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Quorum Sensing and Quorum Quenching: The Yin and Yang of Bacterial Communication

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1. Switch Off the Light When Going Out

Vibrio fischeri is a remarkable Gram-negative marine bacterium. First, it emits light when it colonizes the fish or squid organs dedicated to this purpose. Second, it turns this light on when entering these organs and switches it off when leaving them. In *V. fischeri*, the production of light, also termed bioluminescence, results from the activity of enzymes that are encoded by the *lux* operon.[1] Bioluminescence results from the hydrolysis of decanal, or other fatty aldehydes, by luciferase/flavin reductase enzymes. This process is costly in terms of energy because *lux* genes also encode fatty aldehyde synthesis.[2] Nonetheless, this bioluminescence has been selected and maintained during the course of evolution. One possible reason for this is that the fish–*V. fischeri* association resembles symbiosis.[3] The animal provides the bacteria with an exclusive, carbon-rich ecological niche. The bacterium emits photons, a function that both attracts prey and deters predators in the light-deprived environment of the deep ocean.[4]

Bioluminescence can be produced in vitro in the appropriate culture medium. Remarkably, light emission by *V. fischeri* depends upon the bacterial cell density, and only occurs at high cell densities.[5] This phenomenon, termed "quorum sensing" (QS),[6] explains why *V. fischeri* only produces light in the fish organ where it lives at very high densities. It does not produce light in the open sea where the bacteria are extremely diluted and their cell density is low (Figure 1).[7]

The involvement of QS signaling molecules that are produced by the bacterial population was demonstrated by adding sterilized, spent culture supernatants of *V. fischeri* to fresh, low cell density cultures of this bacterium.[5] The responsible signaling molecule was identified as *N*-(3-oxo)hexanoyl-L-homoserine lactone (3O,C6-HSL).[8] The 3O,C6-HSL is synthesized from *S*-adenosyl methionine and an acyl chain carrier protein (ACP), and is the product of one of the *lux* genes (*luxI*) that encodes the acyl homoserine lactone (AHL) synthase, LuxI.[9,10] As it is produced at a basal level by each cell, the concentration of the signal builds up in the environment as bacteria proliferate, and this indicates the cell density. Once a threshold signal concentration (i.e., a cell density threshold) is reached, it is sensed and bound by the LuxR sensor peptide, which is encoded by *luxR* and also acts as a transcriptional regulator by activating the expression of the *lux* operon (Figure 1).[11] Interestingly, the first gene of this operon is *luxI*. The activation of *lux* gene transcription therefore results in an increased synthesis of the signal synthase and generates a signal burst in the environment. As a consequence, and aside from being a density-dependent regulatory process, QS also allows the synchronization of the expression of QS-regulated genes in a bacterial population. This feature and the very low active concentrations of QS signals (from pM to μM) have led to the coining of the term "quormone" to describe these signals.[12]

Since the discovery of QS regulation in *V. fischeri*, numerous QS systems have been described in bacteria. They regulate diverse functions,[13] such as production of antifungal or antibiotic compounds, motility patterns, and plasmid conjugal transfer, just to name just a few of the known QS-regulated processes (Table 1). Remarkably, various causative agents of animal and plant diseases, such as *Aeromonas* and *Pseudomonas* or *Pectobacterium* and *Agrobacterium*, respectively, exhibit QS-regulated pathogenicity or pathogenicity-related determinants.[14] For instance, in *Pectobacterium,* the production of the plant macerating enzymes, including pectate lyases, pectinase, and cellulase, is QS-regulated, as is the production of harpin, which is a toxic peptide that the bacteria injects into plant cells through a dedicated secretion system.[14,15] The biological rationale underlying this phenomenon is related to the plant's ability to induce strong defensive reactions upon sensing compounds that arise from the partial degradation of the cell walls.[16] By turning off the synthesis of both the harpin and the maceration enzymes at low cell densities, *Pectobacterium* can proliferate in the plant environment without triggering these defensive reactions until the bacteria reach a concentration that allows a massive production of maceration enzymes, which is lethal to the plant.[17] This suggests that disrupting QS regulation could be a valuable approach to develop new phytoprotective agents as well as new therapeutic strategies aimed at animal pathogens that rely upon QS for the regulation of pathogenicity.

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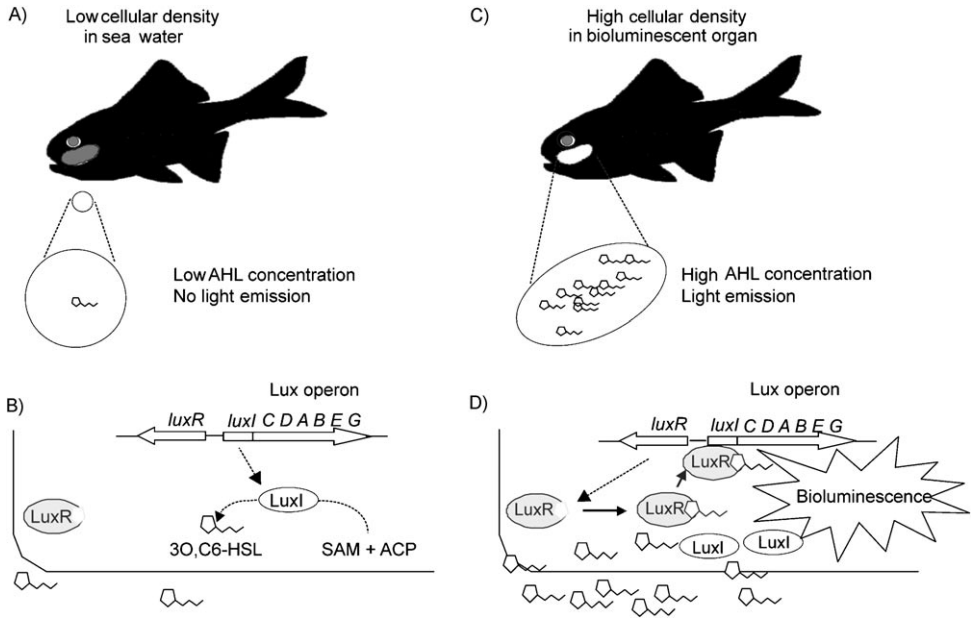

Figure 1. Regulatory scheme of QS in *Vibrio fisheri*. A) and C) schematic view of the fish–*Vibrio* relationship. B) and D) simplified bioluminescence gene regulation in *Vibrio* at B) low cell density and D) high cell density. See text for details.

The first discovered signal, 3O,C6-HSL is a member of the AHL class. This class contains molecules with a conserved homoserine lactone moiety that is N-linked to an acyl chain that ranges from four to 18 carbons in length; the acyl chain may or may not be saturated, and harbors a hydrogen, keto, or hydroxy substitution at the C3 position (Figure 2).[18] These structural differences confer some specificity in signal recognition by the bacterial population that emitted this signal.[19] Irrespective of the bacterial genera investigated, AHLs are most often synthesized by a LuxI-type synthase and are sensed by a LuxR-type receptor;[20] however, AHLs are not the only signal class (Figure 2). In Gram-positive bacteria, QS regulation relies upon signaling peptides and butyrolactone,[21,22] whereas some Gram-negative bacteria, for example *Xanthomonas* and *Ralstonia*, produce and respond to lipid signals such as *cis*-11-methyl-2-dodecenoic acid (also known as the diffusible signal factor or DSF)[23] or 3-hydroxy palmitate methyl ester (3OH-PAME).[24] Complex cyclic molecules, such as 2-heptyl-3-hydroxy-4-quinolone (PQS) and diketopiperazines (DKP) have also been identified as QS signals in pseudomonads.[25,26] Aside from all of these well established QS signal molecules AI-2, a furanosyl borate diester,[27] has been proposed as a "universal" QS signal.[28] Its biological function is still the subject of debate,[29] and it can also be described as a byproduct of a metabolic signaling system.[30] The AHL class is the most common signal class in Gram-negative bacteria, therefore, the discussion below is focused on AHL-dependent QS regulatory process and interference.

2. Quorum Quenching Prevents Bacteria from Communicating

The term "quorum quenching" (QQ) was coined to describe all processes that interfere with QS.[31] QQ strategies do not aim to kill bacteria or limit their growth. Rather, they affect the expression of a specific function. This is an important feature because these strategies exert a more limited selective pressure for microbial survival than biocide treatments. This is a valuable trait for the development of sustainable biocontrol or therapeutic procedures in the present context of rising antibiotic resistance. Three steps of the AHL-based QS regulation mechanism could be targets for QQ procedures: 1) the production of signal molecules, 2) the signal molecule itself, and 3) sensing of the signal molecule by the cognate regulatory protein. The mechanisms that are involved could be either of abiotic or biotic origins. A few examples of the many natural instances of QQ are given below.

Bacteria dazzled by inhibition of AHL signal sensing

The first example of natural inhibition of AHL signal sensing involves AHLs themselves. *N*-decanoyl-HSL (C10-HSL) and *N*-(3-oxo)tetradecanoyl-HSL (3O,14C-HSL) were reported to inhibit the production of the antibiotic pigment violacein by *Chromobacterium violaceum*, a QS-dependent function controlled by *N*-hexanoyl-HSL (C6-HSL).[32] Similarly, the acyl length and the substitution of the acyl chain can perturb QS-regulated func-

Table 1. List of organisms that harbor a QS-regulated function.

Bacterial strain	LuxI/LuxR homologues	AHL produced	Regulated function(s)	Refs.
Aeromonas hydrophila	AhyI/AhyR	C4-HSL	Protease, biofilm	[103]
Agrobacterium tumefaciens	TraI/traR	3O,C8-HSL	Conjugal transfer of pTi	[104]
Burkholderia cepacia	CepI/CepR	C8-HSL	Siderophore, protease	[105]
Pectobacterium carotovorum	ExpI/ExpR CarI/CarR	3O,C6-HSL	Exoenzymes antibiotic	[106,107]
Pectobacterium chrysanthemi	ExpI/ExpR	3O,C6-HSL	Pectinases	[108]
Pseudomonas aeruginosa	LasI/LasR RhlI/RhlR	C4- and 3O,C12-HSL	Exoenzymes, biofilm	[109]
Pseudomonas chlororaphis	PhzI/PhzR	C6-HSL	Phenazine	[110]

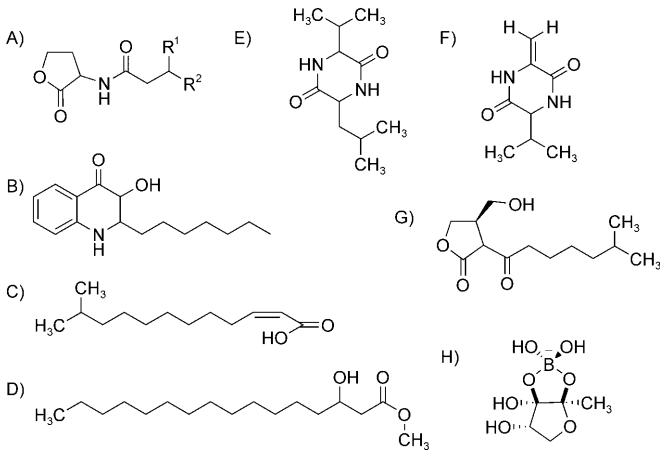

Figure 2. Chemical structure of representative QS signal molecules. A) *N*-acyl homoserine lactone (AHL); B) 2-heptyl-3-hydroxy-4-quinolone (*Pseudomonas* QS signal or PQS); C) *cis*-11-methyl-2-dodecenoic acid from *Xanthomonas* (diffusible signal factor or DSF); D) 3-hydroxy-palmitate methyl ester, (3-OH-PAME) from *Ralstonia*; E) and F) diketopiperazines cyclo-(L-Leu-L-Val) and cyclo(Δ-Ala-L-Val), respectively, from *Pseudomonas*; G) 2-isocapryloyl-3*R*-hydroxymethyl-γ-butyrolactone from *Streptomyces*; H) (2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate (*S*-THMF-borate, AI-2) from *Vibrio harveyi*. Possible variations of the lateral chain of the AHL: R1: H, OH or O; R2: can vary in acyl chain length or be a coumaroyl group.[117]

tions such as *V. fischeri* luminescence[10] or conjugal transfer in *Agrobacterium tumefaciens*.[33]

Other natural compounds are capable of interfering with QS-regulated functions in vivo (Figure 3). Their mode of action

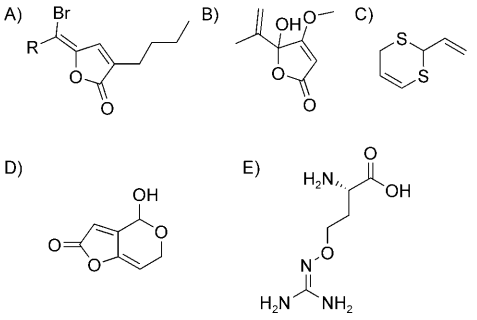

Figure 3. Quorum-sensing inhibitors from natural sources. A) Halogenated furanone from *Delisea pulchra*; B) Penicillic acid from *Penicillium*; C) Cyclic sulfur-containing compound that was isolated from garlic; D) Patulin from *Penicillium* and E) L-canavanine from *Medicago truncatula*.

and chemical structure frequently remain unknown. These compounds often compete with AHL for binding to the LuxR-like receptor. This prevents QS regulation from occurring. In addition, because AHLs are involved in the conformation and stabilization of the receptor, competition can lead to an increased degradation of this peptide. One of the main classes of QS inhibitors is composed of the furanones (Figure 3 E, F). Some of these molecules, which are produced by the red algae *Delisea pulchra*, were shown to inhibit the formation of bacterial biofilms.[34] Some bacterial cyclopeptides (i.e., cyclo-(Ala-Val)) also perturb QS-regulated functions.[26] Penicillic acid (Figure 3 B) and patulin (Figure 3 G) are produced by fungi and were also reported as QS inhibitors.[35] Last, but not least, numerous QS inhibitors were also discovered in the extracts of several plants, such as *Allium sativum*, *Chlamydomonas reinhardtii*, *Fragaria vesca*, *Glycina max*, *Lycopersicum esculentum*, *Medicago truncatula*, *Oryza sativa*, *Pisum stivum,* and *Vanilla*.[35–40] One of these QS inhibitors was identified as L-canavanine (Figure 3 C) in *Medicago sativa*, and appears to be produced in large quantities by alfalfa and other legumes.[41]

Silencing bacteria through limitation of signal accumulation

N-Acyl homoserine lactones are very sensitive to elevated temperatures. Yates et al.[42] reported that *N*-butanoyl-HSL (C4-HSL) and C6-HSL were three and 1.5-times more rapidly degraded at 37 °C than at 22 °C, respectively. As they contain lactones, these molecules are also sensitive to alkaline pH. Schaefer et al.[43] estimated that the half-life (in days) of *N*-(3-oxo)hexanoyl-HSL (3O,C6-HSL) was $1/(1\times10^{7}\times[OH^{-}])^{23}$. More recently, Byers et al.[44] demonstrated that the half-life of this molecule was 30 min at pH 8.5, while it was seven hours at pH 7.8. The mechanism involved in this degradation is lactonolysis, which leads to the generation of acyl-homoserine (Figure 4.1). This reaction can be reversed by acidification of the medium, which allows the restoration of the QS signal molecule. In addition, AHL can undergo a spontaneous Claisen-like alkylation of the β-ketoamide moiety, which leads to the formation of tetramic acids. Tetramic acid derivatives of AHL do not function as QS signal molecules, but might act as antibiotics against Gram-positive bacteria.[45]

Aside from abiotic factors, AHL stability is also affected by biotic activities. The enzymatic degradation of the AHL molecules appears to occur in a very broad range of organisms.

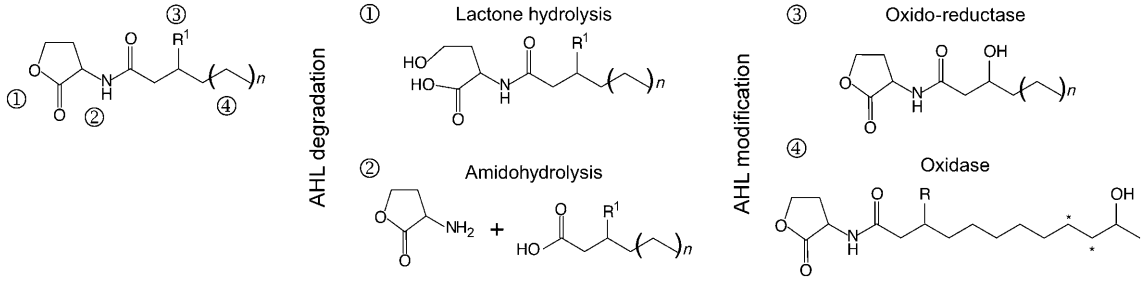

Figure 4. AHL-degradation pathways and degradation products. Degradation pathways (1: lactone hydrolysis; 2: amidohydrolysis) or modification pathways (3: oxido-reductase and 4: oxidase).

Table 2. List of bacterial strains and organisms that are able to degrade or to modify AHL signal molecules.

Taxonomy	Group	AHLase	Gene	Spectrum	Refs.
Prokaryotes					
Acidobacteria	acidobacteria	lactonase	*qlcA*	C6, C7 and C8-HSL	[60]
Acinetobacter sp strain C1010	γ-proteobacteria	n.i.*	unknown	C6- and C8-HSL	[111]
Agrobacterium tumefaciens	α-proteobacteria	lactonase	*attM*, *aiiB*	broad	[58,74]
Agrobacterium tumefaciens XJ01	α-proteobacteria	n.i.	unknown	broad	[54]
Agrobacterium radiobacter K84	α-proteobacteria	lactonase	*aiiS*	broad	Uroz et al. unpublished
Arthrobacter sp. *IBN110*	firmicute	lactonase	*ahlD*	broad	[59]
Anabaena sp. *PCC 7120*	cyanobacteria	acylase	*aiiC*	broad	[49]
Bacillus	firmicute	lactonase	*aiiA*	broad	[57]
Bacillus megaterium CYP102 A1	firmicute	oxidase	P450BM-3 (monooxygenase)	long chains	[72]
Bosea	α-proteobacteria	n.i.	unknown	C6- OC6- and C8-HSL	[55]
Comamonas	β-proteobacteria	acylase	unknown	broad	[47]
Delftia acidovorans	β-proteobacteria	n.i.	unknown	C6 to C8-HSL	[112]
Klebsiella pneumoniae KCTC2241	γ-proteobacteria	lactonase	*ahlK*	C6 and OC6-HSL	[59]
Ochrobactrum sp.	α-proteobacteria	n.i.	unknown	C6- to C8-HSL	[112]
Pseudomonas sp. *PAI-A*	γ-proteobacteria	acylase	unknown	long chains	[114]
Pseudomonas aeruginosa	γ-proteobacteria	acylase	*quiP*	long chains	[115]
			pvdQ (PA2385)	C7 to C12-HSL (C4 to C6 not degraded)	[77,114]
Rhodococcus	actinobacteria	lactonase	*qsdA*	broad	[62]
		oxidoreductase	unknown	long 3O, AHL	[70]
		acylase	unknown	broad	[70]
Ralstonia sp. *XJ12B*	β-proteobacteria	acylase	*aiiD*		[116]
Shewanella sp. *strain MIB015*	γ-proteobacteria	acylase	*aac*	broad with preference for long chain	[56]
Sphingomonas	α-proteobacteria	n.i.	unknown	C6- OC6- and C8-HSL	[55]
Sphingopyxis	α-proteobacteria	n.i.	unknown	C6- OC6- and C8-HSL	[55]
Streptomyces sp. *strain M664*	actinobacteria	acylase (excreted)	*ahlM*	long chains	[70]
Variovorax	β-proteobacteria	acylase	unknown	broad	[47,69]
Eukaryotes					
mammalian cells		lactonase	*pon1,2,3*	broad but prefer long chains	[51]
Lotus corniculatus		n.i.	n.i.	C6-HSL	[52]
Hordeum vulgare (Barley)		n.i.	n.i.	C6-HSL	[53]
Pachyrhizus erosus (yam bean)		n.i.	n.i.	C6-HSL	[53]
fungi		lactonase	n.i.	C6-HSL	[53]
pork		acylase	n.i.	broad	[71]

n.i.: Not identified.

Degradation of AHLs was first demonstrated in bacteria, and has been reported in members of nearly 20 genera (Table 2). This ability was also reported for complex bacterial consortia;[46] this suggests a possible functional complementation between the bacterial isolates. When investigated, the range of AHL molecules that were degraded varied from organism to organism and was influenced by environmental conditions.[47–49]

Many eukaryotes, such as the mycorrhizal and nonmycorrhizal fungi of the *Ascomycota* and *Basidiomycota* lineages also degrade AHLs.[50] Mammalian cells, such as human epithelial or kidney cells are able to degrade AHLs, and have a preference for long chain substrates.[51] Interestingly, cells with strong bacterial interactions, such as lung epithelial cells, show a greater ability to degrade AHLs than cells from other tissues; this indicates a possible co-evolution of the host and pathogen. Several plants, including barley, clover, *Lotus* and yam bean, also prevent accumulation of AHLs with acyl chain length from C6 to C10 in their environment.[52,53]

All organisms that are known to degrade AHLs are potentially in contact with sizeable AHL-producing bacterial communities; this is a situation first demonstrated in a biofilm that developed in a water reclamation system.[54] Bacteria that produce and degrade AHLs also co-exist in the rhizosphere of tobacco[55] and in the intestine of the Ayu fish (*Plecoglossus altivelis*).[56] All of this information prompted investigations into the use of natural bacterial isolates to quench QS-regulated functions, especially in deleterious organisms. A summary of the available reported information is given in Table 3.

Quorum-quenching enzymes

Three main enzymatic mechanisms have been clearly described: lactone hydrolysis (see also above), amidohydrolysis (Figure 4.2) and oxidoreduction (Figure 4.3 and 4.4).

1) Lactone hydrolysis: *AHL lactonase*: AHL-lactonases induce the hydrolysis of the homoserine lactone ring of the AHLs; this leads to the generation of acyl homoserine. This hydrolysis is identical to the pH-mediated lactonolysis, and as such, the re-

Table 3. Examples of QQ due to the introduction of bacteria that are naturally capable of degrading AHLs.

Quenched	Quencher	Function quenched	Refs.
Agrobacterium tumefaciens	*Rhodococcus erythropolis* W2 *Bacillus sp A24*	pTi transfer	[47, 98]
Aeromonas	*Shewanella* sp.	exoprotease production	[56]
Burkholderia glumae	*Acinetobacter* sp. C1010 *Bacillus* sp. 240B1	n.d.*	[111]
Chromobacterium violaceum	*Rhodococcus erythropolis* W2 *Comamonas* sp. D1 *Bacillus* sp. A24	violacein production	[47, 98]
Pectobacterium carotovorum	*Rhodococcus Comamonas Ochrobactrum* sp. strain A44 *Bacillus* sp. strain A24 *Arthrobacter* sp IBN110 *Bacillus* sp. 240B1	maceration of potato tuber tissue antibiotic production	[47, 112] [59, 111]
Pseudomonas chlororaphis PCL1391	*Bacillus* sp. A24 *Acinetobacter* sp. C1010	phenazine production	[98, 111]
Yersinia enterolitica	*Bacillus cereus* 720	n.d.*	[113]

* Not done. The impact of coinoculation was only tested on the AHL degradation. The functions regulated by QS in *B. glumae* and *Y. enterolitica* were not tested.

action can be reversed by acidification of the medium. Lactonase activities have been demonstrated in several bacterial genera and also in eukaryotic cells. The first demonstration of AHL-lactonase activity was obtained in *Bacillus* sp.[31] This lactonase hydrolyzes a large range of AHLs, from C4- to C14-HSL, with or without a substitution at the C3 position. These enzymatic activities have also been identified in Gram-positive and -negative bacteria, and possibly in Acidobacteria (Table 3).

To date, two families of lactonases have been identified in prokaryotes. The first and most well-studied group is represented by the AiiA lactonase.[57–60] The prototypic AiiA is a metallohydrolase of the β-lactamase family[31] that requires two Zn^{2+} ions for full functionality.[61] The AiiA-like lactonases are not affected by differences in acyl chain length and substitution in the AHLs. AiiA-like lactonases are widespread in bacteria and have been characterized in Gram-positive and -negative prokaryotes (Figure 5 B). The second type of AHL-lactonase is represented by the QsdA lactonase from *Rhodococcus erythropolis*. It is specific to this genus (Figure 5 A) and is not related to the AiiA AHL lactonase family, although both are Zn-dependent metalloproteins.[62] QsdA belongs to the phosphotriesterase (PTEase) family. Phosphotriesterases were first described for their ability to cleave the P–O bond in phosphotriesters, such as the nerve gas parathion, but were later shown to be promiscuous enzymes that harbor PTEase, lactonase, or amidohydrolase activities.[62,63] QsdA is more closely related to the so-called phosphotriesterase-like lactonase (PLL) proteins, such as SsoPox from *Sulfolobus solfataricus*. It shares an extra loop 7 and a shorter loop 8 with SSoPox, and these have recently been shown to create a perfectly fitting pocket where the lactone ring and the acyl chain interact.[64]

AHL-lactonase activities have also been reported in different eukaryotes and in mammalian serum.[65] In contrast to the AHL-lactonase, the serum lactonases are Ca^{2+}-dependent, a trait that is consistent with these enzymes being paraoxonases with lactonase activity. Three paraoxonases, Pon1, Pon2, and Pon3, have been shown to be responsible for AHL degradation in mammalian serum[66,67] and to interfere with QS regulation in *Pseudomonas aeruginosa* biofilms.[68]

2) Amide bond hydrolysis: *AHL acylase*: AHL acylases catalyze the complete and irreversible degradation of the AHLs through the hydrolysis of their amide bond; this releases homoserine lactone and the corresponding fatty acid (Figure 4.2). To date, this enzymatic activity has been described in prokaryotes and eukaryotes (Figure 5). It was first demonstrated in *Variovorax paradoxus*,[69] and later in other bacterial genera (Table 3). Six genes that encode AHL-acylase have been characterized (Figure 5). All of these AHL-acylases degrade long-chain AHLs more efficiently than short-chain forms. Certain enzymes, such as Aac, PvdQ, AhlM, and QuiP appear to be unable to degrade AHL that has an acyl chain shorter than eight carbons. Among these examples, AhlM is the only one that is secreted.[70] Finally, the porcine kidney acylase, which was previously recognized for its ability to hydrolyze a variety of N-acyl-L-amino acids, was shown to degrade AHLs with an acyl chain that ranged from four to eight carbons in length.[71]

3) Modification of the acyl chain: *AHL oxidase and reductase*: To date, two occurrences of AHLases with oxidative or reducing activities have been reported, but only in bacteria. In contrast to the previous QQ enzymes, these activities do not lead to the degradation of the AHL molecules into QS-inactive molecules. Instead, they catalyze a modification of the chemical structure of the signal. This is nevertheless important, because this modification might affect the specificity and recognition of the AHL signal, and therefore interfere with QS-regulated functions.[72] The first AHL oxido-reductase activity was reported in a strain of *Rhodococcus erythropolis*.[73] It reduces the keto group of 3-oxo-AHLs, which have an acyl chain that is between eight and 14 carbons long, to the corresponding 3-hydroxy derivative. It is inactive against hydroxylated and fully reduced AHLs. The second AHLase is a P450 monooxygenase from *Bacillus megaterium* that was previously reported for its ability to oxidize fatty acids and *N*-fatty acyl amino acids.[72] This enzyme oxidizes long-chain AHLs such as dodecanoyl-HSL (C12-HSL) and *N*-(3-oxo)dodecanoyl-HSL (3O,C12-HSL) at the ω-1, ω-2, and ω-3 positions, with the highest affinity for the 3-oxo derivative.

What are the biological functions of the AHLases?

1) AHL degradation as an antibiotic resistance process: A recent study revealed that rearrangement compounds arising from 3O-AHLs generated tetramic acids that are toxic to several Gram-positive strains, including *Bacillus* sp. Interestingly, nu-

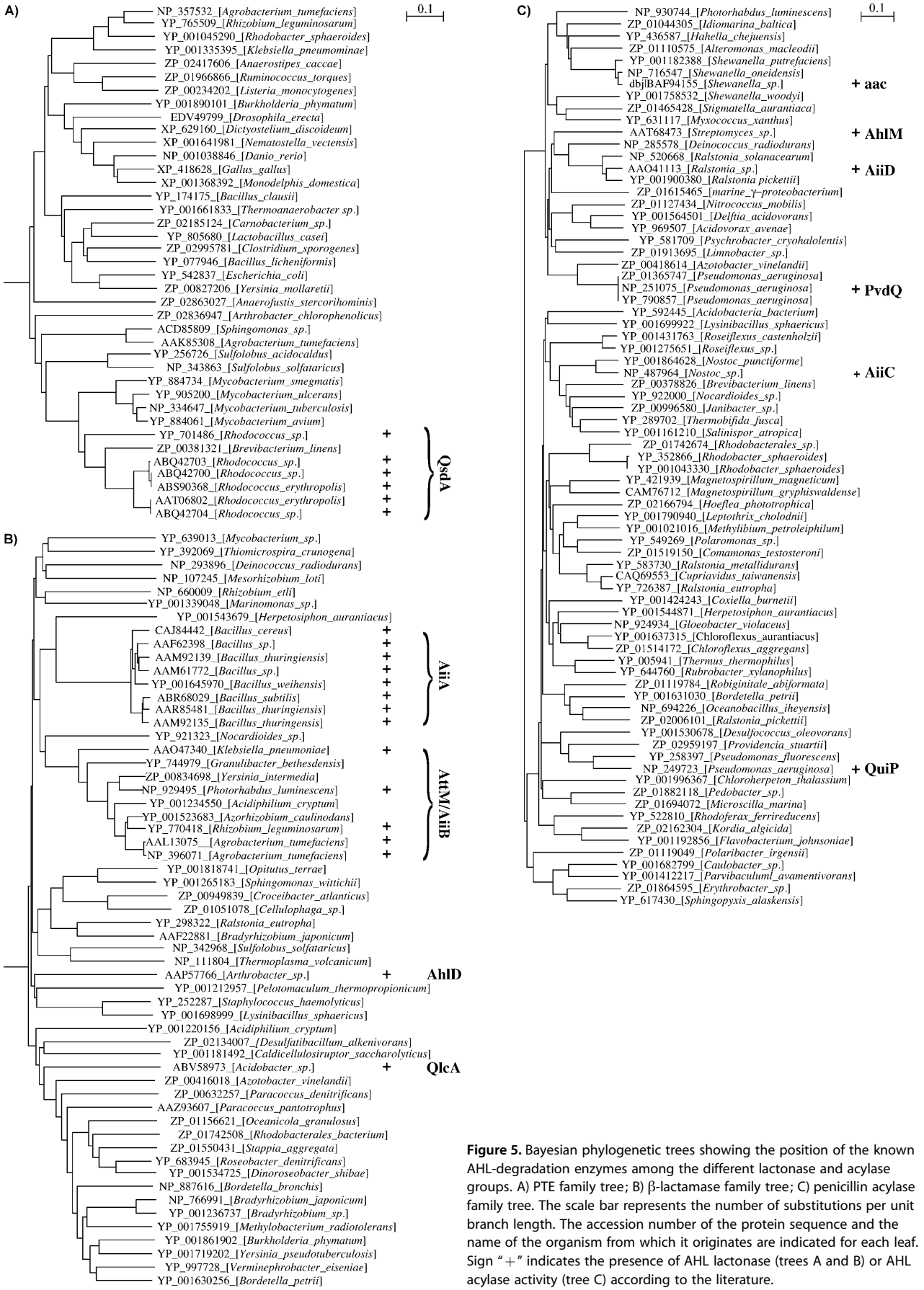

Figure 5. Bayesian phylogenetic trees showing the position of the known AHL-degradation enzymes among the different lactonase and acylase groups. A) PTE family tree; B) β-lactamase family tree; C) penicillin acylase family tree. The scale bar represents the number of substitutions per unit branch length. The accession number of the protein sequence and the name of the organism from which it originates are indicated for each leaf. Sign "+" indicates the presence of AHL lactonase (trees A and B) or AHL acylase activity (tree C) according to the literature.

merous *Bacillus* sp. strains harbor genes encoding AHL lactonases that are able to degrade AHLs. As a consequence, one role of the AiiA lactonase in *Bacillus* might be to detoxify the 3-oxo-AHL derivatives, as proposed by Kaufmann et al.[45] Whether AHL-lactonases efficiently degrade AHL tetramic acid derivatives remains to be verified.

2) AHL degradation as a regulatory process: Remarkably, degradation of AHLs also occurs in bacterial strains that produce these molecules. The best-described example is that of the pathogen *Agrobacterium,* for which Ti plasmid transfer and copy number are regulated by QS. Interestingly, *Agrobacterium* also harbors two genes, *attM* and *aiiB* that encode AHL-degrading lactonase enzymes.[58,74] Whereas the role of AiiB is not yet understood, the role of AttM has previously been elucidated. The *attM* gene is part of the *attKLM* operon,[74] and AttM plays a dual role. First, it can degrade γ-butyrolactone to γ-hydroxybutyrate; this latter compound is then degraded to succinic acid semialdehyde by the AttL and AttK peptides.[75] Second, it allows the degradation of AHL in response to γ-aminobutyrate (GABA), which is a plant compound that is produced at high concentrations following wounding and induces the expression of the *attKLM* operon.[76] AHL degradation by AttM in *Agrobacterium* therefore appears to counteract AHL-mediated induction of QS-regulated functions.

3) Functional promiscuity of AHL degradation enzymes: The possibility that AHL degradation might not be the primary role of AHL acylases, lactonases, and oxidases cannot be excluded. As stated above, in *Agrobacterium*, the *att*KLM operon is also involved in the dissimilation of GBL. In *Streptomyces* spp., the AhlM acylase degrades penicillin.[70] In *P. aeruginosa*, PvdQ is part of an operon that is responsible for the synthesis of the siderophore pyoverdine.[77] A null-mutation in PvdQ abolishes pyoverdine synthesis; this supports the view that the PvdQ acylase is required for the synthesis of pyoverdine. In *Mesorhizobium loti*, the MLR6805-encoded lactonase is part of an operon that is involved in pyridoxine degradation, a free form of the B6 vitamin.[78] In *Rhodococcus* the *qsd*A gene is part of a four-gene operon, in which two genes show a strong homology to genes that are involved in lipid metabolism in other bacteria.[62] In mammalian cells, paraoxonases were first identified as organophosphate-detoxifying enzymes. They also have thiolactonase activities and exhibit antioxidant properties, mostly towards sterols and lipid-like molecules.[79] Finally, P450 monooxygenase is able to modify fatty acids, *N*-acyl amino acids, as well as D-isomers of C12-HSL.[72] The *Rhodococcus* oxidoreductase also modifies 3-oxododecanamide (3O,C12-NH_2, which lacks the homoserine lactone ring) and *N*-(3-oxo-6-phenyl)hexanoyl-HSL (3O,6Ph,C6-HSL, which contains an aromatic acyl-chain substituent), as well as D-isomers of 3O,C12-HSL.[73] All of these data suggest that AHL degradation by bacteria and eukaryotes might not be the primary function of AHL degradation enzymes, but might play an important role in AHL turnover.

3. Engineering the Quorum-Quenching Process

As mentioned above, interference with QS regulation could affect three points in bacterial communication, all of which have been observed in nature. Strategies to engineer the QQ process as a novel therapeutic tool take the same routes, but the strategies differ with respect to plant or animal pathogenesis.

Development of synthetic chemical inhibitors

1) Blocking signal synthesis: This QQ strategy relies upon the inhibition of AHL synthesis by the LuxI-like protein. AHLs are synthesized from two precursors, *S*-adenosyl methionine (SAM) and the cognate acyl-ACP. A few substrate analogues, such as holo-ACP or butyryl-*S*-adenosylmethionine (butyryl-SAM), have been found to interfere efficiently in vitro with the synthesis of the QS signal. The most effective inhibitor, *S*-adenosyl-DL-homocysteine, reduces the activity of the RhlI protein by 97%.[80] Due to the central role that is played by SAM and acyl-ACP in the catabolism of fatty acids and amino acids, however, these analogues might have deleterious effects on the bacterial or the eukaryotic cells, which limits the value of this approach.

2) Blocking the receptor: Blocking the receptor with an analogue of AHL is a classical pharmacological approach. It is also likely to be more specific than the AHL synthesis inhibition approach that is described above because it relies upon the use of analogues of two central metabolites. This is probably the reason why blocking the recognition of AHL is an intensively investigated strategy for the inhibition of QS-regulated functions. The molecules that are designed to this end fall into two classes: analogues of the cognate substrate (AHL analogues) and the analogues of natural agonists of the luxR regulatory protein.

The first attempts to generate analogues with inverse agonist properties targeted the acyl chain moiety of AHL. Two research teams screened an analogue library against the Lux system of *V. fisheri* and the Tra system of *Agrobacterium*.[10,33] Schaefer et al.[10] identified analogues of 3O,C6-HSL with a modified acyl chain that were able to displace AHL from its cognate sensor, the LuxR protein; however, these analogues acted as competitive agonists. In contrast, Zhu et al.[33] showed that most analogues acted as antagonists when TraR was not overexpressed, and as potent agonists when the regulator was overexpressed. This strategy was further explored by Reverchon et al.[81] A series of acyl-chain end-modified 3O,C6-HSL analogues harboring alkyl, cycloalkyl, or aryl moieties were synthesized. Four of the alkyl-substituted analogues showed agonist activity. In contrast, all the analogues that harbored an aryl moiety were found to be strong antagonists, with IC_{50} (the concentration necessary to obtain 50% inhibition) values that ranged from 2 to 15 μM.

Another strategy to generate QS-sensing antagonists has been to substitute the HSL ring with an alternative ring structure. By focusing on the *P. aeruginosa* signaling molecules C4-HSL and 3-O,C12 HSL,[82,83] or the *V. fischeri* signaling molecule 3O,C6-HSL[10], the authors produced AHL analogues in which

the homoserine lactone was replaced by an aminocyclic ketone containing a five or six-membered ring,[81] or various cyclic functional groups, including thiolactones, aza-HSL, caprolactam, or the succinimidyl group.[10] None of the latter groups generated potent agonist or antagonist effects. Interestingly, the compound containing the cyclohexanol (Figure 6B) moiety

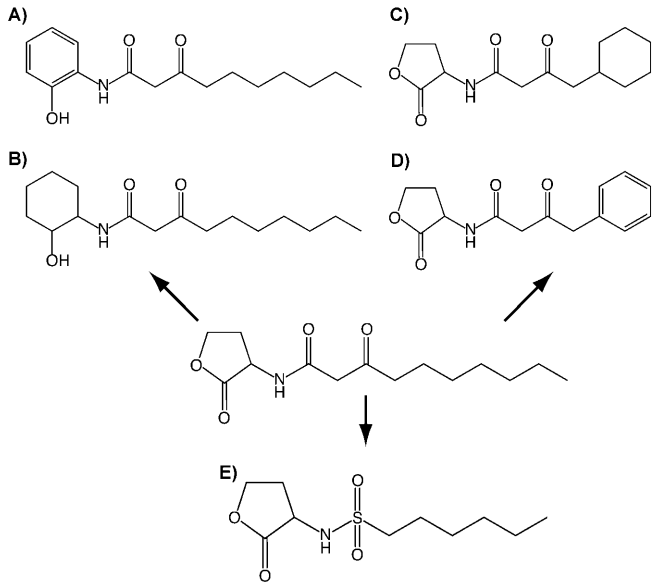

Figure 6. Synthetic quorum-sensing inhibitors. AHL analogues that have a substituted HSL moiety: A) 3O,C12-aminophenol; B) 3O,C12-aminocyclohexanol. AHL analogues with an acyl-end modification: C) 3O,cyclohexanyl C6-HSL; D) 3O,benzyl C6-HSL). AHL analogue with a carbonyl-to-sulfonyl substitution at C1: E) *N*-sulfonyl-HSL. Molecules A, D and E are strong antagonists whereas molecules B and C are strong agonists.

was the strongest agonist, whereas the structurally related, 3O,C12-aminophenol (Figure 6A), which differs from the former compound only by the unsaturation of the aromatic ring, is a strong antagonist. Nonetheless, the concentrations that were required to obtain significant inhibition with these analogues were in the 100 μM range, which is rather high from a therapeutic perspective.

A more recent strategy has targeted the central portion of the molecule by substituting a sulfonamide bond for the carboxamide at position 1 in the acyl chain.[84,85] This led to the generation of very potent antagonists, such as C5-sulfonyl-HSL; its IC_{50} is similar to the most efficient aryl-substituted AHL antagonists. Substituting a sulfonyl urea group at C1[86] did not further improve the QQ ability of the AHL analogues. It should be noted that these assays have only been performed in vitro, and that the effect of the acyl-sulfonyl-HSL on cell growth parameters is yet to be determined. Nonetheless, the activities of these molecules at low doses make them potential therapeutic candidates.

Finally, the strategy of creating chemical modifications in natural inhibitors, such as the furanones that were identified in red algae, or the garlic QQ inhibitor molecules, has met with limited success. In fact, none of the derivatives proved to be more efficient than the natural form, and none were active at concentrations low enough to validate their use as therapeutic molecules.[87] However, these furanones are sometimes used in boat paintings to limit biofouling.

Development of innovative biological tools

1) Immunotherapy: a vaccine against AHL-based quorum sensing: The 3O,C12-HSL has been shown not only to mediate bacterial QS, but also to exert cytotoxic effects on mammalian cells.[88,89] These findings allowed the development of new strategies to combat microbial infections by targeting the QS signaling molecules. Kaufmann et al.[90,91] reported an immunopharmacotherapeutic approach for the attenuation of AHL-dependent QS in the Gram-negative human pathogen *P. aeruginosa*. Monoclonal antibodies (mAbs) raised against the 3O,C12-HSL hapten demonstrated an affinity (K_d range between 150 nM and 5 μM) in vitro for 3O,C12-HSL, and exhibited high specificity as they did not recognize any of the short-chain 3O-AHLs. These mAbs exerted a clear inhibitory effect on the production of pyocyanin by *P. aeruginosa*, a QS-controlled virulence factor. More recently, Park et al.[92] have reported a similar immunotherapeutic approach for the attenuation of QS in the Gram-positive human pathogen *Staphylococcus aureus*. Remarkably, the antibody that was raised against the peptidic QS signal suppressed *S. aureus* pathogenicity in an abscess-formation mouse model in vivo, and provided complete protection against an otherwise lethal *S. aureus* challenge. These findings provide a strong foundation for further investigations of immunotherapy for the treatment of bacterial infections in which QS controls the expression of virulence factors.

2) QQ bacterial strains as biocontrol agents: When expressed in a heterologous system, such as *Burkholderia glumae*, *Pectobacterium carotovora*, *Pseudomonas aeruginosa* or *Agrobacterium tumefaciens*, some of the known AHL-degrading enzymes have been shown to limit or abolish the accumulation of AHL in the environment. As a consequence, this process also significantly reduces the expression of QS-regulated functions of the host bacteria.[62,93,94] Therefore, in these systems, the expression of an AHL-degradation enzyme by an AHL-producing strain led to the same phenotype as a *luxI* null-mutation. However, this approach is barely exploitable in biological control experiments.

A more suitable approach is based upon the selection of bacteria that are naturally able to degrade AHL or AHL analogues (such as γ-caprolactone), either as single isolates or as consortia. Co-inoculation experiments involving the AHL-producing pathogen and the AHL-degrading quencher(s) revealed that this approach is often able to significantly reduce, and in some cases abolish, the expression of the QS-regulated function of the pathogens.[46,47] AHL-degrading strains might therefore have great potential as biocontrol agents to protect plants from pathogens. To date, however, there have been no reports indicating that any of these biocontrol agents has been tested successfully under field or field-like conditions.

3) Engineering plants with QQ abilities: As stated earlier, some plant pathogens rely upon QS regulation to circumvent the

host defense by launching a massive concerted attack on the host cell only at high cell densities. Furthermore, they remain "invisible" to their host at low cell densities, thus preventing the premature production of pathogenic factors that could trigger local and systemic defense responses by the plant. Therefore, QS-regulated functions could be quenched either by overproducing the pathogenic AHL signals in transgenic plants or by engineering the plant to degrade AHLs.

By focusing on plant–*Pectobacterium* interactions, two groups[95–97] have generated transgenic plants that produce high levels of C6-HSL and/or 3O,C6-HSL. To do so, the *yenI* gene from *Yersinia enterocolitica* and the *expI* gene from *P. carotovorum* pv. *carotovora* were introduced into potato or tobacco plants. Whereas the *expI* transgenic tobacco exhibited enhanced resistance, the *yenI* transgenic potatoes exhibited greatly enhanced susceptibility to the pathogen when compared to the untransformed control plants, even at low-challenge cell densities. Although it is not yet clear why the two plants reacted in such drastically different ways, this observation might not be so unexpected. In fact, the threshold levels of the challenging pathogen population, QS signal concentration, or plant elicitor concentration to obtain a successful interaction are likely to vary between the two plant models.

Plants have also been engineered to produce AHL-degradation enzymes. The bacterial AHL lactonase AiiA from *Bacillus* was expressed in tobacco and potato plants.[57] Soluble proteins that were extracted from the plant tissues were able to degrade 3O,C6-HSL; this confirms that the plant expressed the *aiiA* gene in its active form. When challenged with *P. carotovora*, AiiA-expressing plants showed an elevated tolerance to the pathogen. Today, this strategy appears to have great biotechnological potential; however, it remains to be demonstrated that the same tolerance is observed in the field against natural populations of the pathogen, and under more stressful climatic conditions.

4) Impact of quorum quenching on beneficial bacteria: While the QQ strategy is generally viewed as a promising way to contain diseases, it is important in the context of ecology and biotechnological development to remember that QQ might have a detrimental role on nonpathogenic or beneficial microbes. In soil and plant environments, several bacteria evolved QS regulation to control a number of functions (rhizosphere fitness, antibiotic production, plasmid transfer, siderophore synthesis, etc.) that are essential to their survival, or to the establishment of beneficial interactions with the plant. Importantly, these bacteria and the pathogens might rely upon related or identical AHL signal molecules. As a consequence, QQ that is directed at the pathogen might also affect these beneficial bacteria. This question was first investigated by Molina et al.[98] They focused on the biocontrol of *Fusarium oxysporum* by using *Pseudomonas chlororaphis*, which mostly involved the QS-dependent production of the antifungal compound phenazine. Inoculation of a bacterial isolate that was naturally or genetically modified to degrade AHL with *P. chlororaphis* led to loss of the pseudomonad biocontrol ability. The impact of QQ was also investigated by Gao et al.[99] on root nodule induction by *Sinorhizobium meliloti*. The introduction of the *aii*A gene from *Bacillus* led to a significant decrease in the number of nodules that were induced on *Medicago truncatula,* and to strong modifications of the AHL-mimicking compounds that were produced by this plant. These few examples strongly indicate that the impact of QQ procedures on nontargeted bacterial populations should not be neglected. The use of highly specific QQ enzymes, however, could reduce the risks. For example, the AHL acylases of *Pseudomonas* and *Streptomyces*, which degrade long-chain AHL more efficiently than short-chain AHLs could be used to selectively interfere with pathogenic strains that are known to produce long-chain AHLs. Specific efforts should therefore be targeted towards the identification of such highly specific QQ enzymes.

5) And beyond AHL? Of course, QQ procedures might integrate signal degradation approaches with chemical antagonist ones, or one of these two approaches with a more classic process. In this respect, Zhao et al.[100] recently combined the production of an antibiotic and of an AHL-lactonase in the same bacterial strains to prevent *Erwinia carotovora* virulence. They observed that the addition of the two mechanisms increased prevention efficacy.

Furthermore, several lines of evidence indicate that the QQ strategy might not be restricted to the only AHL-dependent QS regulation. As stated earlier, the immunological approach yields valuable results by interfering with the peptide signal of *S. aureus*. The degradation of β-hydroxypalmitate methyl ester (3OH-PAME) involved in the regulation of virulence in *Ralstonia* was also recently reported by Shinohara et al.[101] They isolated an *Ideonella* sp. strain that was capable of hydrolyzing 3OH-PAME and quenching exopolysaccharide production of *Ralstonia solanacearum*. Several bacterial isolates from the *Bacillus*, *Paenibacillus*, *Microbacterium*, *Staphylococcus* and *Pseudomonas* genera are able to degrade the DSF produced by *Xanthomonas* and *Xylella* species (Figure 2E and F).[102] Coinoculation of pathogenic *Xanthomonas campestris* pv *campestris* or *Xylella fastidiosa* (two strains producing DSF) with the quencher strain significantly reduced their pathogenicity.[102] Finally, we observed the degradation of the QS signal cyclo-dipeptides (DKPs) by *Rhodoccocus erythropolis* (Uroz et al., unpublished data). This strain rapidly hydrolyzes the cyclo(Ala-Val) DKP by using amidohydrolase activity. Therefore, the QQ approach does not appear to be limited to the AHL-dependent QS systems. Interestingly, several bacterial species are now known to use several types of the QS signaling molecules that are described above. Therefore, the combination of different QQ procedures should permit control of bacterial pathogens that regulate expression of their virulence genes by using different types of QS signal molecules (AHL and 3OH-PAME) such as *Ralstonia solanacearum*.[24]

4. Conclusions

From the above, it appears that QQ strategies have a multifaceted value. In the present context of fighting antibiotic resistance, QQ appears to be a novel and promising strategy to prevent or limit the impact of bacterial diseases in plants, animals, or humans. One of the most interesting aspects of QQ lies in

the fact that it is a nonlethal approach. As a consequence, the QQ strategy exerts a limited selective pressure for the survival of bacteria. The second most interesting aspect is the versatility of this procedure. As explained above, several molecular targets exist: the signal synthase, the signals themselves, and the signal sensor. These targets are not exclusive. Recent data also suggest that QQ is not limited to only AHL-based QS regulation. Last but not least, the procedure involves "environmentally friendly" components. The AHL degradation enzymes are found in bacteria that commonly occur in soil or plant environments, and most of them are nonpathogenic isolates. The sensor antagonists are natural compounds that originate from various plants, including edible plants. The AHL analogues used to select strains or consortia that degrade AHL are nontoxic compounds, and are most often rapidly degraded by these bacteria. These features are very important in the context of the increasingly demanding registration procedures of chemical compounds (e.g. the REACH directive).

In addition to the above-mentioned considerations, the biological significance of the QQ phenomenon remains intriguing. This role is often only inferred from partial clues, for example, the mammalian cells with AHL-degradation ability are those that are most often in contact with microbes. A biological role for QQ can, however, be assigned in several species of the Gram-positive *Bacillus* genus and in the Gram-negative *Agrobacterium* genus, but these still remain exceptions rather than a rule. Nevertheless, the widespread quenching ability of various prokaryotic or eukaryotic microbes and that of plants strongly suggests that the quenching phenomenon must be re-introduced in the global QS regulation scheme. One obvious reason to do so is related to the very nature of a biological signal. To be perceived as such, a molecule must appear and disappear, either by being unstable or by undergoing some kind of turnover. The QS regulation scheme must therefore include, in between synthesis and sensing, the various quenching processes that will most likely limit the accumulation of the signals or their sensing, and possibly favor the obligatory degradation of these signals in nature. In other words, QS and QQ, which are opposite but complementary processes, can be described as the yin and yang of bacterial communication.

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Keywords: antibiotics · biological activity · microbiology · *N*-acyl homoserine lactone · quorum sensing · signal degradation

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