Quorum Sensing and Quorum-Quenching Enzymes

Yi-Hu Dong¹ and Lian-Hui Zhang¹2,*

¹Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteus, Singapore 138673
²Department of Biological Sciences, The National University of Singapore, 10 Kent Ridge Crescent, 119260, Singapore

(Accepted November 3, 2004)

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 of 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 dodecenolic acid (DSF) (Wang et al., 2004), most of which are involved in the regulation of bacterial virulence (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

* To whom correspondence should be addressed.
(Tel) 65-6586-9686; (Fax) 65-6779-1117
(E-mail) lianhui@imcb.a-star.edu.sg
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 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; Hentzer and Givskov, 2003; Zhang et al., 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, Ralstonia 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 PA01, 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. Phylogenetic 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, K. pneumoniae and Arthrobacter sp., and share about 30-58% homology in their peptide sequences. Amazingly, the AHL-lactonases from...
the two clusters may share less than 25% homology, for example AiiA and AiiM (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., 2000; Draganov et al., 2002; Reimann et al., 2002). Moreover, its biological effectiveness against C4-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.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Gene*</th>
<th>Enzyme</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prokaryotes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus sp. 240B1</td>
<td>aiIA</td>
<td>AHL lactonase</td>
<td>Dong et al., 2000</td>
</tr>
<tr>
<td>B. thuringiensis</td>
<td>aiIA homologues</td>
<td>AHL lactonase</td>
<td>Dong et al., 2002; Lee et al., 2002</td>
</tr>
<tr>
<td>B. cereus</td>
<td>aiIA homologues</td>
<td>AHL lactonase</td>
<td>Dong et al., 2002; Reimann et al., 2002</td>
</tr>
<tr>
<td>B. myoides</td>
<td>aiIA homologues</td>
<td>AHL lactonase</td>
<td>Dong et al., 2002</td>
</tr>
<tr>
<td>B. anthracis</td>
<td>aiIA homologues</td>
<td>AHL lactonase</td>
<td>Ulrich, 2004</td>
</tr>
<tr>
<td>Agrobacterium tumefaciens</td>
<td>attM, aiIB</td>
<td>AHL lactonase</td>
<td>Zhang et al., 2002; Carlier et al., 2003</td>
</tr>
<tr>
<td>Arthrobacter sp. IBN110</td>
<td>ahlD</td>
<td>AHL lactonase</td>
<td>Park et al., 2003</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>ahlK</td>
<td>AHL lactonase</td>
<td>Park et al., 2003</td>
</tr>
<tr>
<td>Variovorax paradoxus VAI-C</td>
<td>ND</td>
<td>AHL acylase?</td>
<td>Leadbetter and Greenberg, 2000</td>
</tr>
<tr>
<td>Ralstonia strain XJ12B</td>
<td>aiID</td>
<td>AHL acylase</td>
<td>Lin et al., 2003; Hu et al., 2003</td>
</tr>
<tr>
<td>Pseudomonas strain PAI-A</td>
<td>pvdQ</td>
<td>AHL acylase</td>
<td>Huang et al., 2003.</td>
</tr>
<tr>
<td><strong>Eukaryotes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (airway epithelia)</td>
<td>PONs</td>
<td>Lactonase</td>
<td>Chun et al., 2003; Greenberg et al., 2004</td>
</tr>
<tr>
<td>Porcine (kidney)</td>
<td>ACY1</td>
<td>Acylase I</td>
<td>Xu et al., 2003</td>
</tr>
</tbody>
</table>

*ND, not determined.*
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 Zn2+ -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 Ca2+ for lactonase activity (Billecke et al., 2000). It is highly likely that PON-type lactonases and AHL-lactonases use different catalytic mechanisms for AHL-degradation. This is consistent with the phylogenetic 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
acids (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 (Brammigan 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. tumefaciens.
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 conjugation 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...
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 therapeutic 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 microbiome and pathogen-host interactions. As of now, it cannot be certain to 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.

**References**


Dong, Y.H., J.L. Xu, X.Z. Li, and L.H. Zhang. 2000. AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia caroto-


Pihonen, M., D. Flego, R. Heikinheimo, and E. Palva. 1993. A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in the plant pathogen Erwinia carotovora. EMBO J. 12, 2467-2476.


