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Review

Quenching of acyl-homoserine lactone-dependent quorum sensing by enzymatic disruption of signal molecules

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Many Gram-positive and Gram-negative bacteria communicate using small diffusible signal molecules called autoinducers. This process, known as quorum sensing (QS), links cell density to the expression of genes as diverse as those associated with virulence factors production of plant and animal pathogens, bioluminescence, antibiotic production, sporulation or biofilm formation. In Gram-negative bacteria, this communication is mainly mediated by *N*-acyl-homoserine lactones (AHLs). It has been proven that inactivation of the signal molecules attenuates many of the processes controlled by QS. Enzymatic degradation of the signal molecules has been amply described. Two main classes of AHL-inactivating enzymes were identified: AHL lactonases which hydrolyse the lactone ring in AHLs, and AHL acylases (syn. AHL amidases) which liberate a free homoserine lactone and a fatty acid. Recently, AHL oxidoreductase, a novel type of AHL inactivating enzyme, was described. The activity of these enzymes results in silencing the QS-regulated processes, as degradation products cannot act as signal molecules. The ability to inactivate AHL (quorum quenching, QQ) might be useful in controlling virulence of many pathogenic bacteria.

Keywords: quorum sensing, quorum quenching, acyl-homoserine lactones, AHL degradation

OVERVIEW OF QUORUM SENSING MECHANISM

Cell-to-cell communication is widely spread in bacteria and controls a broad range of activities (*via* modulation of gene expression) that result in bacterial phenotype changes and better adjustment to environmental conditions during growth (for review see: Swift *et al.*, 1993; Kaiser & Losick 1993; Fuqua *et al.*, 2001; Whitehead *et al.*, 2001; Podbielski & Kreikemeyer, 2004; Reading & Sperandino, 2006; Turovskiy *et al.*, 2007). Quorum sensing (QS), the term introduced in 1994 by Fuqua *et al.* (1994), is an example of cell-to-cell communication and depends on the production, secretion and response to small, diffusible signal molecules also called autoinducers (Kaplan & Greenberg, 1985). The signal molecules are produced and secreted during bacterial growth. Their concentration in the environment increases as the bacterial population expands, and when it reaches a threshold level (quorum level), it induces phenotypic effects by regulating QS-dependent target gene expression. Bacterial QS mechanism is based on two groups of signal molecules: peptide derivatives typical for Gram-positive bacteria, and fatty acid derivatives exploited by Gram-negative bacteria.

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Abbreviations: acyl-ACP, acyl-acyl carrier protein; AHL, *N*-acyl-homoserine lactone; Blc, gamma-butyrolactone catabolism; C4-HSL, *N*-butanoyl-L-homoserine lactone; GBL, gamma-butyrolactone; GHB, gamma-hydroxybutyrate; 3-oxo-C6-HSL, *N*-3-oxo-hexanoyl-L-homoserine lactone; 3-oxo-C8-HSL, *N*-3-oxo-ocatnoyl-L-homserine lactone; 3-oxo-C12-HSL, *N*-3-oxo-dodecanoyl-L-homoserine lactone; QQ, quorum quenching; QS, quorum sensing; SA, succinate; SAM, *S*-adenosylmethionine; SSA, succinic semialdehyde.

QS is omnipresent in many known bacteria species. It has been reported that most of bacteria, not only free-living in various environments but also those associated with higher organisms (symbionts or pathogens), use a sort of quorum sensing mechanism for controlling different 'social' activities.

Many human and plant pathogenic Gram-negative bacteria, including the genera *Agrobacterium, Brucella, Bukholderia, Erwinia, Enterobacter, Pseudomonas, Ralstonia, Serratia, Vibrio* and *Yersinia* (for review see: Fuqua & Greenberg, 2002; Loh *et al.*, 2002; Williams *et al.*, 2007) utilize the QS mechanism for regulation of the virulence factors synthesis. Symbiotic bacteria of legumes belonging to the *Rhizobium* genus mediate their physiological processes connected with nodulation efficiency, symbiosome development as well as nitrogen fixation by means of complex regulatory systems based on signal molecules and QS (Rosemeyeer *et al.*, 1998; Wisniewski-Dye *et al.*, 2002; Marketon *et al.*, 2002; Gonzalez & Marketon, 2003; Hoang *et al.*, 2004). This phenomenon was also observed in extremophilic microorganisms, such as the haloalkaliphilic archeon *Natronococcus occultus* (Paggi *et al.*, 2003) or the acidophilic proteobacterium, *Acidithiobacillus ferrooxidans* (Farah *et al.*, 2005; Rivas *et al.*, 2007).

Gram-positive bacteria have developed cell–to–cell communication as well. Bacteria from the genera *Bacillus, Enterococcus, Staphylococcus, Streptococcus* and *Streptomyces* exploit this mechanism to develop genetic competence, produce antimicrobial peptides or exotoxins and to form biofilm (Kleerebezem *et al.*, 1997; Mayville *et al.*, 1999; Claverys & Havarstein, 2002; Knutsen *et al*., 2004; for review see: Podbielski & Kreikemeyer, 2004).

Nowadays the QS mechanisms of Gram-positive and Gram-negative bacteria are being extensively studied as a new and promising target for reducing bacterial infections in humans, animals and plants.

In this review, we briefly overview the acyl-homoserine lactone (AHL)-based QS mechanism of Gram-negative bacteria and focus on enzymatic degradation of AHLs resulting in silencing of the QS process, named quorum quenching.

AHL-DEPENDENT QUORUM SENSING MECHANISM OF GRAM-NEGATIVE BACTERIA

The best known and understood QS mechanism is the one described for Gram-negative bacteria. This mechanism involves production, secretion and response to the concentration changes of small signal molecules belonging to the *N*-acyl-homoserine lactones (AHLs) family (Eberhard *et al.*, 1981; Gray, 1997; Fuqua *et al.*, 1994; 2001). The signal molecules of the AHL-type contain a homoserine lactone moiety and a fatty acyl side chain which, depending on the type of the signal molecule, varies in length, oxidation state and saturation level of the carbon chain (Fig. 1). For synthesis of AHL, *S*-adenosylmethionine (SAM) and acyl-acyl carrier protein (acyl-ACP) are required (Hanzelka & Greeneberg, 1995).

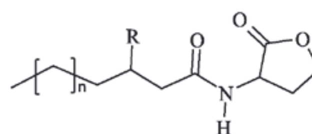


Figure 1. *N*-acyl homoserine lactone (AHL) — the main QS signal molecule of Gram-negative bacteria.
All AHLs are composed of the homoserine lactone ring and the acyl side chain that varies in length, oxidation state and presence of substituents. R – substituent at third carbon in the acyl side chain, hydrogen (H), oxygen (O) or the hydroxy group (HO) in the known AHLs.

An AHL-dependent QS mechanism was first described in the marine bacterium *Vibrio fischeri* (*Photobacterium fischeri*), where it is responsible for bioluminescence in a population density-dependent manner (Nealson *et al.*, 1970; Eberhard, 1972; Kaplan & Greenebrg, 1985). The paradigm of QS, at the molecular level, consists in the activity and cooperation of two components. The first is an AHL synthase (usually LuxI or LuxI homologue) which is responsible for constitutive synthesis of signal molecules (Fuqua & Winans, 1996). The second one is a regulatory protein (LuxR and/or LuxR homologues) which promotes (in most cases) transcription of target genes, when bound with AHL (Fuqua *et al.*, 1994). AHL binding requires three-dimensional changes of the regulatory protein and in turn allows its interactions with specific DNA regions enabling transcriptional activation of target genes (Fig. 2) (Hanzelka & Greenberg, 1995). In most cases AHLs freely diffuse to the surrounding environment; however, AHL molecules with longer acyl side chains (over 10 carbons) are transferred from cells to the environment by an active or carrier-assisted transport system (Pearson *et al.*, 1999).

Similar and homologous QS systems have been described in more than 70 different Gram-negative bacteria species (Bainton *et al.*, 1992; Jones *et al.*, 1993; Pirhonen *et al.*, 1993; Zhang *et al.*, 1993; Milton *et al.*, 1997; Pearson *et al.*, 1997; Cha *et al.*, 1998; von Bodman *et al.*, 2003; and more, for review see: Williams, 2007; Williams *et al.*, 2007). The QS systems are responsible for controlling various activities, e.g. antibiotic production and resistance, conjugation, replication, virulence determinant production, exoenzyme synthesis, swarming, biofilm formation, bioluminescence and many others (Swift *et al.*, 1993;

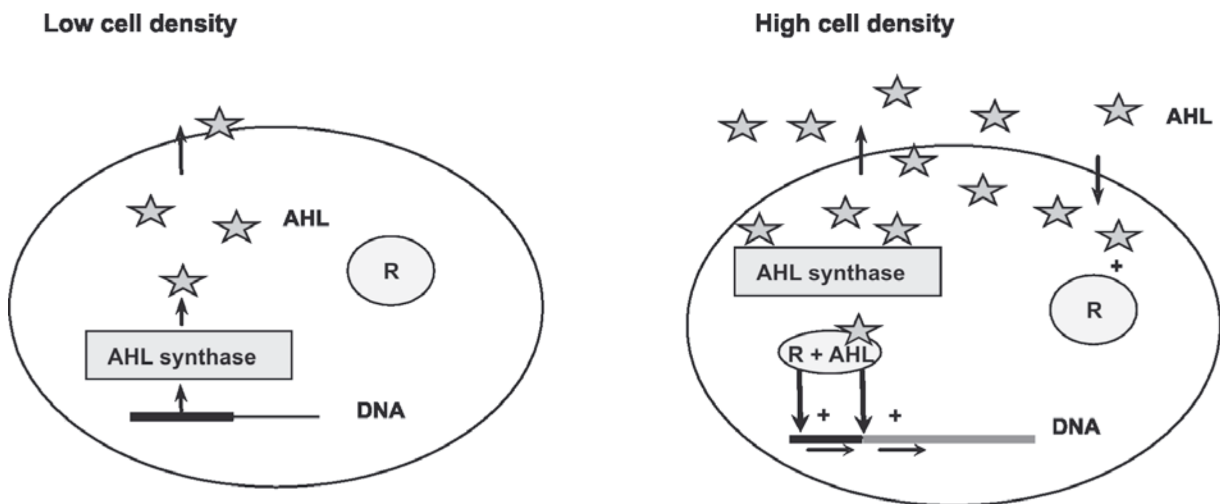


Figure 2. Overview of quorum sensing mechanism exploited by Gram-negative bacteria.
Bacterial cells produce signal molecules — *N*-acyl-homoserine lactones (AHLs) using specific enzyme — AHL synthase. The signal molecules are transferred into the environment and back to the cells by either passive diffusion or active ATP-dependent transport systems (low cell density). With an increasing density of bacterial population the AHLs accumulate in the environment and in the bacterial cells. When, the concentration of AHLs reaches a threshold level (quorum state), the signal molecules interact with the regulatory protein (R). The regulatory protein (R), in most cases, acts as a positive transcription regulator. The complex of R protein and AHL binds to promoters of target genes and initiates their expressions in a cell density-dependent manner (high cell density).

1996; Whitehead *et al.*, 2001; Henke & Bassler, 2004; Reading & Sperandio, 2006).

Abundant AHL-dependent QS systems require a variety of signal molecules. Although various bacterial species produce the same type of AHLs, these molecules are responsible for the regulation of different processes, e.g. 3-oxo-C6-HSL is involved in exoenzyme and carbapanem production by *Pectobacterium carotovorum* (former: *Erwinia carotovora*), exopolysaccharide production of *Pantoea* (former *Erwinia*) *stewartii* and bioluminescence of *V. fischeri* (Eberhard *et al.*, 1981; Bainton *et al.*, 1992; Pirhonen *et al.*, 1993; von Bodman & Ferrand, 1995; von Bodman *et al.*, 1998). Concurrently, bacteria of the same species produce distinct AHLs in strain-dependent manner. Such a phenomenon has been observed for various isolates of *Rahnella aquatilis*. One of the isolates from the tomato rhizosphere produces only 3-oxo-C6-HSL, whereas another one 3-oxo-C6-HSL and 3-oxo-C8-HSL (Steidle *et al.*, 2001). A similar strain-dependent AHLs synthesis has been observed for different strains of *P. carotovorum* subsp. *carotovorum* (Chatterjee *et al.*, 2005; Jafra *et al.*, 2006a) and *Burkholderia cepacia complex* (Gotschilich *et al.*, 2001).

Some QS systems are organized in a hierarchical way and control metabolism at more than one level. This phenomenon was detected in the human pathogen *Pseudomonas aeruginosa* (de Kievit & Iglewski, 2000; Diggle *et al.*, 2002) and the marine bacterium *Vibrio anguillarum* (Milton *et al.*, 1997; 2001). In *P. aeruginosa* two distinct types of signal molecules (the long side-chain signal molecule 3-oxo-C12-HSL and the short side-chain one C4-HSL) are involved in regulation of pathogenicity determinants, such as elastase and protease synthesis or swarming motility (de Kievit & Iglewski, 2000). The synthesis of the short side-chain molecules is dependent on the QS mechanism mediated by the long side-chain one. Such a double QS system assures that targeted genes would be expressed only under very specific environmental conditions and at an appropriate threshold density of bacterial populations. However, detailed studies concerning regulation of synthesis of pathogenicity factors in *P. aeruginosa* cells have revealed that AHL-mediated QS is not the sole mechanism involved in this regulation and the regulation of expression of pathogenicity determinants is more complex (Reimmann *et al.*, 1997; de Kievit & Iglewski, 2000; Diggle *et al.*, 2003).

During the last decade the number of publications concerning the QS mechanism in the bacterial world has been growing rapidly and it is next to impossible to present the complete data here. Instead, our review focusses on presenting only the current state of knowledge concerning quorum quenching (QQ) mechanism of Gram-negative bacteria. For further readings we strongly recommend following the original experimental work carried out on the subject.

INTERFERENCE IN QS

As the QS mechanism, among others, controls virulence factor synthesis in many pathogen-

ic bacteria, it is now generally accepted that the ability to inactivate autoinducers and suppress QS signal generation and/or response might be useful in controlling infection development and persistence of human, animal and plant bacterial pathogens (Mäe *et al.*, 2001; Fray, 2002; Hentzer *et al.*, 2003; Zhang, 2003; Ozer *et al.*, 2005).

Interference in the QS mechanism can be achieved in a variety of ways. First, many natural substances can disturb the signal perception by imitating AHLs structure. The AHL analogues block the AHL receptor (regulator) protein and therefore prevent activation of the target gene expression (Manefield *et al*., 1999). Halogenated furanones produced by red alga *Delisea pulchra* were able to inhibit AHL-dependent carbapenem antibiotic synthesis and extracellular plant cell wall degrading enzyme production in *P. carotovorum* subsp. *carotovorum* (Manefield *et al*., 2001).

Many studies showed that also higher plants produce and secrete secondary metabolites that interfere with the microbial QS systems (Teplitski *et al.*, 2000; Gao *et al*., 2003). During the plant development, different compound that mimic bacterial signal molecules are secreted through the plant root system affecting significantly the rhizosphere bacterial gene expression (Gao *et al.*, 2003). Those active plants compound have not been identified yet, so the mechanism of their interference with bacterial QS remians unknown. Recently, Adonizio *et al.* (2008) reported an inhibitory effect of aqueous extracts of plants *Conocarpus erectus*, *Callistemon viminalis* and *Bucida buceras*, on *P. aeruginosa* QS resulting in the inhibition of virulence factor production. Also here, the mode of action of active compounds is not known, however, their bacteriocidal or bacteriostatic activity has been excluded.

The growing potential of synthetic QS inhibitors is well-documented, too (Reverchon *et al.*, 2002; Wu *et al.*, 2004; Rasmussen *et al.*, 2005; Muh *et al.*, 2006). Synthetic analogues of *N*-acyl-homoserine lactones, such as *N*-acyl-3-amino-5*H*-furanone effectively block LuxR protein preventing cognate signal molecules binding. A review concerning the influence of natural and synthetic analogues of AHLs on QS of Gram-negative bacteria has been recently presented by Geske *et al.* (2008).

Any factor that interferes with the QS of pathogenic bacteria by inactivating of signal molecules and affecting the expression of the target genes, might be an excellent tool for the development of antibacterial therapies. Since the low molecular weigth compounds mentioned above enables inhibition of the QS mechanism, an alternative strategy is offered by AHL-degrading enzymes, which seems to be one of the most potent approaches for silencing QS.

Table 1. AHL-degrading enzymes described for various bacterial species

Bacterial strain	Gene/protein	Protein size (kDa)	Number of amino acids	Enzymatic activity	REFERENCES
Bacillus sp.	*aiiA*/AiiA	28	250	AHL lactonase	Dong *et al.*, 2000; 2002 Wang *et al.*, 2004
B. thuringiensis	*aiiA*/AiiA	28	250	AHL lactonase	Dong *et al.*, 2002 Lee *et al.*, 2002
Arthrobacter sp.	*ahlD*/AhlD	31	273	AHL lactonase	Park *et al.*, 2003
A. tumefaciens	*attM*/AttM	29	264	AHL lactonase	Zhang *et al.*, 2002
A. tumefaciens	*aiiB*/AiiB	nd.	nd.	AHL lactonase	Zhang *et al.*, 2002 Carlier *et al.*, 2003
Rhodococcus sp.	nd.	nd.	nd.	AHL lactonase	Park *et al.*, 2006
R. erythropolis	*qsdA*/QsdA	36	323	AHL lactonase	Uroz *et al.*, 2008
V. paradoxus	nd.	nd.	nd.	AHL acylase	Zhang *et al.*, 2002 Carlier *et al.*, 2003
R. eutropha	*aiiD*/AiiD	84	794	AHL acylase	Lin *et al.*, 2003
P. aeruginosa	*pvdQ*/PvdQ	80	762	AHL acylase	Huang *et al.*, 2003 Sio *et al.*, 2006
P. aeruginosa	*quiP*/QuiP	90	847	AHL acylase	Huang *et al.*, 2006
Anabaena sp.	*all3924*/AiiC	nd.	847	AHL acylase	Romero *et al.*, 2008
Streptomyces sp.	*ahlM*/AhlM	86	804	AHL acylase	Park *et al.*, 2005
Comamonas sp.	nd.	nd.	nd.	AHL acylase	Uroz *et al.*, 2006
R. erythropolis	nd.	nd.	nd.	AHL acylase	Uroz *et al.*, 2005
Acinetobacter sp.	nd.	nd.	nd.	nd.	Kang *et al.*, 2004
Delftia sp.	nd.	nd.	nd.	nd.	Jafra *et al.*, 2006b
Ochrobactrum sp.	nd.	nd.	nd.	nd.	Jafra *et al.*, 2006b

nd., not determined

ENZYMATIC DEGRADATION OF AHLS

Many different bacteria belonging to various genera have been reported to express activity degradating AHLs (Table 1). The chemical structure of AHLs suggests that the degradation of such molecules may occur in four different ways (Fig. 3). Two of them lead to the degradation of the homoserine lactone ring mediated by lactonase or decarboxylase. Two others cause cleavage of AHL to a homoserine lactone and a free fatty acid moiety as reaction products of acylase (syn. amidase) or deaminase (Dong & Zhang, 2005).

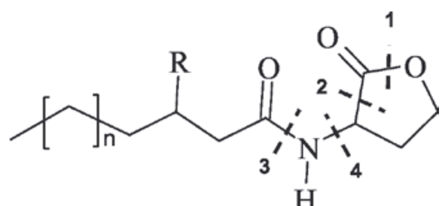


Figure 3. The possible ways of enzymatic degradation of AHLs.
Broken lines mark position of possible cleavages by following enzymes: **1,** lactonase; **2,** decarboxylase; **3,** acylase; **4,** deaminase (according to Dong & Zhang, 2005).

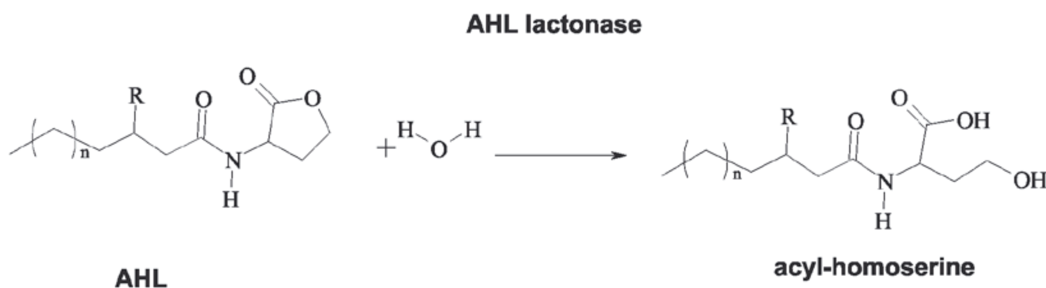
Only two groups of AHL-degrading enzymes of the four mentioned above have been identified so far: AHL lactonases, the second AHL acylases (syn. AHL amidases) [EC 3.1.1.81]. The mechanism of action of AHL acylases is based on the cleavage of the amide carbon–nitrogen bond between fatty acid and homoserine lactone moiety, therefore biochemically the enzyme is an amidase [EC 3.5.1.4] and according to the proper biochemical nomenclature, should be called AHL amidase. However, researchers working on AHL degradation commonly use term AHL acylase for this particular group of enzyms; for this reason in this paper we use this generally accepted name as well.

AHL LACTONASES

AHL lactonases hydrolyze the lactone ring in the homoserine moiety of AHLs, whithout affecting the rest of the signal molecule structure (Fig. 4) (Dong *et al.*, 2000; 2001; Leadbetter, 2001).

The occurrence of AHL lactonases does not seem to be connected with taxonomic classification, as they have been found in poorly related species of bacteria (see Table 1).

One of the first described and the best characterized AHL-lactonases is $AiiA_{24B1}$, the product of the *aiiA* gene from *Bacillus* sp. 24B1 (Dong *et al.*, 2000). At the time of study, the amino-acid sequence of this enzyme showed low similarity to enzymes of known sequence. Yet, the obtained data was sufficient to identify two main amino-acid motifs in the AiiA amino acid sequence (Dong *et al.*, 2000). The first one, ^{104}HLHFDHAG^{111} is characteristic of metal-dependent β-lactamases, whereas the second one, ^{165}HTPGHTPGH^{173}, is similar to the zinc-binding motif typical of metal-dependent hydrolases.



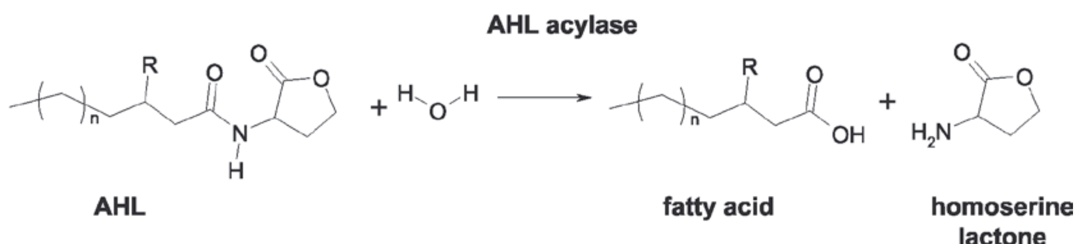


Figure 4. Mechanism of AHL-degrading enzymes.
A. Mode of AHL lactonase action. **B.** Mode of AHL acylase action.

The studies of Wang *et al.* (2004) indicated that this motif does not bind metal ions and represents a new catalytic module responsible for AHL degradation (unlike in metal-dependent hydrolases). The crystal structure of AHL lactonase of *B. thuringensis* subsp. *kurstaki* (AiiA $_{BTK}$) (Kim *et al.*, 2005; Liu *et al.*, 2005) demonstrated, however, two zinc ions in the active centre of the enzyme. Therefore the first indication of Wang *et al.* (2004) suggesting that the AiiA$_{24B1}$ might not be a metalloprotein is rather unlikely. The presence of the 'His^{106}-X-Asp^{108}-His^{109}-59X-His^{169}-21x-Asp^{191}' motif is essential for the enzyme activity and allows classification of the enzymes into the AHL lactonase family. Homologues of AiiA lactonase were discovered in many bacteria belonging to the *Bacillus* genus. All of them show high nucleotide sequence similarity, greater than 90% (Dong *et al.*, 2002; Lee *et al.*, 2002; Kim *et al.*, 2005).

During recent years more bacteria possessing AHL-lactonase activity were described: *Agrobacterium tumefaciens* (Zhang *et al.*, 2002; Carlier *et al.*, 2003), *Arthrobacter* sp. and *Rhodococcus* sp. (Park *et al.*, 2003; 2006) and *R. erythropolis* (Uroz *et al.*, 2008).

The AHL lactonase activity observed in *Arthrobacter* sp. (Park *et al.*, 2003) depends on the *ahlD* gene product. AhlD degrades various AHLs regardless of the length of the acyl side chain or oxidation state (Park *et al.*, 2003). A search of amino-acid sequence databases has revealed that the AhlD amino-acid sequence is in 27% similar to a sequence of *Bacillus stearothermophilus* and in 23% to one of *Klebsiella pneumoniae* (Park *et al.*, 2003). The presence of AHL-degrading activity in cells of these bacteria was confirmed by bioassay for AHL-degradation (Park *et al.*, 2003).

An AttM AHL lactonase was identified in *A. tumefaciens* (Zhang *et al.*, 2002). This bacterium is known to employ QS for cell–to–cell conjugal plasmid transfer (Fuqua & Winans, 1994). The expression of the *attM* gene is growth phase-dependent, and controlled by several different factors (Zhang *et al.*, 2002; 2004). Initially, the control of QS-dependent Ti plasmid transfer by titration of the signal molecules was proposed as the main role of AttM lactonase (Zhang *et al.*, 2002). The *attM* gene is located in the pAt plasmid (Carlier *et al.*, 2003) and its product might be involved in recycling of AHLs molecule, as *A. tumefaciens* C58 derivatives lacking the pAt plasmid excreted a much higher level of the AHL (oxo-C8-HSL) in comparison with the plasmid-bearing C58 strain (Zhang *et al.*, 2002). However, recent work of Chai *et al.* (2007) suggests that AttM plays a more general role in *A. tumefaciens* metabolism. Furthermore, the most recently published work of Khan and Farrand (2009) has excluded the role of AttM lactonase in conjugal Ti plasmid transfer. Both aspects will be discussed further.

According to phylogenetic analyses, all described lactonases and their homologues can be classified into two clusters: the AiiA-like cluster and the AttM-like cluster (Dong & Zhang, 2005). Members of the AiiA-like cluster are more than 90% identical while the AttM-like cluster members show only 30–58% homology in peptide sequence and are less than 25% identical with the AiiA-like cluster members. Recently Uroz *et al.* (2008) reported a novel class of AHL-lactonases from *Rhodococcus erythropolis* strain W2, described later.

AHL ACYLASES

These enzymes hydrolyze the amide bond between the acyl side chain and the homoserine lactone in the AHL molecules generating the free fatty acid and the homoserine lactone (Fig. 4) (Leadbetter & Greenberg, 2000). In most cases degradation products are further metabolized and finally used by the bacteria as carbon, nitrogen or energy sources.

To the present, nine AHL acylases from various groups of bacteria have been reported. One of the first to be described was an enzyme from Gram-negative bacterium *Variovorax paradoxus* VAI-C strain (Leadbetter & Greenberg, 2000). Initial investigations indicated a wide range of AHLs degradation capacity of this bacterium. *V. paradoxus* when grown at the presence of AHL, released into a growing medium a homoserine lactone as a main degradation product while a fatty acid was utilized to generate energy. Untill now, however, the gene responsible for AHL degradation has not been cloned and the enzymatic activity involved in AHL decay by *V. paradoxus* is poorly characterised.

Of the nine AHL acylases reported, five have been described in detail: AiiD from *Ralstonia eutropha* (Lin *et al.*, 2003), PvdQ and QuiP from *P. aeruginosa* (Huang *et al.*, 2003; 2006; Sio *et al.*, 2006), AhlM from *Streptomyces* sp. (Park *et al.*, 2005) and AiiC from *Anabaena* sp. PCC7120 (Romero *et al.*, 2008).

Another well studied, the AHL acylase, AiiD, was found in *R. eutropha* (Lin *et al.*, 2003). AiiD is most similar to aculeacin A acylase from *Actinoplanes utahensis* and shows some sequence homology with cephalosporin acylase and other N-terminal nucleophile (Ntn) hydrolases (Lin *et al.*, 2003). Data presented by Brannigan *et al.* (1995) and Oinonen and Rouvinen (2000) suggested that such N-terminal hydrolases are synthesized as inactive proenzymes. The enzyme precursor undergoes autoproteolysis and becomes a mature active AHL acylase composed of two subunits. AiiD shows high substrate specificity and degrades only AHLs and, above all, exhibits preference for the degradation of long chain AHLs (Lin *et al.*, 2003).

In *P. aeruginosa* a complex QS mechanism is involved in the regulation of pathogenicity factors synthesis, so that determining the AHL-degrading activity was of particular interest. During preliminary studies Huang *et al.* (2003) isolated and analyzed a close homologue of AiiD acylase, PvdQ protein. PvdQ possesses strong preferences for the decomposition of long-chain AHLs (with the acyl side chain longer than 8 carbons) and does not degrade the short ones. PvdQ activity is sufficient for AHLs degradation, although the *pvdQ* negative mutants remains capable of expressing AHLs degrading activity. Such observations have led to the conclusion that *P. aeruginosa* has to express another AHL-degrading factor to enable signal molecule disintegration during its growth. In 2006, the same group and the group of Sio *et al.* (2006) described a second AHL acylase present in *P. aeruginosa,* namely QuiP (the *PA2385* gene). Further study has revealed that QuiP is expressed constitutively during growth and its activity is sufficient to inactivate long-chain AHLs produced by *Pseudomonas*. Mutants in *quiP* gene exhibit growth defects during cultivation in medium supplemented with 3-oxo-C12-HSL as the only carbon and/or nitrogen source (Huang *et al.,* 2006; Sio *et al.,* 2006).

For a long time the presence and activity of AHL acylase was observed and investigated only in Gram-negative bacteria. The first example of such activity in Gram-positive bacteria was revealed in *Streptomyces* sp. (Park *et al.,* 2005). The study resulted in isolation of *Strepotmyces* sp. strain able to degrade AHLs by the secreted AHL-degrading enzyme. The protein encoded by the *ahlM* gene was identified as AHL acylase on the basis of mass spectrometry analyses of the product of AHL degradation. The AhlM acylase is able to degrade not only AHLs but also penicillin G, that suggests a low specificity of this enzyme. Many AHL acylases are subject to post-transcriptional processing from a modified inactive proenzyme into a native, mature enzyme. Heterologous *ahlM* gene expression in *E. coli* showed that the synthesized protein is inactive, does not undergo any processing and is not secreted outside the cell. Therefore the *ahlM* gene was cloned and expressed in *Streptomyces lividans* which, by itself, does not show an AHL-degrading activity. The maturation of AhlM acylase in *Streptomyces* still needs to be carefully investigated.

Most recently, the AHL acylase was described in the cyanobacterium *Anabaena* sp. PCC7120 (Romero *et al.,* 2008). The gene *all3924* whose product showed AHL-degrading activity was cloned and named *aiiC*. The recombinant protein was proven to have the AHL acylase activity by standard tests. Genomic analyses have revealed the presence of *aiiC* sequences in the genomes of various nitrogen-fixing cyanobacteria.

The investigation of the AHL-degrading activity in various bacteria is ongoing. Studies of Uroz *et al.* (2006) have revealed that an amidolytic activity of *Comamonas* sp. strain D1 is involved in AHL decay, but the gene responsible for this activity has not been cloned so far. Interestingly, the AHL-degradation activity of *Comamonas* sp. D1 strain requires active growth of the bacteria as has been also reported for *R. erythropolis* (Uroz *et al.,* 2005; Park *et al.,* 2006). However, in the case of *R. erythropolis* W2 another AHL-degrading activity towards 3-oxo-C12-HSL was observed in the crude cell extract. Studies of *R. erythropolis* strain W2 have revealed the presence of two distinct types of AHL-degrading enzymes (Uroz *et al.,* 2005; 2008), as discussed in the following chapter. Furthermore, our study has shown, that a bacterium from the genus *Ochrobactrum* strain A44 has an ability to degrade AHLs (Jafra *et al.,* 2006b). The AHL lactonase activity was excluded in this case, indicating that this bacterium employs a different type of AHL-degrading enzyme, possibly an AHL acylase. *Ochrobactrum* sp. A44 cells are able to break down a wide spectrum of AHLs with or without an oxo-substituent and varying in the acyl-side chain length.

Summerising, the AHL acylases described to date belong to the N-terminal hydrolase superfamily and are composed of two or more subunits. Usually, four domains may be distinguished in their amino-acid sequences: signal peptide, α-subunit, linear spacer and β-subunit. AHL acylase proenzyme has to be processed into an active enzyme by autoproteolysis (Kim *et al.,* 2000). Only one of the characterized enzymes, AhlM from *Streptomyces* sp., was reported to be secreted outside the bacterial cell, the remaining ones are known to act intracellularly (Park *et al.,* 2005). The genes encoding AHL acylases are widespread in bacteria, both Gram-negative (AiiD from *R. eutropha* (Lin *et al.,* 2003), PvdQ and QuiP from *P. aeruginosa* (Huang *et al.,* 2003; 2006)) and Gram-positive (AhlM from *Streptomyces* (Park *et al.,* 2005)) as well as cyanobacteria *Anabaena* sp. (Romero *et al.,* 2008).

A UNIQUE AHL DEGRADATION SYSTEM IN *RHODOCOCCUS* sp.

A very interesting AHL-degrading system has been found in *Rhodococcus* sp. So far, three various AHL-degrading enzymatic activities have been described in this genus (Uroz *et al.,* 2005; 2008; Park *et al.,* 2006). For the first time, Uroz *et al.* (2003) observed an AHL-degrading activity of

R. erythropolis strain W2 with a preference for 3-oxo-substituted AHLs. The following studies have revealed that 3-oxo-AHLs are converted into their 3-hydroxy-derivatives by means of an oxidoreductase activity (Uroz *et al.,* 2005). This type of AHL degradation was observed only during bacterial growth, but not when cell extract was tested. The oxidoreductase activity is not specific solely to AHLs with a 3-oxo-substituent. This enzyme is also able to reduce AHL derivatives such as *N*-(3-oxo-6-phenylhexanoyl) homoserine lactone (which contains an aromatic acyl chain substituent) or 3-oxododecanamide (which lacks the homoserine lactone ring). Further investigation of AHL decomposition by *R. erythropolis* W2 has resulted in the discovery of another type of enzymatic activity — AHL acylase (Uroz *et al.*, 2005). A year later Park *et al.* (2006) reported a third enzymatic activity involved in AHL decomposition by *Rhodococcus* strains —an AHL lactonase. Two *Rhodococcus* isolates — strains LS31 and PI33 — were able to utilize AHLs, but with different AHL-degrading spectra. The most recent studies of Uroz *et al.* (2008) described the genetic background of the AHL lactonase activity in this genus. The *qsdA* gene, first described for *R. erythropolis* strain W2, encodes a novel class of wide-spectrum substrate AHL lactonases. The QsdA lactonase belongs to the phosphotriesterase (PTE) family of zinc-dependent metalloproteins. It is not related to any known Zn-dependent AHL lactonases from the glyoxylase family, or to any known AHL acylase from the β-lactamase family. The *qsdA* gene was found in almost all tested strains from the *Rhodococcus* genus but not in *R. erythropolis* DCL14, although this strain is still able to degrade AHLs. The PTE lactonase is exclusively found in the *Rhodococcus* genus, in strains capable of hydrolizing AHLs (Uroz *et al.*, 2008). At the moment *Rhodococcus* is the unique species of bacteria which possesses three different types of AHL-degrading activities.

DEGRADATION OF BACTERIAL AHLs IN MAMMALIAN CELLS

The AHL lactonase activity has also been reported in mammalian cells. Eukaryotic lactonases, named paraoxonases (PONs), isolated from human airway epithelia behave in a different way than the previously described bacterial enzymes (Billecke *et al.*, 2000; Draganov *et al.*, 2000; Teiber *et al.*, 2003; Chun *et al.*, 2004). Three types of PONs, including PON1, PON2 and PON3, are known to hydrolyze a wide range of chemicals (Draganov & La Du, 2004). They share 60% of amino-acid sequence identity and require Ca^{2+} ion for activity. Further analyses have shown that PON1, PON2 and PON3 differ in their substrate preferences, the latter two lacking the paraoxonase activity. Still all three enzymes are able to hydrolyze the lactone ring of AHL (Draganov *et al.*, 2005). Studies of Ozer *et al.* (2005) have revealed a strong activity of purified PON1 from human serum against 3-oxo-C12-HSL of *P. aeruginosa*. PONs display the highest degrading activity against long-chain AHL molecules, such as 3-oxo-C12-HSL and are less effective with short-chain AHLs (Chun *et al.*, 2004; Yang *et al.*, 2005). As 3-oxo-C12-HSL plays an important role in QS of *P. aeruginosa,* a PON activity towards this molecule indicates the importance of these enzymes in host cell protection against microbial infection. The observation of a PON AHL-degrading activity in serum of several mammal supports this hypothesis.

SUBSTRATE PREFERENCES OF AHL-DEGRADING ENZYMES

The AHL-degrading bacteria usually show some degree of substrate preference. These preferences are a direct result of the AHL-degrading enzymes amino-acid structure and/or co-factor involvement. Also the occurrence of different types of AHL produced by the various bacteria present in the natural environment influence the substrate preferences (Huang *et al.*, 2003; 2006; Carlier *et al.*, 2003; Lin *et al.*, 2003).

Most AHL lactonases known which hydrolyse the homoserine lactone ring do not display any preferences for the length of the carbon acyl side chain (Wang *et al.*, 2004; Dong & Zhang, 2005). Good examples are the AiiA and AhlD lactonases from *Bacillus* sp. (Dong *et al.*, 2000; Wang *et al.*, 2004; Lui *et al.*, 2005; Kim *et al.*, 2005) and *Arthrobacter* sp. (Park *et al.*, 2003), respectively. The AhlD lactonase from *Arthrobacter* sp. is able to degrade a great variety of AHLs, including C4-HSL (Park *et al.*, 2003). The same was observed for *Rhodococcus* sp. AHL lactonases (Uroz *et al.*, 2005; Park *et al.*, 2006).

Among AHL acylases, only one example of broad range AHL-degrading enzyme has been described so far (Romero *et al.*, 2008). The AiiC acylase from *Anabaena* sp. can hydrolyze a set of AHLs, that differ in the acyl chain length and substitution, including C4-HSL, however, it shows preference for long-chain AHLs (C10-HSL and above).

Such a broad degradation ability is not usually observed in the case of other known AHL-degrading enzymes which usually show preference for one particular signal molecule or a specific group of AHLs. QuiP and PvdQ, the AHL acylases from *P. aeruginosa* active exclusively against 3-oxo-C12-HSL

(Huang *et al.*, 2003; 2006) are excellent examples. Some substrate preference has been observed in *R. eutropha* producing AiiD, the AHL acylase which only degrades unsubstituted and 3-oxo-substituted AHLs with acyl side chains at least 8 carbons long (Lin *et al.*, 2003). AHL-degrading bacteria, even if their AHL-degrading enzymes do not exhibit preference for the AHL side chain length, usually break down AHLs with the acyl side chains containing at least 6 carbons (Uroz *et al.*, 2003; Zhang 2003; Dong & Zhang 2005; Jafra *et al.*, 2006b).

LOCALIZATION OF AHL-DEGRADING ACTIVITY

Most of the AHL-utilizing bacterial strains were reported as soil or rhizosphere inhabitants (Lee *et al.*, 2002; Huang *et al.*, 2003; Wang *et al.*, 2004; 2006; Park *et al.*, 2005; Yoon *et al.*, 2006). Their living conditions and nutrient availability are functions of the occupied soil environment. This environment shows large variability in the availability of nutrients, water, vital ions, and oxygen. Such an unstable environment would not usually create optimal conditions for the AHL-degrading enzymes. Therefore intercellular AHL-degrading enzymes which act in fairly constant, optimal reaction conditions and are isolated from the external environmental appeared in evolution and predominate in bacteria (Leadbetter & Greenberg, 2000; Dong *et al.*, 2000; 2001; 2002; Leadbetter, 2001; Zhang *et al.*, 2002; Carlier *et al.*, 2003; Park *et al.*, 2003; Huang *et al.*, 2003; Lin *et al.*, 2003; Molina *et al.*, 2003; Uroz *et al.*, 2003; 2005; Wang *et al.*, 2004; Liu *et al.*, 2005; Kim *et al.*, 2005; Jafra *et al.*, 2006b).

Both AHL lactonases and AHL acylases were reported to be cytoplasmic, although in the case of the recently reported AiiC acylase from cyanobacteria (Romero *et al.*, 2008) the predicted secondary structure of a putative transmembrane domain in the N-terminal region indicates its periplasmic localization. The cytoplasmic, intracellular localization of AHL-degrading enzymes suggests that extracellular degradation is rather an exception. And the first example of the extracellular AHL-degrading enzyme in the literature is the AhlM acylase of *Streptomyces* sp. (Park *et al.*, 2005).

THE ROLE OF SIGNAL MOLECULE-DEGRADING ENZYMES

The examples of AHL-degrading activities described above show that QQ enzymes are wide spread in the natural environment. The reason why bacteria have developed the system allowing them to degrade AHLs is an interesting subject of research. Some data indicate that the AHL degradation products may serve as a source of nitrogen or/and carbon and an energy reservoir (Leadbetter & Greenebrg, 2000; Huang *et al.*, 2003; Park *et al.*, 2003; Sio *et al.*, 2006; Yang *et al.*, 2006; Yoon *et al.*, 2006; Chai *et al.*, 2007). Such a situation is observed for *V. paradoxus* which utilizes the fatty acid released from AHL as an energy source (Leadbetter & Greenberg, 2000). *Arthrobacter* sp. expressing AhlD lactonase (Flagan *et al.*, 2003; Park *et al.*, 2003; Yang *et al.*, 2006) and the soil-borne *Burkholderia* strains (Yang *et al.*, 2006) are other examples. The soil bacterium *Nocardioides kongjuensis* also uses the AHL degradation products as a carbon source (Yoon *et al.*, 2006).

Another case is *P. aeruginosa* expressing the PvdQ enzyme (Huang *et al.*, 2003; Sio *et al.*, 2006). One of the AHL degradation products is toxic for *P. aerugionosa* and as such has to be degraded effectively. The other product of AHL degradation, a fatty acid, is utilized by this bacterium as a source of energy (Huang *et al.*, 2003). The PvdQ AHL acylase thus secures an additional carbon and energy source from AHL hydrolysis when the bacteria grow in a nutrient-poor environment. This AHL acylase synthesis is induced or repressed under the control of environmental conditions (Huang *et al.*, 2003). Additionally, *P. aeruginosa* synthesizes another AHL acylase named QuiP which controls QS-dependent virulence processes (Huang *et al.*, 2006). The QuiP activity is sufficient for AHL utilization during *P. aeruginosa* growth, both in natural environments and in *in vitro* tests. Unlike PvdQ, the QuiP is expressed constitutively (Huang *et al.*, 2003; 2006).

Expression of signal molecule-degrading enzymes could also ensure success in competition for the limited natural resources, as has been reported in the case of *Bacillus thuringiensis* and *Pectobacterium carotovorum* mutual interactions. *B. thuringiensis* does not produce factors that could affect the *P. carotovorum* growth but biosynthesis of AiiA lactonase by *B. thuringiensis* significantly reduces *P. carotovorum* adaptability in the environment. *Bacillus* strains without an AHL-degrading activity do not exhibit such ability (Dong *et al.*, 2004). This example leads to the conclusion that switching the QS systems on and off by various AHL degraders might play a great role in the environment in mutual, both symbiotic and pathogenic, bacterial interactions.

Recently, Park *et al*. (2008) revealed that the AiiA lactonase of *B. thuringensis* is essential for rhizosphere colonization by this bacterium and its survival in the soil. A mutant defective in AiiA lactonase synthesis was unable to successfully colonize the test plants' rhizosphere and its viability was significantly reduced. The limited persistence of *B. thuringiensis aiiA* mutant in natural for *B. thuringnesis* niche was demonstrated. This finding sug-

gested that AiiA lactonase might be involved in microbial competitivenss in the rhizosphere and helps bacteria to survive on the plant root. These authors also suggested that AiiA lactonase can participate in bacterial metabolism.

An interesting concept explaining the role of AHL degradation in the environment has been posted by Kaufmann *et al.* (2005). The 3-oxo substituted AHLs are subject to an unusual Claisen-like condensation reaction resulted in the tetramic acid derivatives formation. Both, the 3-oxo AHLs and their nonenzymatically-formed products possess bacteriocidal activity towards Gram-positive, but not Gram-negative bacteria. These tetramic acid derivatives are also able to complex metal ions (e.g., iron) important for bacterial growth in natural environment. This finding supported the hypothesis that AHL-degrading enzymes of Gram-positive bacteria, such as AHL lactonases from *Bacillus* evolved in order to control the antibacterial activity of AHLs and to enhance bacterial survival in natural environment.

Another example of the use of AHL-degrading enzymes by bacteria comes from *A. tumefaciens*. The AttM AHL lactonase from this bacterium has been proposed to help control the conjugation and Ti plasmid transfer between members of its own population (Zhang *et al.*, 2002; Carlier *et al.*, 2003). For conjugation a high concentration of AHLs is required. It is essential to complete conjugation while the population is still expanding. At the stationary phase the number of bacteria does not change and conjugation, even if it occurs, does not ensure further Ti plasmid transfer to new cells. Expression of the *attM* gene is negatively regulated by the AttJ repressor, which is switched off when *A. tumefaciens* population enters to the stationary phase (Zhang *et al.*, 2002). Taking this into acount, it was suggested that time-dependent AttM synthesis and activity lead to the degradation of autoinducers and inhibition of Ti plasmid conjugation (Zhang *et al.*, 2002). Another explanation for the role of AttM AHL-degrading activity in *A. tumefaciens* cells was raised by Chai *et al.* (2007). The AHL-degrading enzymes, such as AttM, evolved by selection for degradation of the compounds found in the environment, and the AHL degradation is purely coincidental. Chai *et al.* (2007) confirmed biochemically the role of the *attKLM* operon in the conversion of γ-butyrolactone (GBL) to γ-hydroxybutyrate (GHB) then to succinic semialdehyde (SSA) and finally to succinate (SA) which was previously observed by Carlier *et al.* (2003). The final product of this metabolic pathway, SA, enters the tricarboxylic acid cycle of the bacterium. The results showed, that *attM* expression is not induced by the presence of any type of AHL, but is strongly induced by GHB and/or SSA. This finding indicated that the AttM enzyme evolved by selection for substances other than AHLs and AHL degradation is a side effect. Further studies revealed that some plant seedling exudates, GHB-like compounds, which might induce the transcription of *attM* by releasing AttJ from the gene promoter, interfere in cell–to–cell signaling mediated by AHLs (Chai *et al.*, 2007).

Recently published studies of Khan and Farrand (2009) addressed the question of the role of AttM lactonase (named by those authors BlcC for γ-butyrolactone catabolism) in conjugative transfer of Ti plasmid of *A. tumefaciens*. The *blcC* mutatation did not affect the AHL signal molecules accumulation or the conjugal ability of these bacteria in plant tissue (Khan & Farrand, 2009). These findings counter the previous hypothesis of the AttM (BlcC) lactonase role in controling Ti plasmid transfer, support the concept of 'accidental' degradation of AHL and prove the major activity of BlcC in degradation of plant-generated GHB-like compounds.

One might conclude that the AHL-degrading enzymes can play different biological role in the various bacteria producing them. Most recent studies indicate, that this role is not always dedicated specifically to AHL-degradation but aids the bacteria to benefit or/and survive in the natural environment.

PERSPECTIVES OF QQ APPLICATION

The biotechnological applications of AHL degradation as a new, promising method for fighting detrimental bacteria have foccused researchers' attention. As many of the human and plant bacterial pathogens employ the AHL-based QS mechanism for regulation of the pathogenicity determinant synthesis or biofilm formation, the application of QQ strategy may be an alternative approach for fighting these microorganisms.

Fighting bacterial plant pathogens remains difficult and ineffective. The most extensive studies of QQ application have been performed on the plant pathogenic bacteria from the former pectolytic *Erwinia* genus. These bacteria, which nowadays are classified into three different species (*Pectobacterium atrosepticum, P. carotovorum* subsp. *carotovorum* and *Dickeya* sp.) are the causative agents of soft rot diseases of many economically important plants. They are also responsible for the blackleg disease of potato plants in temperate climate (Pérombelon & Kelman, 1980; Pérombelon, 2002; Ma *et al.*, 2007; de Haan *et al.*, 2008). As the AHL-dependent QS mechanism is involved in bacterial pathogenesis (for review see: Whitehead *et al.*, 2002), QS interference strategies

are proposed as a new, alternative approach to the attenuation of bacterial infection development in plants. The first strategy relies on introduction of a gene coding AHL synthase directly to the plant cells and its stable expression in the plant tissue (Fray *et al.*, 1999; Mäe *et al.*, 2001; Fray, 2002; Toth *et al.*, 2004); the second one employs AHL-degrading bacteria for plant protection against *Pectobacteria* (Uroz *et al.*, 2003; 2005; Dong *et al.*, 2004; Jafra *et al.*, 2006b). Yet another approach involves heterologous expression of genes encoding AHL-degrading enzymes in pathogen cells or in plant tissue (Dong *et al.*, 2001). The success of preliminary research has enabled a new and alternative strategy for controlling bacterial infections.

As bacterial populations use signal molecules to sense cell density and coordinate their own behavior, the artificially increased level of AHLs (due to expression of AHL-synthase gene in the plant tissue) makes bacteria to misinterprete the population size. Such misinterpretation leads to the production of virulence determinants long before the pathogen population is large enough to sustain infection in the plant which in turns would enable the pathogens invasion to be overcome (Mäe *et al.*, 2001). However, Toth *et al.* (2004) reported that AHL production by transgenic plants not only can induce bacterial infection development but also makes the plants more sensitive and susceptible to infection caused by pectolytic *Erwinia* (*Pectobacteria*). This is in contradiction to earlier results obtained by Mäe *et al.* (2001).

Application of bacterial cells producing AHL-degrading enzymes prevents maceration of plant tissue by the tested pathogens (Uroz *et al.*, 2003; 2005; Jafra *et al.*, 2006b). This strategy gives the opportunity for direct application of AHL-degrading bacteria as biological control agents of plant bacterial diseases.

Transgenic potato and tobacco plants expressing the gene encoding AiiA lactonase manifested strong resistance against infection by *P. carotovorum* subsp. *carotovorum* (Dong *et al.*, 2001). This was the first example of purposeful usage of the AHL-degrading enzyme for attenuation of infection symptoms development in plants, however, this strategy requires the genetic modification of higher organisms which is not universally accepted.

Introduction of genes encoding AHL-degrading enzymes resulted in a decrease of the AHL level in the surrounding environment and hampered the ability of *P. carotovorum* to prolong infection (Dong *et al.*, 2000). Heterologous expression of the *aiiA* gene encoding the AiiA lactonase from *Bacillus* sp. in *P. carotovorum* cells, impedes production of exoenzymes, and disease symptoms development. A similar approach was used for two other important plant pathogens *Burkholderia thailandensis* and *Erwinia amylovora* where AHL lactonase encoded by *aiiA* gene homologues was expressed (Ulrich, 2004; Molina *et al.*, 2005). The same approach was employed to study the silencing of the hierarchical QS system of *P. aeruginosa*. In this case, it resulted in reduction of signal amount of the first QS system (3-oxo-C12-HSL) and in consequence prevented accumulation of the second QS system signal molecule (C4-HSL) (Reimmann *et al.*, 2002). The pathogenicity factor synthesis and swarming motility were markedly reduced. However, the fading of the signal did not influence the bacterial cell adhesion capacity and did not interfere with surface colonization by these bacteria. Park *et al.* (2005) demonstrated that addition of purified AhlM protein to the growth medium of *P. aeruginosa* eliminated 3-oxo-C12-HSL and reduced and delayed C4-HSL accumulation and strongly decreased virulence factor synthesis.

The above examples show that, the AHL-degrading enzymes together with QS inhibitors may be successfully used to disrupt bacterial cell to cell communication and to control bacterial infections.

INFLUENCE OF QQ ON SOIL ENVIRONMENT

For the reason, that a signal molecule degradation does not always lead to the suppression of negative effects and sometimes the benefits do not outweigh the risks, the subject needs further and deeper studies. This can occur, especially in the natural environment of the soil, where *Rhizobium, Sinorhizobium* and *Bradyrhizobium* use the AHL-mediated QS mechanism for controlling plant nodulation. Nodulation is important not only for plants but also in agriculture to increase the crop production in areas of poor soil nitrogen availability as this process is the only possibility to bind air-borne nitrogen, transform it into inorganic salts and ensure its utilization. Nodulation cannot occur if the QS is suppressed in these bacteria (Rosemeeyer *et al.*, 1998; Marketon & Gonzalez, 2002; Hoang *et al.*, 2004; for review: Gonzalez & Keshavan, 2006).

The soil-borne bacterium *Pseudomonas aerofaciens* competes with soil fungi from the genus *Fusarium* by quorum sensing-dependent and AHL-based production of an antifungal antibiotic phenazine which suppresses *Fusarium* growth. *P. aerofaciens* is used as a protective agent against *Fusarium* infections in plants (de Boer, 2000). Since phenazine can not be synthesized in an AHL-deficient environment, AHL degradation could cause problems rather than bring benefits in this particular case (Whistler & Pierson, 2003).

FINAL REMARKS

Numerous reports show that bacteria become resistant to a great variety of antibiotics, which results in the reduction in the potential use of pharmaceuticals and their availability for humans. Such observations lead straightforwardly to the conclusion that new therapies, alternative to antibiotic usage, ought to be invented in the near future.

QS inhibition by enzymatic degradation of signal molecules is one such option. It has been reported that most, if not all bacteria, both Gram-negative and Gram-positive, use a sort of QS for controlling population-dependent behavior. Therefore even if the signal molecules exploited in these processes have many distinct structures, it is usually possible to find an enzyme which could destroy any one of them.

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