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Quorum-quenching microbial infections: mechanisms and implications

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The discovery of antibiotics early in the past century marked the beginning of active control and prevention of infectious microbial diseases. However, extensive use of antibiotics has also unavoidably resulted in the emergence of 'superbugs' that resist conventional antibiotics. The finding that many pathogens rely on cell-to-cell communication mechanisms, known as quorum sensing, to synchronize microbial activities essential for infection and survival in the host suggests a promising disease control strategy, i.e. quenching microbial quorum sensing or in short, quorum quenching. Work over the past few years has demonstrated that quorum-quenching mechanisms are widely conserved in many prokaryotic and eukaryotic organisms. These naturally occurring quorum-quenching mechanisms appear to play important roles in microbe–microbe and pathogen–host interactions and have been used, or served as lead compounds, in developing and formulating a new generation of antimicrobials. Characterization of the crystal structures of several types of quorum-quenching enzymes has provided valuable information to elucidate the catalytic mechanisms, as well as clues for future protein tailoring and molecular improvement. The discovery of quorum-sensing signal degradation enzymes in mammalian species represents a new milestone in quorum sensing and quorum quenching research. The finding highlights the importance of investigating their roles in host innate defence against infectious diseases and to determine the factors influencing their *in vivo* concentrations and catalytic activities.

Keywords: quorum sensing; quorum quenching; cell-to-cell communication; AHL-lactonase; AHL-acylase; PONs

1. INTRODUCTION

Microbial pathogens, infecting humans, animals and plants, cause tremendous economic and personal losses. Until the establishment of germ theory and identification of specific microbes as the causal agents of a wide variety of infectious diseases, mankind seemed helpless against these diseases. This landmark finding ultimately led to the discovery and development of vaccines and antibiotics (for a review, see Morens *et al*. 2004). The invention of antibiotics in the 1920s and subsequent developments have rewritten the history of medicine, allowing treatment of infections that were once widely fatal. However, the early optimistic prediction of eradicating infectious diseases has become non-sustainable, as many pathogens have developed resistance to antibiotics. Infectious diseases continue to be the leading causes of death and illness worldwide (for reviews, see Livermore 2004; Morens *et al*. 2004). The rapid emergence of 'superbugs' that resist most commonly used antibiotics has emphasized the need for the development of new antibiotics and novel strategies against microbial pathogens (for reviews, see Williams 2002; Livermore 2004).

Conventional antibiotics kill or stop bacterial growth by interfering with essential housekeeping functions (e.g. DNA, RNA and protein synthesis), hence inevitably imposing selection pressure that results in the emergence of antibiotic-resistant microbial pathogens. The concerns about resistance not only call for better use and administration of conventional antibiotics, but also prompt scientists to look for new disease control strategies. At least in theory, any strategy that can effectively stop pathogenic infection, but does not impose a 'life-or-death' selection pressure, would be a promising alternative to contain infectious diseases and may help to prevent antibiotic resistance in microbial communities. One such promising strategy is the recently demonstrated quorum-quenching approach, also known as antipathogenic or signal interference, which abolishes bacterial infection by interfering with microbial cell-to-cell communication—also known as quorum sensing (for reviews, see Hentzer & Givskov 2003; Zhang 2003; Zhang & Dong 2004).

Why does quenching microbial quorum sensing hold promises in infection control? This novel strategy results from the realization that many single-celled microbial organisms, including bacterial and fungal pathogens, can communicate with each other and act collectively in the regulation of infection-related traits, including expression of virulence genes and production of biofilms. The pathogens produce, detect and respond in a population density-dependent manner to specific small signal molecules, ranging from fatty acid derivatives to oligopeptides and furanones (figure 1), thus synchronizing the expression of virulence genes among family

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One contribution of 12 to a Theme Issue 'Bacterial conversations: talking, listening and eavesdropping'.

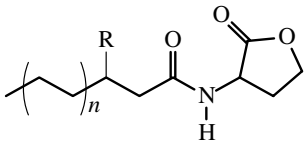
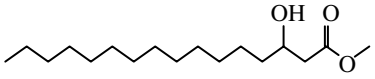
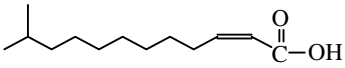
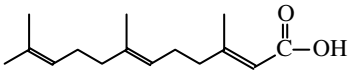
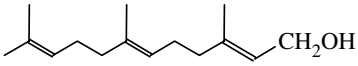
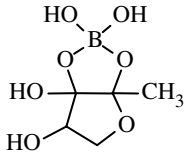
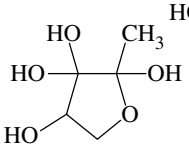
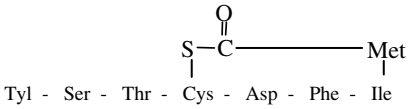
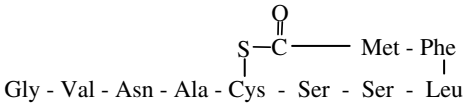
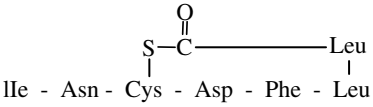
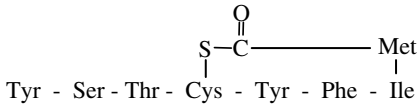
QS system	signal structure	representative organism
AHLs		*Vibrio fischeri* *Agrobacteriium tumefaciens* *Erwinia carotovora* *Pseudomonas aeruginosa* *Burkholderia cepacia*
PAME		*Ralstonia solanacearum*
DSF		*Xanthomonas campestris*
farnesoic acid		*Candida albicans*
farnesol		*C. albicans*
AI-2 (*S*-THMF-borate)		*Vibrio harveyi*
AI-2 (*R*-THMF)		*Salmonella typhimurium*
AIP-I		*Staphylococcus aureus* group I strains
AIP-II		*S. aureus* group II strains
AIP-III		*S. aureus* group III strains
AIP-IV		*S. aureus* group IV strains
CSP	Glu-Met-Arg-Leu-Ser-Lys-Phe-Phe-Arg-Asp-Phe-Ile-Leu-Gln-Arg-Lys-Lys	*Streptococcus pneumoniae*

Figure 1. Examples of microbial quorum-sensing signals. The information was summarized from the following references: Hornby *et al.* (2001), Zhang & Dong (2004) and Waters & Bassler (2005). A range of AHL signals with variation in acyl chain ($n=0$, 1, 2, …; $R=$H, O or OH) have been identified in over 70 Gram-negative bacterial species.

members (for reviews, see Whitehead *et al.* 2001; Zhang & Dong 2004; Waters & Bassler 2005). Over a short period of time, numerous quorum-quenching phenomena have been observed, and the quorum-quenching strategies have been tested with promising results. This review focuses on the principle of quorum quenching, molecular aspects of quorum quenching, the potential implications of quorum quenching in microbe–microbe and pathogen–host interactions and the intriguing possibility of using quorum quenching to control and prevent infectious diseases.

2. THE GENERAL MECHANISMS AND KEY COMPONENTS OF QUORUM SENSING

An understanding of the molecular mechanisms and the key components of quorum sensing is important for designing and developing effective quorum-quenching strategies. Different bacterial species may produce different types of quorum-sensing signals (figure 1), but they appear to adopt only two general mechanisms for detecting and responding to these signals. One general mechanism is represented by acylhomoserine lactone (AHL)-dependent quorum-sensing systems, in

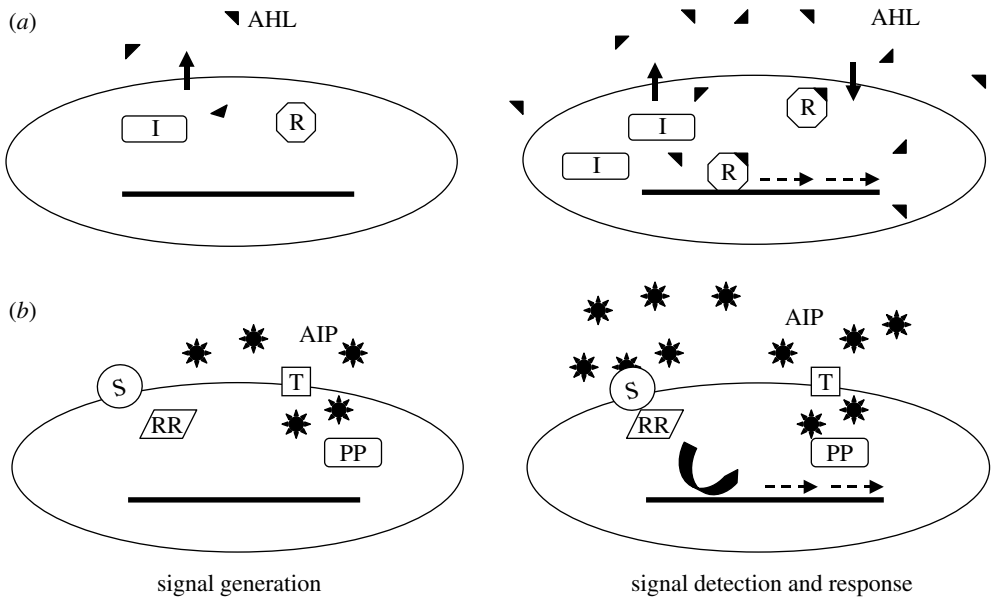


Figure 2. Two general mechanisms of microbial quorum sensing. (*a*) Signal detection by a cytosolic transcription factor, represented by the AHL-type quorum-sensing system. The signals produced by a LuxI-type protein (I) accumulate in intercellular environment, transport into cytosol, bind to LuxR-type transcription factors (R), and initiate expression of the target genes (indicated by dashed lines). (*b*) Signal detection by a two-component sensor and response regulator pair, represented by the AIP-type quorum-sensing system. Precursor peptides (PP) are modified and the resulting AIP signals exported by an ABC transporter (T). The signals are detected by the sensor histidine kinase (S), transduced to the cognate response regulator (RR) by phosphorylation relay (P), which modulates the target gene expression.

Table 1. General steps and key components of AHL-type quorum-sensing systems.

quorum-sensing process		key component	prospective quorum-quenching strategy
low-population density	(1) basal signal generation	proteins and enzymes involved in biosynthesis of acyl chain and *S*-adenosylmethionine (SAM); LuxI-type (I) protein	fatty acid biosynthesis inhibitor; SAM biosynthesis inhibitor; I protein inhibitor
	(2) signal accumulation	proteins involved in long-chain signal active efflux	AHL signal degradation enzyme; active efflux inhibitor
high-population density	(3) signal reception	LuxR-type (R) transcription factor; putative influx system for long-chain AHL signal?	R protein inhibitor; influx inhibitor?
	(4) autoinduction and activation of quorum-sensing regulon	R and I proteins involved in boosted AHL signal production; quorum-sensing-dependent transcription factors	AHL signal degradation enzyme; inhibitors for I and R proteins
	(5) signal decay	AHL degradation enzyme and its regulatory mechanisms	chemical inducing early expression of AHL degradation enzyme

which the quorum-sensing signal is detected by a cytosolic transcription factor (figure 2*a*). In the other mechanism, the quorum-sensing signal such as the autoinducing peptide (AIP) produced by *Staphylococcus aureus*, is detected by a membrane-associated two-component response regulatory system (figure 2*b*). Most of the bacteria seem to use one or other of the above quorum-sensing systems in modulating the target gene expression, but there are also pathogens that recruit the two quorum-sensing mechanisms for the same purpose; for example, *Vibrio harveyi* (for a review, see Waters & Bassler 2005).

AHL-mediated quorum sensing is one of the best characterized cell-to-cell communication mechanisms. More than 70 bacterial species are known to produce AHL-type quorum-sensing signals (Williams *et al.* 2007). Among them many are pathogens; for example, the agriculturally important *Agrobacterium tumefaciens* (Piper *et al.* 1993; Zhang *et al.* 1993) and *Erwinia carotovora* (Pirhonen *et al.* 1993), and the medically important *Pseudomonas aeruginosa* (Passador *et al.* 1993) and *Burkholderia* species (Ulrich 2004; Valade *et al.* 2004). As illustrated in table 1, the AHL-type quorum-sensing process can be arbitrarily divided into

several key steps: (i) basal level signal generation, (ii) signal accumulation, (iii) signal reception, (iv) signal autoinduction and activation of the target genes, and (v) signal decay. While steps (i)–(iv) seem to be the more or less conserved features (for reviews, see Whitehead *et al.* 2001; Zhang 2003), step (v) has so far been reported only in *A. tumefaciens* (Zhang *et al.* 2002, 2004).

At step (i), bacterial population density is low and each cell produces a basal level of AHL signal. The AHL molecules are synthesized by AHL synthase (I-protein) encoded by *luxI* homologue using corresponding acyl chain derived from the common fatty acid biosynthesis pathway and *S*-adensylmethionine (figure 3*a*; Moré *et al.* 1996; Schaefer *et al.* 1996). At this stage, LuxR-type transcription factor (R-protein) may not be required, as knocking out the *luxR* homologue does not affect the basal production of AHL signals (Marketon *et al.* 2002). At step (ii), the short-chain AHL signals are able to diffuse passively across bacterial membranes (Pearson *et al.* 1999; Dong *et al.* 2005), whereas efflux of long-chain AHL signals appears to rely on active transportation mechanisms (Pearson *et al.* 1999; Chan & Chua 2005; Dong *et al.* 2005). The multidrug efflux pump MexAB–OprM was reported to be involved in active transport of 3-oxo-C12 homoserine lactone (HSL) signal in *P. aeruginosa* (Pearson *et al.* 1999). Mutation of the MexGHI–OpmD efflux pump in *P. aeruginosa* drastically reduces the production of AHL signals and virulence factors (Aendekerk *et al.* 2002). Similarly, null mutation of the efflux pump BpeAB–OprB in *Burkholderia pseudomallei* significantly decreases AHL signal accumulation in the medium (Chan & Chua 2005). In step (iii), the R-protein and the AHL signals are the key players. Binding of the cognate AHL signal to TraR, the R-protein of *A. tumefaciens* prolongs the half-life of the receptor-like transcription factor from a few minutes to over 30 min (Zhu & Winans 1999). It was shown recently that LasR, the R-protein of *P. aeruginosa*, also requires AHL signals for correct folding (Schuster *et al.* 2004). In addition, given that efflux of long-chain AHL signals requires active transportation, it seems logical that an active influx system may also exist—but this awaits further investigations. In step (iv), the critical components are the R-AHL complex, I-protein and also probably the downstream transcription factors that contribute to the control of quorum-sensing regulons. The R-AHL complex, which is a dimer, binds to conserved palindromic sequences of the quorum-controlled promoters, including the promoter of the *luxI*-type gene, and boosts AHL production (autoinduction) and expression of other genes in the quorum-sensing regulon (Zhu & Winans 1999; Qin *et al.* 2000; Schuster *et al.* 2004). In step (v), the key components are an AHL-degradation enzyme and the cognate regulatory transcription factor(s). The AHL-degradation enzymes have been identified in several bacterial pathogens that produce AHL signals, such as *A. tumefaciens* and *P. aeruginosa* (Zhang *et al.* 2002; Huang *et al.* 2003). The expression of AHL-lactonase, encoded by *attM* of *A. tumefaciens*, is suppressed by negative transcription factor AttJ at early growth stages, but is induced specifically at the stationary phase (Zhang *et al.* 2002, 2004), and in the process of pathogen–host interactions

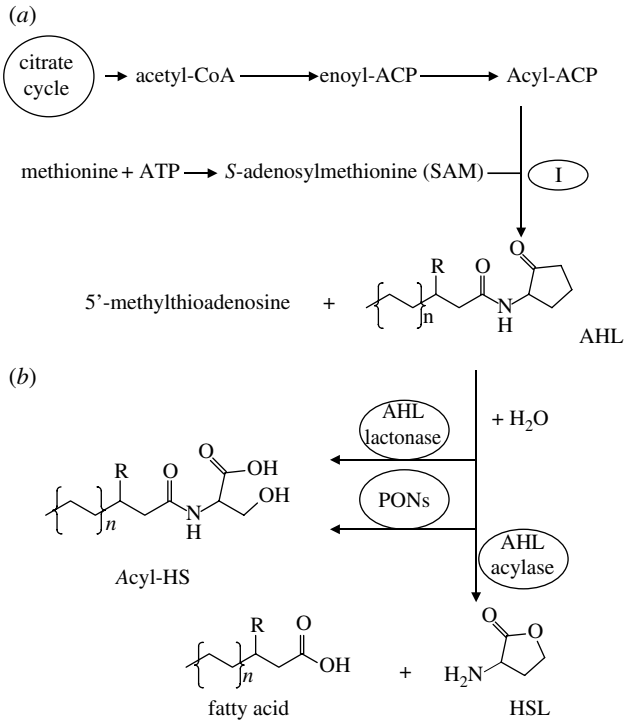


Figure 3. Synthesis and degradation of AHL-type signals. (*a*) I-protein catalyses biosynthesis of AHL signal using substrates acyl-ACP and SAM. (*b*) AHL degradation by AHL-lactonase, PONs and AHL-acylase.

(Rosen *et al.* 2003), which degrades AHL signals and switches off the quorum-sensing-dependent gene expression (Zhang *et al.* 2002, 2004). However, the role of the AHL-acylase encoded by *pvdQ* of *P. aeruginosa* in signal decay has not yet been established (Huang *et al.* 2003).

The two-component system, mediated quorum sensing has been documented in both Gram-positive and Gram-negative bacteria, and these have been extensively reviewed recently (e.g. Novick 2003; Waters & Bassler 2005). Briefly, in this type of quorum-sensing system, the quorum-sensing signal, such as the AIP signal produced by *S. aureus*, is transported to the intercellular environment by an ABC transporter. The accumulated signals are then detected by a two-component sensor, which transfers the sensory information to its cognate response regulator (figure 2*b*). The activated response regulator modulates the expression of quorum-sensing regulon through regulatory RNAs and intracellular transcription factors (for a review, see Novick 2003). As with the AHL-type quorum-sensing system (table 1), the AIP-type quorum-sensing process can also be divided into steps (i)–(iv), i.e. basal level signal generation, signal accumulation, signal reception, and signal autoinduction and activation of quorum-sensing regulon. However, it is not clear whether the last step, signal decay, exists or not in the AIP-type quorum-sensing system.

3. PROSPECTIVE AND DEMONSTRATED QUORUM-QUENCHING STRATEGIES

In theory, any mechanism that can effectively interfere with any one of the key processes in quorum sensing, for example those listed in table 1, could be potentially used for quenching quorum sensing and preventing

microbial infections. In the past few years, several groups of potent quorum-quenching chemicals and enzymes have been identified, including the halogenated furanone compounds produced by the seaweed *Delisea pulchra* and the synthetic derivatives that target R proteins (Givskov *et al.* 1996; Hentzer *et al.* 2003), the synthetic AHL and the AIP analogues that may compete with the corresponding quorum-sensing signals (Lyon *et al.* 2000; Smith *et al.* 2003), and the quorum-quenching enzymes, including AHL-lactonase, AHL-acylase and paraoxonases (PONs), which degrade AHL signals (figure 3*b*; Dong *et al.* 2000; Lin *et al.* 2003; Draganov *et al.* 2005; Ozer *et al.* 2005; Yang *et al.* 2005). Given that research in quorum sensing and quorum quenching has been progressing so rapidly in recent years, no one may doubt that more novel quorum-quenching mechanisms will be unveiled in future.

(a) *Mechanisms of small quorum-sensing inhibitors*

The known small chemicals that inhibit quorum sensing can be roughly grouped into two categories according to their structures and functions. One group is the structural mimics of quorum-sensing signals, such as the halogenated furanones and the synthetic AIPs that are similar to AHL and AIP signals, respectively (Lyon *et al.* 2000; Hentzer *et al.* 2003). Evidence shows that these inhibitors act by interfering with the corresponding signal binding to the receptor (Lyon *et al.* 2000) or decreasing the receptor concentration (Manefield *et al.* 2002). The other group of small chemicals is the enzyme inhibitors. For example, triclosan inhibits enoyl-ACP reductase whose product is the essential intermediate in AHL biosynthesis (figure 3*a*; Hoang & Schweizer 1999), and closantel is a potent inhibitor of histidine kinase sensor of the two-component system (Stephenson *et al.* 2000). Several recent reviews (e.g. Hentzer & Givskov 2003; Zhang & Dong 2004), as well as Bjarnsholt & Givskov (2007), have provided detailed discussions on the molecular mechanisms and potential impacts of these small quorum-sensing inhibitors.

(b) *Mechanisms of AHL-lactonase*

AHL-lactonases, which hydrolyse the homoserine lactone ring of AHL signals (figure 3*b*), have now been identified from a range of bacterial species (for a review, see Dong & Zhang 2005). The first AHL-lactonase, encoded by the *aiiA* gene of a *Bacillus* sp. isolate 240B1 (hereafter referred to as $AiiA_{240B1}$), was identified by functional cloning in *Escherichia coli* using AHL signals as substrates. The enzyme was proposed as a member of the metallo-hydrolase superfamily as it contains a 'His^{104}-X-His^{106}-X-Asp^{108}-His^{109}' motif that resembles the zinc-binding motif of several metalloenzymes, including glyoxalase II, arylsulfatase and β-lactamase (Dong *et al.* 2000). Site-directed mutagenesis based on sequence alignment of the AiiA homologues has established the motif 'His^{106}-X-Asp^{108}-His^{109}−59X-His^{169}-21X-Asp^{191}', which is essential for the enzyme activity of AHL-lactonase (Dong *et al.* 2000, 2002). The recent crystal structure analysis of AHL-lactonase from *Bacillus thuringiensis* subsp. *kurstaki* (hereafter referred to as $AiiA_{BTK}$) by two independent research groups shows that the enzyme contains two zinc ions in the active site (Kim *et al.* 2005; Liu *et al.* 2005), which agrees with the recent biochemical analysis that AHL-lactonase is a metalloprotein (Thomas *et al.* 2005). The two zinc ions are coordinated to a number of ligands, including His^{104}, His^{106}, Asp^{108}, His^{109}, His^{169} and His^{235}, as well as a single oxygen of a bridging carboxylate from Asp^{191} and a bridging water/hydroxide ion. All residues directly involved in metal coordination are completely conserved in all AHL-lactonases. The data of the crystal structural analysis of $AiiA_{BTK}$ are highly consistent with the previous mutagenesis study on $AiiA_{240B1}$ (Dong *et al.* 2002). The only inconsistency is that the substitution of His^{104} with serine was found to be non-essential for the $AiiA_{240B1}$ activity (Dong *et al.* 2000), but critical for $AiiA_{BTK}$ based on structural analysis and by substitution with alanine (Kim *et al.* 2005). We have confirmed recently that replacement of His^{104} with alanine in $AiiA_{240B1}$ basically abolished the enzyme activity (L.-H. Wang & L.-H. Zhang 2006, unpublished data). Therefore, it is unlike that, as previously proposed, $AiiA_{240B1}$ might not be a metalloprotein (Wang *et al.* 2004), and the enzyme could also contain zinc ions. It is also worth noting that $AiiA_{240B1}$ and $AiiA_{BTK}$ share a high 90% amino acid identity (Dong *et al.* 2000; Kim *et al.* 2005).

Crystal structure analysis of AHL-lactonase has revealed a $\alpha\beta/\beta\alpha$ sandwich-fold in overall structure with two zinc ions in their active sites, located in a loop-rich region on top of the $\alpha\beta/\beta\alpha$-fold (Kim *et al.* 2005; Liu *et al.* 2005). Somewhat expectedly, these structural features are remarkably similar to those of glyoxalase II (Cameron *et al.* 1999) and RNase Z proteins (de la Sierra-Gallay *et al.* 2005), members of the metallo-β-lactamase superfamily, in despite of limited sequence similarity. On the basis of the three-dimensional structures of AHL-lactonase, with and without L-homoserine lactone, and the suggested reaction mechanism for binuclear metal-binding glyoxalase II (Cameron *et al.* 1999) and RNase Z (de la Sierra-Gallay *et al.* 2005), a catalytic mechanism of AHL-lactonase has been proposed (Kim *et al.* 2005). A nucleophilic water/hydroxide bridging the two Zn^{2+} ions attacks the substrate's carbonyl carbon. The lactone ring and carbonyl oxygen of AHL interact with Zn1 and Zn2 ion, respectively, resulting in enhanced polarization of the carbonyl bond, making it more susceptible to a nucleophilic attack. The nucleophilic attack on the substrate's carbonyl carbon results in formation of a negatively charged intermediate that may be stabilized primarily by the interactions with Zn1 ion. The C–O bond of the lactone ring of AHL then breaks to yield the ring-opened product; in this process, Tyr^{194} may act as a general acid for protonation of the leaving group.

In contrast to AHL-acylase and PON enzymes, which have variable substrate spectra as discussed below, AHL-lactonase is by far the most specific AHL-degradation enzyme. It hydrolyses both short- and long-chain AHL signals with similar efficiency, but shows no or little residue activity to other chemicals, including non-acyl lactones and aromatic carboxylic acid esters (Wang *et al.* 2004). Further determination of the crystal structure of AHL-lactonase/AHL

complex would be essential to elucidate its intriguing substrate specificity.

(c) *Mechanisms of AHL-acylase*

Several bacterial species, including *Variovorax paradoxus*, a *Ralstonia* isolate, *P. aeruginosa* PAO1, and a *Streptomyces* sp. have been reported to encode AHL-acylase for degradation of AHL signals by hydrolysing the amide bond of AHLs and producing corresponding fatty acids and homoserine lactone (figure 3*b*; Leadbetter & Greenberg 2000; Huang *et al.* 2003; Lin *et al.* 2003; Park *et al.* 2005). The three identified AHL-acylases, i.e. the AiiD from *Ralstonia* sp. XJ12B (Lin *et al.* 2003), the PvdQ from *P. aeruginosa* PAO1 (Huang *et al.* 2003) and the AhlM from *Streptomyces* sp. (Park *et al.* 2005), share many of the known characteristics of Ntn hydrolases, including a signal peptide followed by an α subunit, spacer sequence and β subunit (Hewitt *et al.* 2000). However, there are also notable differences in the substrate specificities among AHL-acylases. AiiD effectively degrades long-chain AHLs and also short-chain AHLs, albeit with less efficiency (Lin *et al.* 2003). PvdQ is unable to degrade AHLs with acyl chains shorter than eight carbons (Huang *et al.* 2003). Similarly, AhlM shows only residue activity in degrading AHLs shorter than eight carbons (Park *et al.* 2005). Furthermore, AiiD fails to degrade penicillin G and ampicillin (Lin *et al.* 2003), while AhlM is able to catalyse the hydrolysis of penicillin G, suggesting a broader substrate specificity. These three AHL-acylases are structurally similar to the cephalosporin acylase (abbreviated hereafter as CAD) from *Pseudomonas diminuta* (Lin *et al.* 2003; Park *et al.* 2005). Crystal structure analysis of CAD reveals a side-chain binding pocket, in which the residues Gln^{50} and Arg^{57} have been proposed as the key components determining the substrate specificity (Kim *et al.* 2000). Even if the catalytic activity of CAD on AHL signals has not been demonstrated, sequence similarity between the CAD and the three AHL-acylases suggests that they may share a similar three-dimensional structure. Interestingly, sequence alignment of the three AHL-acylases with CAD shows that these four acylases have different residues in the two corresponding positions (Ile^{50} and Ser^{57} in AiiD, Leu^{50} and Asp^{57} in PvdQ, and Leu^{50} and Ser^{57} in AhlM). Further mutagenesis and crystal structure analysis of these AHL-acylases would be critical for elucidating the molecular mechanisms implicated in substrate specificity and catalysis.

(d) *Mechanisms of paraoxonase enzymes*

Strong AHL inactivation activity was first observed in human epithelial cells (Chun *et al.* 2004). Later, it was found to be widely conserved in the sera of all six of the tested mammalian species—human, rabbit, mouse, horse, sheep and bovine (Yang *et al.* 2005). The characteristics of these AHL inactivation enzyme(s), such as dependence on Ca^{2+} ion and lactonase-like activity, are reminiscent of those of PONs (Ozer *et al.* 2005; Yang *et al.* 2005). PONs, including PON1, PON2 and PON3, exhibit a wide range of physiologically important hydrolytic activities, including drug metabolism and organophosphate detoxification (for reviews, see Draganov & La Du 2004; Ng *et al.* 2005). The quorum-quenching enzyme activity of PONs has been demonstrated recently by three independent laboratories. The purified recombinant human PON2 efficiently hydrolyses several tested AHL compounds (Draganov *et al.* 2005). The recombinant animal CHO cells expressing mouse PON1, PON2 and PON3, respectively, display strong AHL degradation activity (Yang *et al.* 2005). Hydrolytic activity of the PON1 purified from human serum against *P. aeruginosa* 3-oxo-C12 HSL signal has also been demonstrated (Ozer *et al.* 2005). These PON enzymes seem to be most active with long-chain AHL signals, such as 3-oxo-C12 HSL, but less efficient with short-chain AHL signals (Chun *et al.* 2004; Yang *et al.* 2005). As is the case with AHL-lactonase, PON enzymes also hydrolyse the homoserine lactone ring of AHL signals (figure 3*b*).

Interestingly, although PON enzymes, particularly PON1, are known to catalyse the hydrolysis of many synthetic chemicals including organophosphate insecticides, nerve agents, aromatic carboxylic acid esters, cyclic carbonate esters, aromatic lactones and alkyl lactones (Billecke *et al.* 2000; Draganov *et al.* 2005), the physiological substrates for these proteins have not been identified previously. Given that host–pathogen interactions are ubiquitous, and that certain AHL signal such as *N*-(3-oxodecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) produced by the human pathogen *P. aeruginosa* have a demonstrated physiological role in interference with host immune systems (Telford *et al.* 1998; Ritchie *et al.* 2003, 2005), the AHL quorum-sensing signals can now be cited as the first index of natural substrates of these fascinating PON enzymes.

The first crystal structure of a PON family member, a variant of PON1 (designated as rePON1) obtained by directed evolution, has been solved recently (Harel *et al.* 2004). PON1 is a six-bladed β-propeller with two Ca^{2+} ions in its central tunnel. One calcium atom lies at the bottom of the active site and is postulated to play a role in catalysis, while the inner calcium is largely buried and appears to have a structural function. The catalytically important Ca^{2+} ion seems to interact with five amino acid residues, i.e. Asn^{224}, Asn^{270}, Asn^{168}, Asp^{269} and Glu^{53}, and one water molecule and one oxygen of a phosphate ion. Based on the structural similarity to secreted phospholipase A2 (Sekar *et al.* 1997), and the pH-rate profiles of rePON1 using 2-naphthyl acetate and paraoxon as substrates, a catalytic pathway has been proposed (Harel *et al.* 2004). The reaction first step involves deprotonation of a water molecule by the His^{115}–His^{134} dyad to generate a hydroxide anion, followed by a nucleophilic attack at the ester carbonyl centre of the substrates that results in production of an oxyanionic intermediate. The negative charge of the resulting intermediates is probably stabilized by the catalytic calcium. In the reaction last step, the C–O bond of the ester intermediate breaks down.

Amazingly, PON1 is able to hydrolyse a wide range of substrates and shows at least three types of enzyme activity, i.e. organophosphatase, arylesterase and lactonase (Billecke *et al.* 2000; Draganov *et al.* 2005). The chemical structures of these substrates are so different that one may question whether the enzyme uses the same mechanism for catalysis. The crystal structure analysis has, however, revealed only one active site. Moreover, the essential role of the newly

Table 2. Examples of quorum-quenching molecules against microbial infections.

quorum-quenching molecules		host	effect	reference
AHL-lactonase	*aiiA*	*Erwinia carotovora*	decreases extracellular pectolytic enzyme activities, and attenuates soft rot symptom on the plants inoculated	Dong *et al.* (2000)
		tobacco, potato	transgenic plants are resistant to *E. carotovora* infection	Dong *et al.* (2001)
		Pseudomonas aeruginosa	decreases production of elastase, rhamnolipids, hydrogen cyanide and pyocyanin, and inhibits bacterial swarming	Reimmann *et al.* (2002)
		Escherichia coli	attenuates the pathogenicity of *E. carotovora* when co-inoculated	Lee *et al.* (2002)
		Bacillus thuringiensis	the efficiency of biocontrol against *E. carotovora* infection is dependent on AHL-lactonase	Dong *et al.* (2004)
		Burkholderia thailandensis	reduces the bacterial swarming and twitching motility, prevents the β-haemolysis of sheep erythrocytes	Ulrich (2004)
		Erwinia amylovora	impairs extracellular polysaccharide production and tolerance to hydrogen peroxide, and reduces the fire blight symptom on apple leaves	Molina *et al.* (2005)
	attM, *aiiB*	*Erwinia carotovora* subsp. *Atroseptica*	decreases maceration in potato tubers	Carlier *et al.* (2003)
paraoxonase	*PON1*	*P. aeruginosa*	the serum containing PON1 prevents bacterial biofilm formation *in vitro*	Ozer *et al.* (2005)
AHL-acylase	*aiiD*	*P. aeruginosa*	decreases swarming ability, elastase and pyocyanin production, and attenuates nematode paralysation	Lin *et al.* (2003)
synthetic AIP-II		mouse	treated mice show resistance to *S. aureus* infection	Mayville *et al.* (1999)
3-oxo-C12-(2-aminocyclohexanone)		*P. aeruginosa*	reduces the production of virulence factors and biofilm formation	Smith *et al.* (2003)
furanone		mouse	attenuates the virulence of *P. aeruginosa* in mouse models	Hentzer *et al.* (2003)
DSF		*Candida albicans*	inhibits the fungal dimorphic transition that is associated with virulence	Wang *et al.* (2004)

identified His^{115}–His^{134} dyad in enzyme activity has been confirmed by site-directed mutagenesis and assay using two classes of substrates, phenyl acetate and paraoxon (Harel *et al.* 2004). These structural features and the findings from the mutagenesis study described above suggest that PON1 may also rely on the same catalytic mechanisms in hydrolysis of AHL signals.

PON1 and the other two members, PON2 and PON3, share about 60% homology at the peptide level. By sequence alignment, we found that all the important residues, including Glu^{53}, His^{115}, His^{134}, Asn^{168}, Asn^{224}, Asp^{269} and Asn^{270}, are perfectly conserved in the three PONs, indicating that these enzymes most probably share the same catalytic mechanism. These three enzymes share overlapping substrates, but also display distinct substrate specificities (Draganov *et al.* 2005). Future investigations may reveal the intriguing mechanisms of these enzymes that determine substrate specificity. More importantly, several polymorphisms have been detected in the coding region of PON1 and some of the amino acid variations showed significant influence on the enzyme activity on synthetic substrates (for reviews, see Deakin & James 2004; Ng *et al.* 2005). It remains to be determined whether these variations could also affect the catalytic activity of PONs against AHL signals.

4. QUORUM QUENCHING IN BASIC RESEARCH AND BIOTECHNOLOGICAL APPLICATIONS

Quorum-quenching molecules have proved to be valuable tools in addressing both the basic and the conceptional questions (table 2). Skin lesions on inoculated mice were reduced when AIP-II, the group-specific cell-to-cell communication signal produced by group-II *S. aureus*, was included in the inoculum mixture of group-I *S. aureus* bacterial cells (Mayville *et al.* 1999). The experiment identified the key structural features of the signals involved in activation and antagonism, and led to the design of a global inhibitor of the virulence response in *S. aureus* (Lyon *et al.* 2000). Since the discovery of the first quorum-quenching enzyme encoded by *aiiA* (Dong *et al.* 2000), the prokaryotic-origin AHL-lactonases and AHL-acylases have been frequently used in investigations of the role of AHL signals owing to the convenience in cloning and expression (table 2). More recently, the importance of AHL quorum-sensing signalling in the regulation of virulence and other physiological functions in *Burkholderia thailandensis* and *Erwinia amylovora* has been demonstrated by expression of the AHL-lactonases encoded by the *aiiA* homologues in these two pathogens, respectively (table 2; Ulrich 2004; Molina *et al.* 2005).

The quorum-quenching enzymes, along with other small naturally quorum-sensing inhibitors and synthetic derivatives, have been explored and evaluated as novel antimicrobial agents against different pathogens with promising results (table 2). Expression of AiiA in transgenic potato and tobacco plants conferred strong resistance to the bacterial pathogen *E. carotovora*, which required AHL quorum-sensing signals to activate the expression of virulence genes (Dong *et al.* 2001). Similarly, natural or recombinant AHL-lactonase-producing bacterial strains, including *B. thuringiensis*, *Arthrobacter* sp. and *Pseudomonas fluorescens*, protected potato from *E. carotovora* infection when co-inoculated with the pathogen (Molina *et al.* 2003; Park *et al.* 2003; Dong *et al.* 2004). Treatment of mice with synthetic furanones, the derivatives of the natural furanones produced by the seaweed *D. pulchra*, significantly decreased the cell number of *P. aeruginosa* in the infected lung tissues and the disease symptoms (Hentzer *et al.* 2003; Wu *et al.* 2004).

5. IMPLICATIONS OF QUORUM QUENCHING IN HOST DEFENCE

The intriguing findings that the PONs from human and other mammalian species have high catalytic activities against long-chain AHL signals suggest that these quorum-quenching enzymes could be active components of mammalian innate immune systems (Chun *et al.* 2004; Draganov *et al.* 2005; Ozer *et al.* 2005; Yang *et al.* 2005). However, somewhat unexpectedly, *Pon1*-knockout mice are protected from infection by quorum-sensing-dependent *P. aeruginosa* pathogen cells that were introduced intraperitoneally; in sharp contrast, wild-type mice show a high percentage of mortality (Ozer *et al.* 2005). Subsequent analysis showed that the transcriptional expressions of PON2 and PON3 are significantly enhanced in the PON1-deficient mice (Ozer *et al.* 2005). This compensation-like phenomenon may explain the enhanced resistance to *P. aeruginosa* infection in PON1-deficient mice. Moreover, it may be worthy to note that in humans, PON1 expression is limited to the liver and PON3 primarily to liver and kidney, whereas PON2 is found in most tissues including the heart, kidney, liver, lung, placenta, small intestine, spleen, stomach and testis (for a review, see Ng *et al.* 2005). Thus, if the expression pattern of PONs in mouse is similar to that in humans, one may speculate that PON2 may be more important in defence against intraperitoneally injected pathogens than the two tissue-specific members. This is the case even though PON2 and PON3 can circulate and reach different tissues through blood vessels (for a review, see Ng *et al.* 2005). Among the three members of the PON family, PON1 is the best characterized, but very little is known about PON2 and PON3. The overlapping lactonase activity and the potential compensation mechanisms of these PONs suggest that coordinated investigation is essential to unveil the role and implications of these endogenous quorum-quenching enzymes in the host innate defence against microbial pathogens.

Pseudomonas aeruginosa is an opportunistic pathogen. Patients with cystic fibrosis, severe burns, or immunosuppression are at particularly high risk of *P. aeruginosa* infection. In addition, the pathogen also frequently causes nosocomial bloodstream infections, which cause significant patient mortality and increased health care costs (Wisplinghoff *et al.* 2003; Osmon *et al.* 2004; Micek *et al.* 2005). The finding that quorum-quenching enzyme activity is abundant in the sera of mammalian species is highly intriguing (Yang *et al.* 2005). It has been known that many factors, including genetic variation, environmental changes, aging and even pharmaceutical drugs, can cause significant changes in the serum concentrations and activities of the PON1 enzyme, which is also known as serum PON enzyme (for a review, see Deakin & James 2004). Future investigation on whether and to what extent these PON1-modulating factors are implicated in the *P. aeruginosa* associated bloodstream infections may aid the development and formulation of new health and clinical practices to prevent and control these costly and dangerous infections.

Enzyme-related inactivation of the AIP quorum-sensing signals produced by the Gram-positive pathogen *S. aureus* has also been documented recently (Rothfork *et al.* 2004). AIP signals are group-specific thiolactone peptides (table 1). *Staphylococcus aureus* strains can be classified into four groups based on the sequence variations of the AIP peptides produced (Novick 2003). The mice-lacking phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which produces reactive oxygens, are more susceptible to *S. aureus* infection than the wild-type control (Pollock *et al.* 1995; Guide *et al.* 2003; Rothfork *et al.* 2004). *In vitro* analysis showed that HOCl and $ONOO^-$, the end products of phagocyte NADPH oxidase, inactivate AIP signals by oxidation of the C-terminal methionine of the peptides (Rothfork *et al.* 2004). However, this type of phagocyte NADPH oxidase-mediated resistance may not be effective against all *S. aureus* strains, as not all AIP signals contain a methionine residue (figure 1).

6. CONCLUSION

The emergence of antibiotic resistance in microbial pathogens highlights why it is important to explore new ways to prevent and control infectious diseases. Previous work, in particular the research progress over the past decade or so, has outlined how single-celled bacterial pathogens use quorum sensing, a community genetic regulatory mechanism, to synchronize microbial activities among family members so as to gain an upper hand in microbe–microbe and pathogen–host interactions. In addition to this rapid progress in understanding quorum-sensing, novel quorum-quenching mechanisms have been discovered that interfere effectively with microbial quorum sensing; these have been consecutively found in a wide range of organisms, including both prokaryotes and eukaryotes. These naturally occurring quorum-quenching mechanisms act by blocking the key steps of quorum sensing, such as signal generation, signal accumulation or signal reception. They have promising potential in both basic research and biotechnological applications. More recently, crystal structure analysis has provided valuable clues on the catalytic mechanisms of several types of quorum-quenching enzymes. Such information is important for understanding substrate

specificity and future tailoring of these intriguing enzymes. The findings that PON enzymes, which are widely conserved in eukaryotic species, are potent quorum-quenching enzymes suggest that quorum quenching could also be a host innate defence mechanism. However, these exciting findings are merely the beginning of understanding of quorum quenching in microbe–microbe and host–pathogen interactions; much remains to be investigated in future studies.

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