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Quorum Sensing and Quorum-Quenching Enzymes

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To gain maximal benefit in a competitive environment, single-celled bacteria have adopted a community genetic regulatory mechanism, known as quorum sensing (QS). Many bacteria use QS signaling systems to synchronize target gene expression and coordinate biological activities among a local population. *N*-acylhomoserine lactones (AHLs) are one family of the well-characterized QS signals in Gram-negative bacteria, which regulate a range of important biological functions, including virulence and biofilm formation. Several groups of AHL-degradation enzymes have recently been identified in a range of living organisms, including bacteria and eukaryotes. Expression of these enzymes in AHL-dependent pathogens and transgenic plants efficiently quenches the microbial QS signaling and blocks pathogenic infections. Discovery of these novel quorum quenching enzymes has not only provided a promising means to control bacterial infections, but also presents new challenges to investigate their roles in host organisms and their potential impacts on ecosystems.

Key words: quorum sensing, quorum quenching enzymes, AHL-lactonase, AHL-acylase, paraoxonase, signal interference

It has become clear that single-celled bacteria can communicate with each other and respond collectively to a changing environment. Such a cell-cell communication mechanism, also known as quorum sensing (QS) in many cases, plays essential roles in synchronizing gene expression and functional coordination among bacterial communities (For reviews, see Whitehead *et al.*, 2001; Fuqua and Greenberg, 2002; Federle and Bassler, 2003). The QS bacteria release, detect and respond to accumulation of small signal molecules, in a cell density-dependent manner, thereby regulating the expression a set of target genes. Several types of bacterial cell-cell communication signals have been identified in the last two decades, such as acylhomoserine lactone (AHL) (Eberhard *et al.*, 1981; Zhang *et al.*, 1993; Pearson *et al.*, 1994); cyclic thiolactone (AIP) (Ji *et al.*, 1995), hydroxyl-palmitic acid methyl ester (PAME) (Flavier *et al.*, 1997), furanosylborate (AI-2) (Chen *et al.*, 2002) and methyl dodecenoic acid (DSF) (Wang *et al.*, 2004), most of which are involved in the regulation of bacterial virulence (for reviews, see Whitehead *et al.*, 2001, Zhang and Dong, 2004). The QS phenomenon is not only limited to the prokaryotic kingdom; single-celled eukaryotic fungal pathogens can also use QS signals to coordinate biological functions. It has recently been reported that the fungal pathogen *Candida albicans* produces farnesoic acid (FA) to regulate the yeast-to-mycelium transition that is important for the fungal virulence (Oh *et al.*, 2001).

Among these QS signals, AHLs are one family of the best-characterized cell-cell communication signals. More than a dozen AHL derivatives, which vary in length or substitution at the acyl side chain, have been identified in a range of Gram-negative bacterial species (for reviews, see Whitehead *et al.*, 2001; Fuqua and Greenberg, 2002). In general, these bacteria have a conserved QS system, with two central components, where a LuxR-type (R) regulator and LuxI-type (I) protein serve as the signal receptor and an AHL synthase, respectively (Fig. 1). At a low population density, bacteria produce a basal level of AHL signals, which are then released from the cells. As the bacteria proliferate, the AHL signals accumulate. When an adequate AHL concentration is reached, the signaling molecules interact with R protein to form the R-AHL complex, which interacts with target promoters, inducing the expression of the target genes. It has been found that AHL signals are involved in the regulation of a range of important biological functions, including luminescence, antibiotic production, plasmid transfer, motility, virulence and biofilm formation (for reviews, see Whitehead *et al.*, 2001; Fuqua and Greenberg, 2002; Zhang, 2003). There are still many bacterial species known to produce AHL signals, but the corresponding biological functions remain

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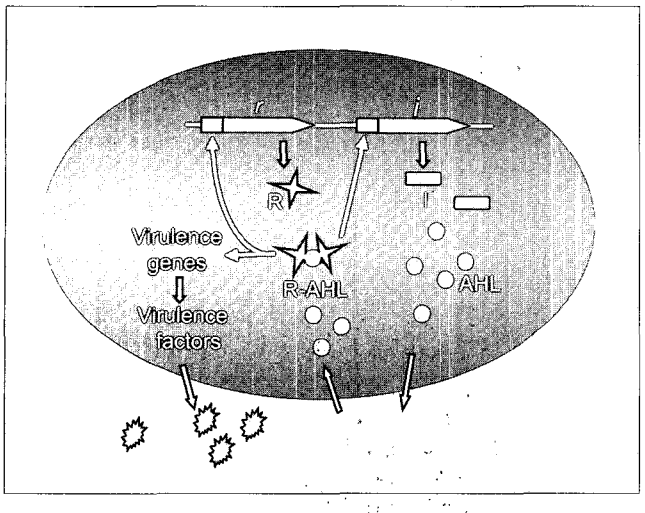

Fig. 1. Schematic representation of the AHL-dependent QS system in Gram-negative bacteria. Symbol: r, the gene encoding the LuxR-type transcription factor (R); i, the gene encoding the LuxI-type AHL synthase (I).

to be unveiled.

Prokaryote-prokaryote and prokaryote-eukaryote interactions are ubiquitous in natural ecosystems. Given that diverse bacterial species use QS-coordinated community biological activities to boost their competitive advantages, for example, production of antibiotics and virulence factors (for reviews, see Whitehead *et al.*, 2001; Zhang and Dong, 2004), it is rational that competitors may also have evolved certain mechanisms to disarm the QS systems of microbes to gain the upper hand in competition. Over the last few years, a range of quorum quenching enzymes and inhibitors have been identified from different sources, including both prokaryotic and eukaryotic organisms (for reviews, see Hentzer and Givskov, 2003; Zhang *et al.*, 2003; Zhang and Dong, 2004). These novel enzymes and inhibitors are the key molecules for establishing the concept of quorum quenching, antipathogenic and signal interference (Dong *et al.*, 2001; 2004; Hentzer and Givskov, 2003; Zhang, 2003, Zhang and Dong, 2004). In this review, the occurrence of quorum quenching enzymes in both prokaryotic and eukaryotic organisms, their roles in microbial physiology and ecology and impacts on the defense and control of bacterial infections are discussed.

Quorum quenching enzymes in prokaryotes

The exciting findings that both plant and human (including animal) bacterial pathogens, such as *Erwinia carotovora, Erwinia chrysanthemi, Erwinia stewartii, Pseudomonas aeruginosa* and *Xenorhabdus nematophilus*, are dependent on AHL QS signals for the production of virulence factors, prompted our laboratory to test whether the microbial QS system is a feasible target for the control and prevention of infectious bacterial diseases. Because the concentration of an AHL signal is a key factor in mediating virulence gene expression, it was reasoned that a strategy could be developed for the control of bacterial infection by degrading the AHL signals produced by pathogenic bacteria (Dong *et al.*, 2000). The first quorum quenching enzyme encoded by the *aiiA* gene was identified from a soil bacterial isolate belonging to a Gram-positive *Bacillus* species (Dong *et al.*, 2000), which was later characterized as an AHL-lactonase (Dong *et al.*, 2001). Shortly after the identification of AiiA, Leadbetter and Greenberg (2000) reported a strain of *Variovorax paradoxus* (VAI-C) capable of using AHL molecules as the sole sources of energy and nitrogen. The presence of homoserine lactone in the AHL metabolic mixture of *V. paradoxus* VAI-C suggests that the bacterium may produce an AHL-acylase, but the gene encoding for the AHL-acylase remains to be cloned and characterized.

Subsequently, a range of other bacterial isolates and strains that produce AHL-degradation enzymes have been identified from soil, plant and biofilm samples as well as from laboratory bacterial culture collections (Dong *et al.*, 2002; Lee *et al.*, 2002; Reimmann *et al.*, 2002; Zhang *et al.*, 2002; Hu *et al.*, 2003; Huang *et al.*, 2003; Lin *et al.*, 2003; Park *et al.*, 2003; Uroz *et al.*, 2003; Ulrich, 2004). Quorum quenching enzyme activity has so far been demonstrated and documented in at least 10 bacterial species, including 4 *Bacillus* species, *Agrobacterium tumefaciens*, *Arthrobacter* sp., *Klebsiella pneumoniae, P. aeruginosa, Rastonia* sp. and *V. paradoxus* (Table 1). The corresponding genes encoding the AHL-degradation enzymes have been cloned and characterized in most cases. Interestingly, these organisms taxonomically belong to three phyla of the Bacteria Kingdom (http://www.ncbi.nlm.nih.gov/Taxonomy/), i.e., Actinobacteria (*Arthrobacter* sp.), Firmicutes (*Bacillus* species) and Proteobacteria (*A. tumefaciens, K. pneumoniae, P. aeruginosa, Ralstonia* sp.. and *V. paradoxus*). Such a diverse distribution suggests that the genes encoding AHL-degradation enzymes might be widely conserved among many prokaryotic organisms.

The taxonomical diversity of these bacterial species is also mirrored in the sequence variations of the AHL-degradation enzymes they produce. The two AHL-acylases from *Ralstonia* sp. XJ12B and *P. aeruginosa* PAO1, respectively, share only a moderate homology, with about 39% identity at the peptide level (Huang *et al.*, 2003; Lin *et al.*, 2003). Similarly, the amino acid compositions of AHL-lactonases from diverse bacterial species also display a large degree of substitutions. Phylogenic analyses have shown these prokaryotic AHL-lactonases can be grouped in two clusters (Fig. 3). The AiiA cluster consists of all the AHL-lactonases from *Bacillus* species, sharing more than 90% peptide sequence identities (Dong *et al.*, 2000, 2002; Lee *et al.*, 2002; Reimmann *et al.*, 2002; Ulrich *et al.*, 2004). The AttM cluster includes enzymes from *A. tumefaciens, Klebsiella pneumoniae* and *Arthrobacter* sp., and share about 30-58% homology in their peptide sequences. Amazingly, the AHL-lactonases from

Table 1. Occurrence of AHL degradation enzymes in prokaryotes and eukaryotes

Species	Gene*	Enzyme	Reference
Prokaryotes			
Bacillus sp. 240B1	*aiiA*	AHL lactonase	Dong *et al.*, 2000
B. thuringiensis	*aiiA* homologues	AHL lactonase	Dong *et al.*, 2002; Lee *et al.*, 2002
B. cereus	*aiiA* homologues	AHL lactonase	Dong *et al.*, 2002; Reimmann *et al.*, 2002
B. mycoides	*aiiA* homologues	AHL lactonase	Dong *et al.*, 2002
B. anthracis	*aiiA* homologues	AHL lactonase	Ulrich, 2004
Agrobacterium tumefaciens	*attM, aiiB*	AHL lactonase	Zhang *et al.*, 2002; Carlier *et al.*, 2003
Arthrobacter sp. IBN110	*ahlD*	AHL lactonase	Park *et al.*, 2003
Klebsiella pneumoniae	*ahlK*	AHL lactonase	Park *et al.*, 2003
Variovorax paradoxus VAI-C	*ND*	AHL acylase?	Leadbetter and Greenberg, 2000
Ralstonia strain XJ12B	*aiiD*	AHL acylase	Lin *et al.*, 2003; Hu *et al.*, 2003
Pseudomonas strain PAI-A *P. aeruginosa* PAO1	*pvdQ*	AHL acylase	Huang *et al.*, 2003.
Eukaryotes			
Human (airway epithelia)	*PONs*	Lactonase	Chun *et al.*, 2003; Greenberg *et al.*, 2004
Porcine (kidney)	*ACY1*	Acylase I	Xu *et al.*, 2003

*ND, not determined.

the two clusters may share less than 25% homology, for example AiiA and AttM (Zhang *et al.*, 2002), but they all contain a highly conserved motif, HXDH~H~D, which has been proven essential for AHL-lactonase activity (Dong *et al.*, 2000, 2002; Wang *et al.*, 2004). The data suggest a highly conserved catalytic mechanism among AHL-lactonases, which will be discussed in the following section.

AHL-degradation enzymes in eukaryotes

As eukaryotic hosts have frequent encounters with microbial pathogens, it may not be surprising that higher organisms have also evolved or exploited existing mechanisms to disarm the QS signaling systems of pathogenic invaders. Until now, only two types of AHL-degradation enzymes of eukaryotic origin have been reported (Table 1). A recent report has shown that the commercial porcine kidney acylase I (EC 3.5.14) is able to deacylate C4-HSL and C8-HSL to produce L-homoserine (Xu *et al.*, 2003). The enzyme seems to prefer short chain molecules, as the rate of C8-HSL deacylation is 6-fold slower than that of C4-HSL. It is not clear whether the enzyme is active against other natural AHL signals, which are usually substituted at the 3 position of the acyl chain (for a review, see Whitehead *et al.*, 2001). Moreover, its biological effectiveness against AHLs may be questionable as the enzyme has a very low activity under both acidic and neutral pH conditions against C4-HSL (Xu *et al.*, 2003). Nevertheless, a BLAST search shows that porcine kidney acylase I is widely conserved in eukaryotes, such as mice, rats and zebrafish, but its role as a quorum quenching enzyme in these organisms remains to be examined.

Interesting observations came from the study on mammalian cells in Greenberg's laboratory. While testing the transgenic expression of the *aiiA* gene in cell lines, they observed a high background of AHL-inactivation activity from the untransformed control cell lines (Greenberg, personal communication). Further study showed that the enzyme activity was associated with the cell membrane of differentiated human airway epithelia (Chun *et al.*, 2004). The enzyme was able to inactivate C6-HSL and 3OC12-HSL, but not C4-HSL. The ability to inactivate 3OC12-HSL varied significantly among different cell types, with tissues likely to be exposed to pathogens showing the highest inactivation of the QS signal, such as A549 cells from human lungs and CaCo-2 cells from human colon. More recent studies have shown that the 3OC12-HSL degradation activity is most likely due to the paraoxonases encoded by the *PON* genes (Greenberg *et al.*, 2004). The finding may not be surprising, as the lactonase activity of the human paraoxonases has been demonstrated over 30 different non-AHL type lactones (Billecke *et al.*, 2000; Draganov *et al.*, 2000; Teiber *et al.*, 2003). In addition, PON enzymes exhibit a range of other physiologically important hydrolytic activities, including drug metabolism and detoxification of nerve agents (for review, see Draganov and La Du. 2004; Mackness *et al.*, 2004). It appears that inactivation of QS signals has now become a new index to the diverse spectrum of the recognized biological functions of PON enzymes.

Mechanisms and specificities of quorum quenching enzymes

The AHL signal molecules produced by different organisms are highly conserved; they have the same homoserine lactone moiety, but may differ in the length and substitu-

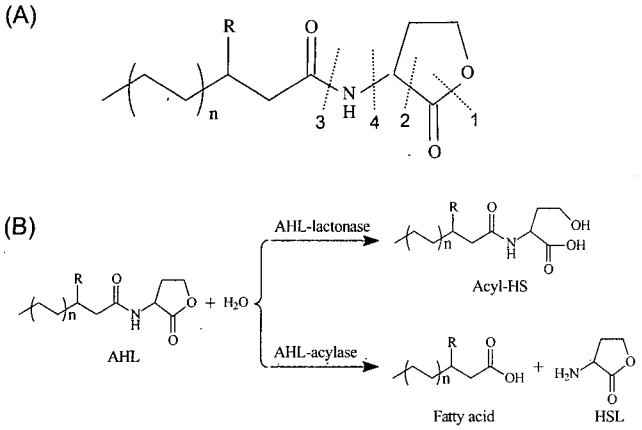

Fig. 2. The general structure of AHL signals and enzymatic degradation products. (A) The AHL structure and its possible enzyme cleavage sites (see text for discussion of potential enzymes); (B) the corresponding degradation mechanisms of AHL-lactonase and AHL-acylase.

tion of their acyl side chain (Fig. 2A). These structural features suggest that there may be at least four types of enzymes that could degrade AHL signals. Fig. 2A illustrates the potential cleavage sites of these enzymes. Among them, lactonase and decarboxylase could hydrolyze the lactone ring at the positions marked as 1 and 2, while acylase and deaminase might separate the homoserine lactone moiety and acyl side chain at sites 3 and 4, respectively (Fig. 2A). To date, only two groups of AHL-degradation enzymes, i.e., the acyl-homoserine lactonase (AHL-lactonase) and acyl-homoserine lactone acylase (AHL-acylase), which degrade AHL by hydrolyzing the lactone bond and the amide linkage (Fig. 2B), respectively, have been demonstrated enzymatically and structurally using AHL substrates (Dong *et al.*, 2001; Zhang *et al.*, 2002; Lin *et al.*, 2003; Park *et al.*, 2003). Although the detailed analysis on AHL signals is not yet available, the lactonase activity of several PON enzymes, such as PON1 and PON3, has been documented against a wide range of lactones (Billecke *et al.*, 2000; Draganov *et al.*, 2000), suggesting an AHL-lactonase-like nature of PON enzymes against AHL signals.

Among these AHL-degradation enzymes, the AHL-lactonase encoded by *aiiA* has been well characterized. Sequence alignment and mutagenesis analyses have led to the identification of a motif of catalytic importance HXDH~H~D (Dong *et al.*, 2000, 2002), which is highly conserved in all AHL lactonases in both AiiA- and AttM-clusters (Fig. 3). The motif, which is similar to the Zn^{2+}-binding motif (HXHXDH) of several metalohydrolases (Dong *et al.*, 2000), could represent a novel catalytic mechanism, as the purified AHL-lactonase does not rely on zinc or other ions for activity (Wang *et al.*, 2004). In contrast, PON enzymes, which do not contain a typical "HXDH~H~D" motif, require Ca^{2+} for lactonase activity (Billecke *et al.*, 2000). It is highly likely that PON-type

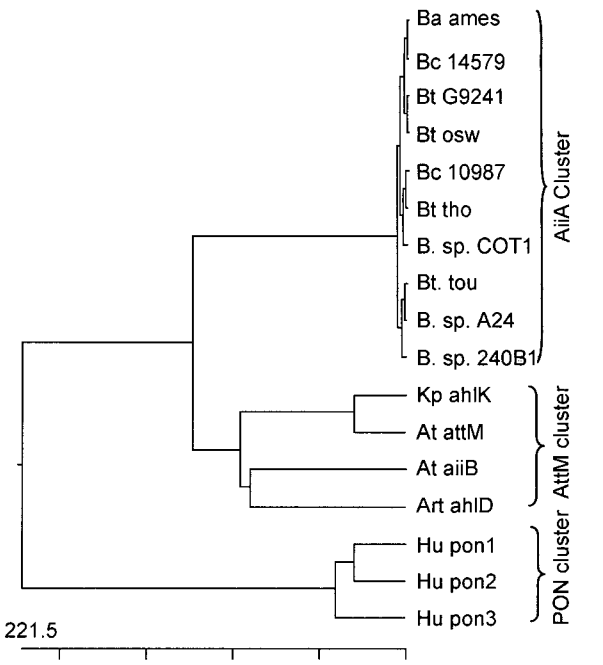

Fig. 3. Phylogenetic analysis of AHL-degrading lactonases. The phylogenic tree was constructed using the Clustal V program of the DNASTAR sequence analysis software package (DNASTAR Inc.). The distance is shown below of the tree. The lactonases (from top to bottom) are from the *Bacillus anthracis* Ames strain (Ba ames, gi30263417), *B. cereus* 14579 (Bc 14579, gi30021556), *B. cereus* G2841 (Bc G9241, gi47564581), *B. thuringiensis* serovar oswaldocruzi (Bt osw, gi28413776), *B. cereus* ATCC10987 (Bc 10987, gi42738443), *B. thuringiensis* serovar thompsoni (Bt tho, gi22095299), *Bacillus* sp. COT1 (B. sp. COT1, gi19773593), *B. thuringiensis* serovar toumanoffi (Bt. tou, gi22095301), *Bacillus* sp. A24 (B. sp. A24, gi21541343), *Bacillus* sp. 240B1 (B. sp. 240B1, gi7416989), *Klebsiella pneumoniae* (Kp ahlK, gi31540969), *Agrobacterium tumefaciens* (At attM, gi17223785), *A. tumefaciens* (At aiiB, gi16119885), *Arthrobacter* sp. IBN110 (Art ahlD, gi31580543), human PON1 (Hu pon1, gi19923106), human PON2 (Hu pon2, gi4505953) and human PON3 (Hu pon3, gi50403778), respectively.

lactonases and AHL-lactonases use different catalytic mechanisms for AHL-degradation. This is consistent with the phylogenic analysis that PON-type lactonases form a unique cluster, distinct from the other two clusters of prokaryotic AHL-lactonases (Fig. 3).

AHL-lactonases may also differ significantly from the PON-type lactonases in substrate specificity. PON enzymes are more like generic hydrolyases, and can hydrolyze various esters and lactones. Among the two best characterized PON enzymes, PON1 from human serum catalyzes the hydrolysis of organophosphate insecticides, nerve agents, aromatic carboxylic acid esters, cyclic carbonate esters, aromatic lactones and alkyl lactones (Billecke *et al.*, 2000); while the PON3 from rabbit serum can also hydrolyze aromatic carboxylic acid esters, cyclic carbonate esters, aromatic lactones and alkyl lactones, but shows less activity against aromatic carboxylic

acid esters (Draganov *et al.*, 2000). In sharp contrast, the AHL-lactonase encoded by *aiiA* from *Bacillus* sp. 240B1 has no or little residue activity to non-acyl lactones and aromatic carboxylic acid esters, but displays strong enzyme activity toward all the tested AHLs, varying in length and nature of the substitution at the C3 position of the acyl chain (Wang *et al.*, 2004). These data suggest that AHL-lactonase is a highly specific enzyme. However, the substrate specificity of other AHL-lactonases, in particular, the enzymes in the AttM cluster sharing low homologies with AiiA, remain to be investigated.

Relatively less is known about the enzymatic mechanism and specificity of AHL-acylases, which break the amide linkage between the fatty acid chain and homoserine lactone moiety. The AHL-acylase encoded by *aiiD* from *Ralstonia* sp. XJ12B is most similar to the aculeacin A acylase from *Actinoplanes utahensis*, and also shares significant sequence similarities with cephalosporin acylases and other N-terminal (Ntn) hydrolases (Lin *et al.*, 2003). Ntn-hydrolases are known to undergo post-translational processing to cleave a primary propeptide into an active, two-subunit form (Brannigan *et al.*, 1995; Oinonen and Rouvinen, 2000). Sequence alignment analysis has shown that AiiD shares the well-conserved key amino acid residues that have been demonstrated to be important to both autoproteolytic processing and catalysis. In addition, site-directed mutagenesis of a few of these key residues has confirmed their essential roles for AHL-acylase activity (Lin *et al.*, 2003). Thus, a similar molecular mechanism of catalysis could be shared between AHL-acylases and Ntn-hydrolases. The AHL-acylases remain to be tested against substrates other than a few AHL signals. AiiD appears to have significantly higher catalytic activity on long chain AHL signals with acyl chains with more than 8-carbons than the shorter chain AHLs with less than 6-carbons (Lin *et al.*, 2003). Further detailed enzymatic and specificity studies should be carried out to characterize this group of interesting enzymes.

The roles of AHL-degradation enzymes in host

AHL-lactobases and AHL-acylases were originally identified because of their activity against AHL signals (Dong *et al.*, 2000; Leadbetter and Greenberg, 2000; Zhang *et al.*, 2002; Lin *et al.*, 2003). All of these known enzymes are of microbial origins (Table 1). Evidence is beginning to accumulate on their roles in microbe-microbe interactions and microbial physiology.

It has recently been shown that *B. thuringiensis* strains, which produce AHL-lactonase, suppress the QS-dependent virulence of the plant bacterial pathogen *E. carotovora* through a new form of microbial antagonism, signal interference, mediated by AHL-lactonase (Dong *et al.*, 2004). *E. carotovora* produces and responds to AHL signals to regulate the antibiotic production and expression of virulence genes; such QS-synchronized functions could be of critical importance for the pathogen in competing for ecological niches in microbe-microbe competition and pathogen-host interactions. Interestingly, the AHL-lactonase producing *B. thuringiensis*, although has no obvious effect on the growth of *E. carotovora*, effectively stops the QS-dependent spreading of the *E. carotovora* cells in plant tissues. In contrast, the *aiiA* mutant of *B. thuringiensis* fails to stop the pathogen QS signaling and the rapid spread of pathogenic bacteria (Dong *et al.*, 2004). Similarly, the expression of AHL-lactonase in isolates of the soil bacterium, *P. fluorescens*, produces a similar effect on the biocontrol of *E. carotovora* (Molina *et al.*, 2003). These data clearly indicate that AHL-lactonase plays a significant role in obtaining competitive advantages for its producer over that of its competitors in natural ecosystems.

Equally fascinating is the role of the AHL-lactonase encoded by *attM* in *A. tumefaciens* QS signal turnover. The QS signal, 3OC8HSL, originally known as conjugation factor, regulates Ti plasmid conjugal transfer in *A.*

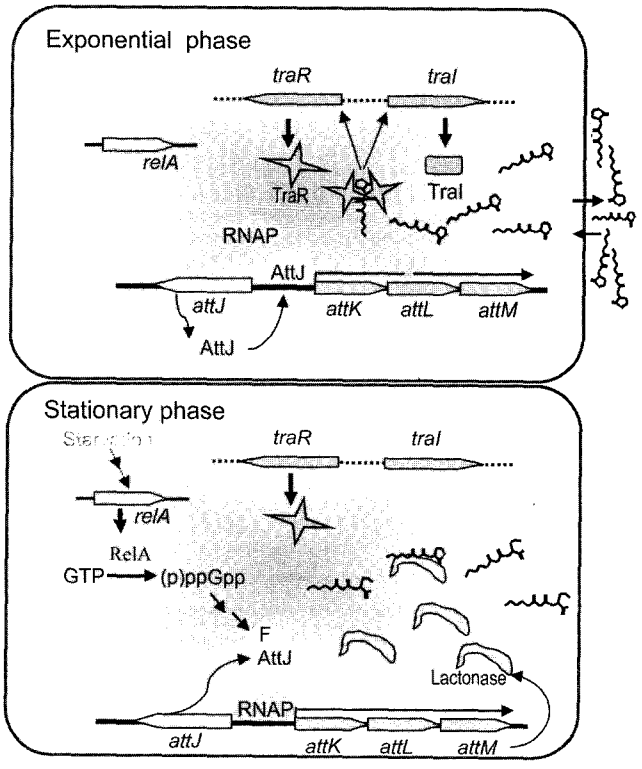

Fig. 4. A model of AHL QS signal turnover in *A. tumefaciens*. During the exponential phase, the occupation of the repressor AttJ to the promoter of the *attKLM* operon prevents the association of RNA polymerase (RNAP); and hence, the expression of the AHL-lactonase encoded by *attM*; the accumulated AHL signals promote the QS-dependent conjugation gene expression in *A. tumefacien* donor cells. During the stationary phase, starvation signal triggers the expression of RelA, producing stress alarmone (p)ppGpp; the later inactivates AttJ through a small ligand (F), allowing binding of the RNAP to the promoter, resulting in the expression of AHL-lactonase (AttM) and elimination of the AHL signals.

tumefaciens (Zhang and Kerr, 1991; Zhang *et al.*, 1993). The production of 3OC8HSL is growth phase dependent; the signal concentration increases following the exponential growth of the bacterial cells, but declines rapidly during the stationary phase (Zhang *et al.*, 2002), which is similar to the pattern of Ti plasmid conjugal transfer. The rapid clearance of the AHL signal is attributed to the expression of AttM (Zhang *et al.*, 2002), which is also growth phase dependent (Fig. 4). During the early stage of bacterial growth, the expression of *attM* is tightly repressed by an IclR-like negative transcriptional factor, AttJ, and AHL signals accumulate following bacterial growth (Zhang *et al.*, 2002). However, during the stationary phase, AttM expression is activated by starvation signals and the stress alarmone (p)ppGpp, resulting in quick degradation of the signal and termination of the QS-dependent Ti plasmid conjugal transfer (Zhang *et al.*, 2004). More recent studies have shown that (p)ppGpp alarmone may induce a factor(s) to inactivate AttJ, resulting in the expression of *attM* for AHL degradation (our unpublished data; Fig. 4). As QS commonly regulates the expression of dozens to hundreds of genes, such a strictly regulated AHL-lactonase-dependent QS signal turnover system may enable *A. tumefaciens* cells to adjust and adapt themselves timely to starvation stress by terminating the energy-consuming conjugation process.

The mammalian paraoxonase family consists of at least three members: PON1, PON2 and PON3. These PON enzymes appear to have multiple protective functions. PON1 was initially identified for its ability to hydrolyze toxic organophosphate. Subsequent investigations have shown that the enzyme has a role in protection against atherosclerosis, by hydrolyzing the derivatives of oxidized cholesterol and phospholipids in oxidized low-density lipoprotein and atherosclerotic lesions (for a review, see Draganov and La Du, 2004). Human PON2 and PON3 lack, or have very limited, activity on organophosphate compounds, but are similar to PON1 in that they both hydrolyze aromatic and aliphatic lactones and have antioxidant properties (Draganov *et al.*, 2000; Draganov and La Du, 2004). The interesting finding that PON enzymes could degrade AHL signals (Chun *et al.*, 2004; Greenberg, meeting report 2004) suggests that these generic hydrolytic enzymes may also contribute to defense against pathogenic invaders. Characterization of their specificity and efficiency in AHL degradation, as well as their expression pattern, would allow for a fair assessment of their roles in pathogen and host interactions.

Biotechnological and pharmaceutical implications of AHL-degradation enzymes

Given that QS deficient mutants of bacterial pathogens are defective in virulence gene expression and become avirulent (Pirhonen *et al.*, 1993; Passador *et al.*, 1993), it might be possible to control bacterial infections by quenching the QS signaling of microbial pathogens. The discovery of quorum quenching enzymes, in addition to quorum sensing inhibitors (for reviews, see Hentzer and Givskov, 2003; Zhang and Dong, 2004), has provided essential tools to assess the feasibility of this novel strategy. The expression of a quorum quenching enzyme, regardless of an AHL-lactonase or AHL-acylase, either in the plant or human pathogens, *E. carotovora* and *P. aeruginosa,* respectively, significantly reduces their virulence (Dong *et al.*, 2000; Reimmann *et al.*, 2002; Lin *et al.*, 2003; Molina *et al.*, 2003). Fig. 5 illustrates highly impressive results; *E. carotovora* or *P. aeruginosa* expressing the *aiiA* gene lost their virulence to infect tobacco or nematode *Caenorhabditis elegans*, respectively. Most excitingly, transgenic plants expressing AHL-lactonase can effectively quench bacterial QS signaling and disintegrate bacterial population density-dependent infections, whereas untransformed control plants develop severe disease symptoms (Dong *et al.*, 2001). These results demonstrate that externally expressed AHL-degradation enzyme is sufficient in eliminating the QS signals of physiological-relevant concentrations and in suppressing the QS-dependent virulence gene expression by pathogens. As the constitutive expression of disease resistant "R" genes might accompany severe yield and biomass penalties, the integration of quorum quenching mechanisms with the inducible plant defense systems could be the most rational way to build

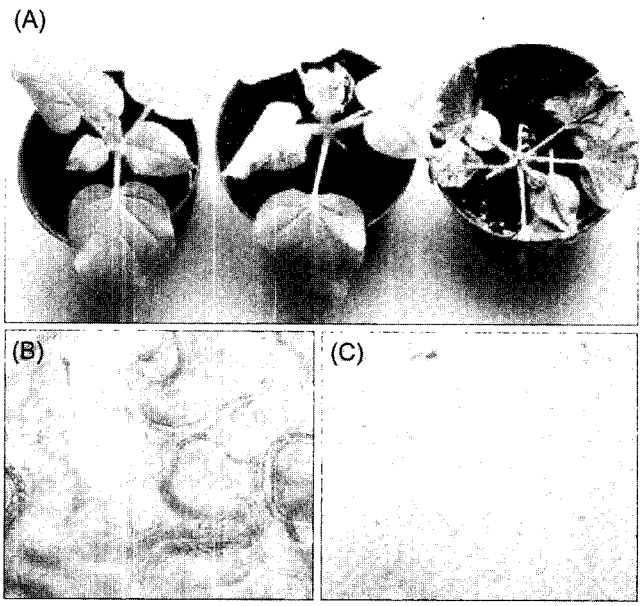

Fig. 5. The expression of *aiiA* encoding the AHL-lactonase in *E. carotovora* and *P. aeruginosa* reduces the pathogenic infections in tobacco and nematodes, respectively. (A) The tobacco plant inoculated with wild-type *E. carotovora* (right), the *E. carotovora* expressing *aiiA* (middle) and water control (left); the photo was taken 2 days after inoculation. (B) Living nematodes on the bacterial lawn of *P. aeruginosa* expressing *aiiA*. (C) Killed nematodes on the bacterial lawn of *P. aeruginosa*. *P. aeruginosa* was grown overnight before the nematode addition. The photos were taken 24 h after inoculation of *C. elegans*.

proactive host defense mechanisms against pathogenic invaders (for a review, see Zhang, 2003). Therefore, the genes encoding these novel quorum quenching enzymes might hold great promise for the genetic engineering of plant disease resistance.

Quorum quenching enzymes could also be explored as a new version of antagonism for the biocontrol of microbial infections. Several natural or engineered AHL-lactonase producing strains, including *B. thuringiensis*, *Arthrobacter* sp. and *P. fluorescens*, significantly reduced potato soft rot when co-inoculated with the pathogen *E. carotovora*, which otherwise causes severe soft rot disease symptoms (Molina *et al.*, 2003; Park *et al.*, 2003; Dong *et al.*, 2004). In contrast, the *aiiA*-deletion mutants of *B. thuringiensis* and the wild-type *P. fluorescens*, which do not produce AHL-lactonase, showed much less or little effect in biocontrol. Antibiotic production has been the major mechanisms of microbial antagonisms commonly exploited in the biocontrol of bacterial and fungal diseases. The finding that QS could be a widely conserved mechanism in the regulation of virulence suggests that quorum quenching mechanisms might have promising potentials in biocontrol.

Interestingly, AHL-degradation enzymes have also been found in AHL-dependent QS bacteria. As discussed in previous sections, the genes encoding AHL-lactonase and AHL-acylase have been identified and characterized from *A. tumefaciens* (Zhang *et al.*, 2002, 2004) and *P. aeruginosa* (Huang *et al.*, 2003), respectively. However, the expressions of these enzymes in the two bacterial species appear to be tightly regulated. For example, the AHL-lactonase encoded by *attM* of *A. tumefaciens* is expressed only when the bacterial cells grow into the stationary phase (Zhang *et al.*, 2002). Further investigation of the molecular mechanisms of genetic regulation may lead to the identification and design of new ways to activate early QS signal degradation, thereby blocking the QS-dependent expression of virulence or virulence-related genes.

It may be premature to discuss the potential implications of quorum quenching enzymes in the context of pharmaceutical applications, as much remains to be done on the enzyme delivery, stability, efficacy, toxicity and side effects. However, as AHL degrading lactonases exist and function in human cells (Table 1), it can be assured that AHL-degradation is not, therefore, a "foreign" function to the human body. Most interestingly, in addition to the degradation of AHL signals, PON enzymes also have other protective functions, as discussed earlier. It would be interesting to determine whether the AHL-lactonases of AiiA and AttM clusters also have similar protective roles against oxidation, and if these quorum quenching enzymes can be developed as generic protective therapeutical proteins.

Conclusion

The exciting findings that many microbial pathogens of agricultural and biomedical importance adopt conserved QS mechanisms to regulate the expression of virulence genes have led to the discovery of various enzymes able to degrade AHL QS signals. Two types of novel and potent AHL-degradation enzymes, *i.e.*, AHL-acylases and AHL-lactonases, including PON-type lactonases, have now been unveiled, but the structural features of AHLs signals suggest that another two types of AHL-degradation enzyme could exist. The wide existence of these quorum quenching enzymes in both prokaryotes and eukaryotes implies potentially important roles for these enzymes in microbe-microbe and pathogen-host interactions. As of now, it cannot for certain be concluded that these AHL-degradation enzymes evolved to degrade AHL signals as their natural substrates, but their impact on AHL-dependent QS bacteria can not be underestimated. These enzymes have been served as important tools for the demonstration of the feasibility of disease control through quenching of the microbial QS signaling, and could also hold great promises in biotechnological and pharmaceutical applications. Although substantial progress has been made in the last few years towards the identification and characterization of these novel AHL-degradation enzymes, more research is needed to gain further insights into the role and regulation of these enzymes in their natural hosts; and importantly, their quorum-quenching efficacy under natural conditions.

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