules, such as hormones that are produced by eukaryotes and hormone-like chemicals that are produced by bacteria.

One of the first bacterial inter-kingdom signalling system was described in enterohaemorrhagic *E. coli* (EHEC).[79,80] Thereby, the hormones epinephrine and norepinephrine, as well as an as-yet unidentified autoinducer molecule named AI-3, are used as an inter-kingdom chemical signal between the bacteria and their hosts. These three signals activate expression of a pathogenicity island, the Shiga toxin, and the flagella regulon. The signals are sensed by the two-component system QseB/

QseC that activates the expression of a second two-component system, QseE/QseF, which is thought to be involved in the expression of the virulence genes by sensing epinephrine, phosphate and sulfate.

It has recently been demonstrated that human gut epithelial cells mimic bacterial QS to communicate with the microbiome.[81] Here, the epithelial cells produce an AI-2 mimicking molecule in response to bacteria or tight-junction disruption. This AI-2 mimic is detected by the bacterial AI-2 receptor (LuxP/LsrB) and activates bacterial QS, including in the enteric pathogen *Salmonella typhimurium*. This AI-2 mimicking activity is induced if epithelia are directly or indirectly exposed to bacteria; thus suggesting that a secreted bacterial component(s) stimulates its production. These findings uncover a potential role for the mammalian AI-2 mimic in inter-kingdom signalling. It is assumed that the gut recruits help from the microbiome for assistance upon damage by pathogens through this widespread bacterial communication pathway.

Another inter-kingdom signalling system has recently been identified in plant-associated bacteria. One of these signalling molecules is HEHEAA, which is formed spontaneously from plant-derived EA and is present in EA solutions (Figure 1). Coutinho et al. found that this plant-derived molecule acted as a signal for the LuxR solo, PipR, which was the co-inducer of *pipA* in the plant root endophytic bacterium *Pseudomonas* GM79.[82] Furthermore, the plant pathogenic *Xanthomonas oryzae* pv. *oryzae* is known to possess the LuxR solo, OryR, with an AHL-binding domain.[83] However, instead of binding AHL, it was shown that OryR responded to an as-yet unknown molecule present in rice macerate.[16]

In insect pathogenic *Photorhabdus* spp., the majority of LuxR solos contains an N-terminal PAS4 binding domain, which is homologous to the insect hormone binding PAS3 domain in the fruit fly *Drosophila melanogaster*.[84] Therefore, the PAS4-LuxR solos are predicted to bind hormones and are proposed to be major players in inter-kingdom signalling through the detection of hormone-specific signals from their eukaryotic hosts.[26,85] However, the chemical nature of these insect hormones has yet to be elucidated.

6. Quorum Quenching (QQ)

The idea that bacteria of different species can communicate through QS systems is not a new one. Several studies have examined this in laboratory settings, with evidence that some LuxR homologues are more promiscuous than others (e.g., refs. [89] and [90]). Structural studies often look to investigate the binding of sensor proteins with their cognate signals. This information could then potentially be used to model inhibitors of such systems.

Inter-species crosstalk is not simply limited to similar systems. In *S. aureus*, 3-oxo-C_{12}-L-homoserine lactone was able to suppress the *agr* locus by acting as an allosteric modulator.[86] A different study explored the ability of 34 different chemical isolates from several marine bacteria to inhibit cell–cell communication systems.[87] The authors identified 7 compounds, which inhibited either the *V. fischeri* LuxI-AHL or the *E. coli* LsrA-AI-2 systems, and 10 compounds with a broader effect that inhibited both systems.[87] This study describes a relatively simple assay that could easily be converted into a high-throughput method to screen other similar compounds in a range of bacteria, including those that are human pathogenic. Despite several other examples in the literature of crosstalk between prokaryotes (see refs. [9], [88], [89] among others), these pathways extend beyond the realms of prokaryote–prokaryote communication and also appear to occur in plant-associated bacteria (Figure 3 A), with some LuxR homologues capable of sensing and responding to plant signals,[90–92] as well as plants or fungi responding to bacterial signals.[51,93,94]

Natural QS antagonists, such as (5*Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(5*H*)-furanone, are able to prevent *Artemia franciscana* infection by inhibiting AI-2 in several *Vibrio* species.[95] In addition, proteins with a broader enzymatic degrading effect on QS signals have been discovered, namely, AHL lactonases, AHL acylases and AHL oxidases.[96–98] Brominated furanones are also present in plants with similar effects.[90,99,100] However, the former enzymes appear to be relatively widespread and act to quench QS signals non-specifically. The genomes of *Photorhabdus* spp. contain many AHL lactonases and AHL acylases.[26] An analysis of these enzymes in bacteria, as in that by Brameyer et al.,[26] shows no clear correlation with the presence or absence of LuxR solos. However, the presence of these enzymes in bacteria that appear to rely heavily on QS during their life cycle suggests that these could be another way to regulate QS, perhaps a switch under adverse environmental conditions with the side effect of disrupting other localised signals. In addition to QQ, the concept of bacterial eavesdropping on signals in a niche environment with many bacterial competitors would give bacterial populations a distinct advantage. Indeed, there are reports of *Burkholderia thailandensis* doing exactly this. A dual-species system was used to demonstrate that, during bacterial competition, promiscuous LuxR homologues provided a fitness advantage by activating the production of antibiotic following activation of an AHL sensor by exogenously produced AHL from *Chromobacterium violaceum*.[101]

7. Summary and Outlook

Although the classical AHL-LuxR detection system has been well defined, it appears that this combination could be the exception rather than the rule for QS. With the huge influx of bacterial genome sequences being made readily available for researchers, it seems that we may have barely scratched the surface of bacterial communication pathways, with an ever-increasing number of peptide signals and potential chemical signals being discovered. With respect to LuxI/LuxR systems, the recent identification of signals for two different LuxR solos has been fundamental in reshaping current hypotheses, suggesting that 1) both LuxI and LuxR homologues are required for these systems to function, or 2) those that lack one system are sensing some unidentified exogenous signal. Exploration of this research area in the future will provide a brand-new perspective on the way we previously thought bacteria communi-

cated. Additionally, as we come to more fully understand bacterial communication pathways, the way in which pathogenic species interact with their respective hosts and those chemicals capable of inhibiting virulence pathways, we might discover new ways to prevent infection by medically relevant bacteria.

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Conflict of Interest

The authors declare no conflict of interest.

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