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REVIEW PAPER

Quorum sensing as a target for developing control strategies for the plant pathogen *Pectobacterium*

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Abstract Quorum sensing is a regulatory mechanism that connects gene expression to cell density in bacteria. Amongst proteobacteria, numerous functions are regulated in this way, including pathogenicity in the *Enterobacteriaceae* genus *Pectobacterium*. In *Pectobacterium*, the signalling molecules involved in this regulatory process belong to the N-acyl-homoserine lactone class. Over the last 6 years, various studies have shown that these signal molecules could be degraded by other bacteria or by plant and animal cells, opening the path to innovative biocontrol strategies. This review explores the various determinants of pathogenicity in *Pectobacterium* and describes approaches that have been developed to quench the quorum-sensing-dependent pathogenicity in *Pectobacterium*. These approaches range from signal degradation by physicochemical constraints to the identification of signal-sensing inhibitors and from the identification of enzymes degrading acyl-homoserine lactones to the construction of transgenic plants tolerant to *Pectobacterium*.

Keywords *Erwinia* · *Pectobacterium* · N-acyl-homoserine lactone · Quorum · Quenching · Virulence

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Abbreviations

3O,C6-HSL	3-Oxo,hexanoyl-homoserine lactone
3O,C12-HSL	3-Oxo,dodecanoyl-homoserine lactone
C4-HSL	Butyroyl-homoserine lactone
C6-HSL	Hexanoyl-homoserine lactone
C12-HSL	Dodecanoyl-homoserine lactone
C14-HSL	Tetradecanoyl-homoserine lactone
GABA	γ-Aminobutyrate
GBL	γ-Butyrolactone
GHB	γ-Hydroxybutyrate
SSA	Succinic semialdehyde
N-AHSL	N-acyl-homoserine lactone
PCWME	Plant-cell-wall-macerating enzymes
PON	Paraoxonase
QS	Quorum sensing
QQ	quorum quenching
SAM	S-adenosyl-methionine
TTSS	Type III secretion system

Introduction

Quorum sensing (QS) regulation

Bacteria have evolved sophisticated mechanisms to coordinate gene expression at population and community levels. For instance, gene expression may depend upon the perception of diffusible molecules that are synthesized by bacterial populations and communities. Because the concentration of the emitted signal in a

confined environment reflects the bacterial cell number and density, such a regulatory pathway was termed 'quorum sensing' (QS) (Fuqua et al. 1994). In an open environment, however, the concentration of the signal reflects the bacterial cell number and the signal diffusion coefficient. In such open environments, the term 'diffusion sensing' was proposed (Redfield 2002). Specific signal sensors and transcriptional factors, the activities of which are modulated by the concentrations of the emitted signals, are involved in QS.

The signal molecules

The structures of QS signals are highly diverse (Whitehead et al. 2001; Waters and Bassler 2005). Oligopeptides and substituted gamma-butyrolactones have been described in Gram-positive bacteria, while other substituted gamma-butyrolactones, the N-acyl-homoserine lactones (N-AHSLs), are synthesized by a large number of Gram-negative bacteria. In this latter bacterial group, 3-hydroxypalmitic acid methyl ester (Flavier et al. 1997), 3,4-dihydroxy-2-heptyl-quinoline (Holden et al. 1999), and a furanosyl borate diester (Chen et al. 2002) can also act as QS signals. Among Gram-negative bacteria, the most common QS signals are N-AHSL (Greenberg 2000; Fuqua et al. 2001; Whitehead et al. 2001). The synthesis of N-AHSL depends upon synthases belonging generally to two classes: the LuxI homologs and the AinS homologs (Fuqua and Greenberg 2002). The perception of the signal relies upon a sensor protein, a LuxR homolog, which is also the transcriptional regulator controlling the expression of QS-regulated genes (Fuqua and Greenberg 1998).

Quorum quenching (QQ)

The term quorum quenching (QQ) encompasses various natural phenomena or engineered procedures that lead to the perturbation and—eventually—the attenuation of the expression of QS-regulated functions (for recent reviews see: Dong and Zhang 2005; Rasmussen and Givskov 2006). Three main steps of the QS regulation could be targeted: the signal synthesis, the stability of signal, or the sensing of the signal. So far, QQ strategies have only dealt with the production, the accumulation or the perception of signals belonging to the N-AHSL class. In theory, however, similar strategies could be developed for QS processes relying upon other molecules. Because QQ targets the expression of virulence functions and does not affect the viability of bacterial pathogens, QQ falls into the family of anti-virulence/anti-disease strategies.

The synthases as targets

Few compounds have been identified as potential inhibitors of bacterial N-AHSL production. N-AHSL synthesis proceeds from S-adenosyl-methionine (SAM) and a fatty acid, linked to an acyl carrier protein. Amongst the synthase inhibitors, the two SAM analogues L-*S*-adenosylhomocysteine and sinefugin (an S-adenosyl-methionine-like antibiotic; Geze et al. 1983) were the most efficient (Parsek et al. 1999). However, other potential targets exist, such as proteins implicated in the synthesis of N-AHSL precursors, SAM or fatty acids. In agreement with this suggestion, mutants of *P. syringae* pv. *tabacci* affected in the acyl-(acyl carrier protein) synthase exhibited a phenotype similar to a *luxI* mutant (Taguchi et al. 2006). The bactericidal molecule triclosan—targeting the enoyl-acyl carrier protein reductase FabI—also affects the synthesis of N-AHSL (Hoang and Schweizer 1999). Whatever the target, this type of approach affects key metabolic compounds in bacteria. It is therefore likely to impair both QS-regulated functions and functions other than those regulated by QS in bacteria, a major drawback in the development of potential, specific inhibitors. Such bactericidal compounds therefore cannot be categorized as anti-virulence molecules.

The sensor as a target

The regulatory proteins of the LuxR family that senses N-AHSL have also been proposed as potential targets for QQ. Such a mechanism occurs in nature. For instance, the red alga *Delisea pulchra* limits bacterial colonization (fouling) by interfering with the QS-controlled motility and biofilm-formation ability of bacteria (Rasmussen et al. 2000). This process is mediated by halogenated furanones produced by the algae (Givskov et al. 1996). These molecules bind the LuxR receptor of potential bacterial colonizers, prevent the binding or displace

the N-AHSL signal (Manefield et al. 1999), and accelerate the degradation of the LuxR protein (Manefield et al. 2002). Similar phenomena have been observed in another alga, *Chlamydomonas reinhardtii*, that produces over a dozen compounds which, most likely, are not furanones and inactivate N-AHSL-mediated QS functions in bacteria (Teplitski et al. 2004). Other inhibitors have been found in plants and more generally in bioproducts. Thus, pea and soybean (Teplitski et al. 2000), *Medicago* (Gao et al. 2003), fruit extracts such as those from grape and strawberry (Fray 2002), garlic (Rasmussen et al. 2005a), vanilla (Choo et al. 2006), lily and pepper (Rasmussen and Givskov 2006), *Clematis vitalba*, *Geranium molle*, and *Tropaeolum majusi* (Karamanoli and Lindow 2006) produce molecules that inhibit QS in bacteria. So far, only a few active molecules have been identified. In garlic, disulfur compounds with QS-antagonistic activity have been reported (Rasmussen and Givskov 2006). In other plant extracts, as in *D. pulchra*, furanones may be involved in the inhibition of QS. These molecules are major constituents of the fruity or spicy aromas of several plant products (Colin Slaughter 1999).

Fungi also produce inhibitors of QS. A screen of 50 *Penicillum* species revealed that about 50% produced inhibitors, two of these being identified as the lactones patulin and penicillic acid (Rasmussen et al. 2005b). Interestingly, patulin naturally occurs in fruits such as apple, pear, peach, apricot, banana, pineapple, and grape (Scott et al. 1972; Frank 1977), where the compound may also contribute to the inhibition of QS.

Aside from the investigations on natural inhibitors, efforts have been made to identify or design chemical compounds that may target the LuxR-like receptor(s). Most of the designs of inhibitors were based on actual structures of the N-AHSL molecules. These studies led to the identification of analogues with either activating or inhibitory activity (Reverchon et al. 2002; Castang et al. 2004; Smith et al. 2003a). Amongst these latter molecules, phenyl-acyl- and chlorophenyl-acyl-homoserine lactone appear to be the most potent inhibitors. Random screening has also permitted the identification of QS inhibitors such as 4-nitro-pyridine-N-oxide, aniline derivatives, N-methyl-iminocycloheptane, N-methyl-N-iminopyrrolidine and complex heterocycles such as ursolic acid (Smith et al. 2003b; Rasmussen et al. 2005a; Ren et al. 2005).

The N-AHSL signals as targets

QQ may also rely upon signal degradation. N-AHSLs being lactone molecules, they are susceptible to lactonolysis, i.e. the opening of the lactone ring under alkaline pH conditions. The resulting compounds, the cognate N-acyl-homoserines, are not recognized as QS signals by bacteria. Alkaline lactonolysis, a chemical reaction, is subject to the Arrhenius law and is therefore temperature-dependent. Both dependences have been demonstrated in vitro (Byers et al. 2002; Yates et al. 2002; Delalande et al. 2005) and most likely occurs *in planta*. Indeed, several elicitors produced by plant pathogenic bacteria induce a multifaceted plant cell response, one aspect of which is a pH increase (e.g. Bourque et al. 1998). Other signals such as bacterial toxins provoke a similar transient pH increase (Boller 1995) that appears to be a key component of the plant defence systems, related to the expression of defence genes as reported in tomato (Schaller and Oecking 1999).

Most of the QQ studies have dealt, however, with the biological degradation of N-AHSL, first observed in bacteria such as *Variovorax* (Leadbetter and Greenberg 2000) and *Bacillus* (Dong et al. 2000). Since these early reports, numerous bacteria inactivating N-AHSL have been identified. Some dissimilate N-AHSL, i.e. use these substrates as growth substrates; some do not. To date, N-AHSL inactivation has been described in α-proteobacteria, e.g. *Agrobacterium*, *Bosea*, *Sphingopyxis* and *Ochrobactrum* (Zhang et al. 2002; Carlier et al. 2003; D'Angelo-Picard et al. 2005; Jafra et al. 2006), β-proteobacteria, e.g. *Variovorax*, *Ralstonia*, *Comamonas*, and *Delftia* (Leadbetter and Greenberg 2000; Lin et al. 2003; Uroz et al. 2003; Jafra et al. 2006), and γ-proteobacteria, e.g. *Pseudomonas* and *Acinetobacter* (Uroz et al. 2003; Huang et al. 2003; Kang et al. 2004). N-AHSL inactivation also occurs in Gram-positive strains, both amongst low-G + C% strains or firmicutes such as *Bacillus* (Dong et al. 2000, 2002; Fray 2002; Lee et al. 2002; D'Angelo-Picard et al. 2005) and high-G + C% strains or actinobacteria, e.g. *Rhodococcus*, *Arthrobacter*, and *Streptomyces* (Uroz et al. 2003; Park et al. 2003, 2005, 2006).

In bacteria, the N-AHSL-inactivating enzymes described to date belong to two enzymatic families: the N-AHSL lactone hydrolases (e.g. AiiA, AttM and

AiiB; Carlier et al. 2003; Dong et al. 2000, 2002; Lee et al. 2002; Zhang et al. 2002) and the N-AHSL acylases/amidohydrolases (AiiD, PvdQ or AhlM; Lin et al. 2003; Huang et al. 2003; Park et al. 2003, 2005; Uroz et al. 2006). N-AHSL lactone hydrolases catalyse a reaction that is identical to pH-mediated lactonolysis, while acylases/amidohydrolases convert N-AHSL to homoserine lactone and a fatty acid. Lactonases generally hydrolyse a large range of N-AHSLs, from short- (C4- or C6-HSL) to long-chain (C12- and C14-HSL) independently of the substitution at carbon 3 (C3). Though not a systematic phenomenon, amidohydrolase may exhibit a more restricted specificity, being specific for long-chain but not short-chain N-AHSL (Park et al. 2005; Sio et al. 2006). Recently, an N-AHSL-modifying activity has been described in *Rhodococcus erythropolis*; in this species, an oxidoreductase converts 3-oxo,N-AHSL to 3-hydroxy,N-AHSL (Uroz et al. 2005). This activity is not a degradative activity *sensu stricto*; it leads, however, to a change in or loss of the signalling capability of the molecules as the substitution at C3 is crucial for signal specificity.

Aside from bacteria, N-AHSL-degradation abilities have been observed in porcine kidney (Xu et al. 2003) and human airway epithelial cells (Chun et al. 2004). N-AHSL degradation has also been detected in the blood sera of various animals: mouse, rabbit, horse and human sera but remained absent from that of fish and chicken, a possible indication of mammalian specificity (Yang et al. 2005). While porcine kidney cells produce an acylase/amidohydrolase (Xu et al. 2003), N-AHSL degradation by airway epithelial cells is mediated by at least three paraoxonases (PON). One of them exhibits a lactonase activity towards N-AHSL (Draganov et al. 2000; Yang et al. 2005). In plants, degradation of the N-AHSL C6-HSL has been demonstrated in vitro, in the growth medium of seedlings of two legume species: clover and lotus. Under similar conditions, seedlings of corn and wheat did not exhibit any C6-HSL-degradative ability (Delalande et al. 2005).

The biological role of N-AHSL degradation

The biological role of N-AHSL-degrading enzymes, with respect to QS-regulated functions, has been investigated only very recently. One of the most-investigated models is the *attKLM* operon in *Agrobacterium*. In this bacterium, QS controls the conjugal transfer of the Ti plasmid in the presence of opines (for review see: Farrand 1998). The *attKLM* operon is expressed under carbon starvation (Zhang et al. 2004; Wang et al. 2006) or, regardless of the growth phase, in the presence of several plant molecules such as γ-butyrolactone (GBL), γ-hydroxybutyrate (GHB), succinic semialdehyde (SSA), and γ-aminobutyrate (GABA) (Carlier et al. 2004; Chevrot et al. 2006). GBL, GHB, and SSA are dissimilated by the *attKLM*-encoded catabolic pathway, while GABA is not. Remarkably GABA, which would be considered a gratuitous inducer of this operon, is produced at elevated concentrations by wounded plants. Under those conditions, i.e. at the very early stages of infection, the expression of the lactonase gene should therefore be induced. This may permit the degradation of the N-AHSL synthesized by the agrobacteria or other bacteria (Chevrot et al. 2006) and prevent any conjugal transfer from occurring, even though very limited amounts of opines could already be produced by a few transformed cells. The lactonase AttM could therefore participate in the fine regulation of QS in the course of the *Agrobacterium*-plant interaction.

Other biological roles for N-AHSL degradation have been suggested, based on the observation that some N-AHSLs may be toxic to other bacteria. The small bacteriocin produced by several *Rhizobium leguminosarum* strains—which has bacteriostatic activities towards some other strains of this species—is indeed the N-AHSL N-(3R-hydroxy-7-cis-tetradecanoyl)-L-homoserine lactone (Schripsema et al. 1996). The N-AHSL lactonase present in some *Rhizobium* strains may therefore be involved in the inactivation of the small bacteriocin. Similarly, the 3-oxo,dodecanoyl-homoserine lactone (3O,C12-HSL) and its spontaneous reorganization derivative, the tetramic acid 3-(1-hydroxydecylidene)-5-(2-hydroxyethyl)pyrrolidine-2,4-dione, are toxic to several Gram-positive, but not to Gram-negative bacteria (Kaufmann et al. 2005). Both N-AHSL lactonase and acylase/amidohydrolase detected in some firmicutes and actinobacteria may therefore play a key role in detoxifying these compounds.

One cannot exclude the possibility that N-AHSL-cleaving enzymes may be implicated in functions unrelated to QQ, because such enzymes are also capable of degrading other molecules. In *Agrobacterium*, the

attKLM operon is involved in the dissimilation of GBL, GHB and SSA (Carlier et al. 2004). In *Streptomyces* spp., the cyclic lipopeptide acylase AhlM also degrades penicillin (Park et al. 2005) and possibly related beta-lactame antibiotics. In mammalian cells, N-AHSL paraoxonases were first identified as organophosphate-detoxifying enzymes. They also have thioloactonase activities and exhibit antioxidizing properties, mostly towards sterols and lipid-like molecules (Draganov et al. 2000; Jakubowski 2000).

Though the biological role of QQ is not fully understood, several authors have proposed to take advantage of quenching to develop novel medical and animal therapies (Cámara et al. 2002; Raffa et al. 2005, Rasmussen and Givskov 2006) or novel biocontrol strategies for plant pathogens (Savka et al. 2002; Von Bodman et al. 2003; Zhang 2003). Amongst plant pathogens, those from the *Erwinia/Pectobacterium* genera have been widely used as model systems to evaluate the validity of the QQ strategy.

Erwinia/Pectobacterium-induced diseases

Overview of *Erwinia/Pectobacterium* taxonomy

The *Erwinia* genus and related genera mostly consist of plant pathogenic bacteria and members of the family *Enterobacteriaceae* (proteobacteria). Like several other microbial groups, the genus *Erwinia* has been reorganized in the light of 16S-based, molecular phylogeny. Four groups have been defined. Group I encompasses species renamed as *Pantoea* species and related bacteria such as *Erwinia herbicola*. Group II includes *Erwinia amylovora* and *Erwinia mallotivora*, group III, species renamed *Pectobacterium*, such as *P. carotovorum* and *P. atrosepticum*, or *Dickeya*, as *D. chrysanthemi*, and group IV consists of strains renamed *Brenneria* but the position of this latter group is still debated (Kwon et al. 1997; Sproer et al. 1999; Gardan et al. 2003; Samson et al. 2005). *Pantoea* species cause bacterial wilt and leaf blight, a disease transmitted by the coleopteran *Chaetocnema pulicaria* (flea beetle). Inoculated to plants, the pathogen produces characteristic water-soaked lesions on young leaves. Eventually, the pathogen colonizes the xylem vessels, leading to subsequent wilting (Von Bodman et al. 2003). Group II *Erwinia* species are the causative agents of bacterial blight, mostly on fruit trees (*E. amylovora*), or bacterial leaf spots (*E. mallotivora*).

Pectobacterium-induced plant diseases

Pectobacterium species are responsible for other disease symptoms known as soft rot, the main symptom being a complete maceration (enzymatic destruction) of plant tissues. Pathogenicity essentially relies upon the production and the secretion by the bacteria of plant-cell-wall-macerating enzymes (PCWME), mostly pectate lyases, pectin methylesterases (which facilitate the action of the first-cited enzymes), pectin lyase, polygalacturonases and oligogalacturonate lyases (for reviews see: Salmond 1994; Hugouvieux-Cotte-Pattat et al. 1996). Other virulence factors of *Pectobacterium* include the production of harpin, a peptide first identified in *Erwinia* in group II *E. amylovora* strains (Wei et al. 1992). Harpin is secreted into plant cells via a type III secretion system (TTSS) encoded by the *hrp* genes (for reviews see: Alfano and Collmer 2004; He 2004). In general, the harpins produced by plant pathogenic bacteria, as well as other TTSS effector peptides such as avirulence gene products, are involved in counter-acting plant defence systems; some of these peptides do exhibit plant-cell-death-inhibiting activity (for review see: Mudgett 2005). In *Pectobacterium*, the precise mode of action of harpin has not been described, though its contribution to pathogenicity has been reported (Bauer et al. 1995; Yang et al. 2002). In addition, harpin could contribute to the aggregative properties of *Pectobacterium* strains (Yap et al. 2006).

Pathogenicity and pathogenicity-related functions in *Pectobacterium* are regulated in a complex manner. Physiologically, environmental parameters affect pathogenicity (Perombelon and Kelman 1980). Pectate lyase synthesis is 20 times higher at 25°C than at 37°C in *D. chrysanthemi* (Hugouvieux-Cotte-Pattat et al. 1992). In several *P. atrosepticum* strains, the emergence of disease symptoms and the expression of maceration enzymes are optimal at temperatures <20°C (Smadja et al. 2004a). Iron deprivation also induces pectate lyase synthesis (Sauvage and Expert 1994), which is also lower under aerobiosis than under reduced oxygen tension, a condition where

plant defences are weak. In potato tubers, for instance, *Pectobacterium* may use the naturally-occurring nitrate as a terminal electron acceptor and produce pectate lyases (Hugouvieux-Cotte-Pattat et al. 1992; Smid et al. 1993).

Aside from environmental parameters, pathogenicity in *P. carotovorum* is controlled by at least three factors: (i) the presence of molecules originating from the plant cell wall, (ii) the general GacA/S system, and (iii) QS (for reviews see: Hugouvieux-Cotte-Pattat et al. 1996; Whitehead et al. 2001, 2002; Von Bodman et al. 2003; Fig. 1). To summarize and simplify, in *P. carotovorum*, in the plant environment and at low cell density, only limited amounts of 3-oxo,hexanoyl-homoserine lactone (3O,C6-HSL) are synthesized by the CarI N-AHSL synthase (Jones et al. 1993). Under those conditions, the expression of the genes encoding plant-cell-wall-maceration enzymes (PCWME) is blocked at: (i) the transcriptional level, a phenomenon mediated by the KdgR repressor (Nasser et al. 1994), and (ii) the post-transcriptional level, a feature mediated by the RsmA protein that binds the PCWME mRNA and accelerates its degradation (Cui et al. 1995). As a consequence, very limited amounts of the PCWME are produced. At high cell density, the presence of an elevated concentration of 3O,C6-HSL is sensed by the regulatory protein ExpR that, as an ExpR/N-AHSL complex, prevents the transcription of *rsmA* which, conversely, is activated in the absence of the cognate N-AHSL (Cui et al. 2005). The existing RsmA protein is further displaced from the PCWME mRNA by the activation of the production of RsmB, a small RsmA-binding RNA, encoded by the eponymous gene *rsmB* (Liu et al. 1998), the transcription of which is controlled by the global regulatory system ExpS/ExpA (analogous to GacS/GacA; Cui et al. 2001; Hyytiainen et al. 2001). PCWME are therefore synthesized, leading to the degradation of plant pectin. Oligomers and degradation products generated by the enzymes, such as polygalacturonate, saturated and unsaturated digalacturonate, galacturonate, and mostly 2-keto, 3-deoxygluconate, recognized by the repressor KdgR (Nasser et al. 1994), further induce the expression of both *rsmB* and PCWME genes, leading to an extensive production of PCWME (Tsuyumu 1977; Collmer and Bateman 1981).

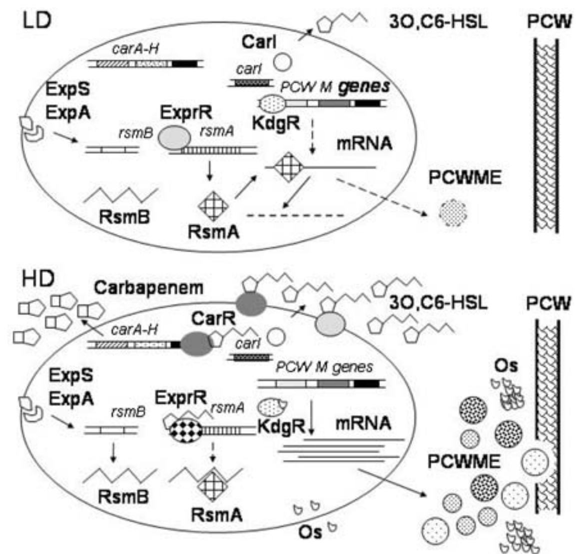

Fig. 1 Regulation of plant-cell-wall-macerating enzymes in *Pectobacterium*. At low cell density (panel LD, top), limited amounts of the N-AHSL 3O,C6-HSL are made via the CarI synthase. Transcription of the genes involved in plant-cell-wall maceration (PCWM) is blocked both by the repressor KdgR and by the RNA-binding protein RsmA. The plant-cell-wall-maceration enzymes (PCWME) are not produced. At high cell density (panel HD, bottom), large amounts of 3O,C6-HSL are made, preventing the synthesis of RsmA via the ExpR-N-AHSL complex. The presence in the cell of the RsmA-binding RNA RsmB, the production of which depends upon the global ExpS/A regulatory system, allows further trapping of RsmA. The PCWM genes are partly expressed leading to the production of PCWME. The enzymatic activities generate oligosides (Os) from the cell wall (PCW). Os release the KdgR-mediated repression of RsmB and PCWM genes. The QS signals also allow the activation of the regulator CarR that controls the expression of the *carAH* genes involved in the synthesis of carbapenem. For additional details, see text

The production of harpin encoded by the *hrpN* gene in *P. carotovorum* is controlled by the same three factors that regulate the synthesis of PCWME, i.e. (i) the presence of molecules originating from the plant cell wall (Liu et al. 1999), (ii) the general GacA/S system (Cui et al. 2001), and (iii) QS via the RsmA/B system (Cui et al. 1995). In addition, harpin production is also modulated by environmental parameters, a response mediated by the product of the *hrpL* regulatory gene (Wei and Beer 1995), which—by analogy with peptides encoded by *hrpL* orthologues—is most likely an alternate sigma factor (Chaterjee et al. 2002). However, the precise regu-

latory mechanism for hairpin production in *Pectobacterium* remains only partly understood.

Aside from pathogenicity, another function is controlled by QS in *Pectobacterium*: the production of the lactame antibiotic carbapenem (for reviews see: Whitehead et al. 2001, 2002; Von Bodman et al. 2003). When accumulated at a sufficient concentration, the signal 3O,C6-HSL produced by the synthase CarI is perceived by the regulator CarR which activates the transcription of the carA-K genes, encoding the enzymes involved in the biosynthesis of the antibiotic (McGowan et al. 1995, 1996). Carbapenem production most likely confers a fitness advantage to *Pectobacterium*, possibly by reducing the number of competing bacteria (Whitehead et al. 2001, 2002) in soil or in plants, macerated or not. However, resistance to β-lactame in bacteria living in the plant environment is widespread (Ogawara 1981). It is therefore possible that another advantage linked to carbapenem production lies in the resistance of *Pectobacterium* to carbapenem and related antibiotics produced by competing bacteria.

QQ of *Pectobacterium* pathogenicity

The above description of the molecular mechanisms underlying pathogenicity in *Pectobacterium* highlights the central role played by QS regulation in pathogenicity. Targeting the QS regulatory elements to develop biocontrol strategies for *Pectobacterium* is therefore a pertinent option (Dong et al. 2000; Smadja et al. 2004b). Two research strategies have been developed: one aimed at producing transgenic plants interfering with QS, the other at isolating plant-associated bacteria naturally interfering with QS in *Pectobacterium*.

The plant-genetic-engineering approach

Plants were genetically modified to gain the capacity to produce or inactivate N-AHSL signals. A first series of these transgenic plants were developed to activate QS functions of pathogens at an inappropriate time; a second type of plants was designed to block the initiation of the QS regulatory cascade. Transgenic tobacco plants, into which the *yenI* gene of *Yersinia enterolitica* encoding N-AHSL synthase was introduced, were able to produce C6-HSL and 3O,C6-HSL (Fray et al. 1999). The N-AHSL-producing plants was able to complement the virulence of an N-AHSL-defective mutant of *P. carotovorum*, as well as the biocontrol activity of an N-AHSL-defective mutant of *Pseudomonas aureofaciens*. However, while a decrease in the virulence of a wild-type *P. carotovorum* strain on the non-host tobacco plant expressing *expI* was reported (Mäe et al. 2001), an increase in the virulence was observed when wild-type *P. carotovorum* was inoculated on the host potato plant expressing the *yenI* gene (Toth et al. 2004). Quite different results were obtained with transgenic tobacco and potato plants expressing the lactonase AiiA of *Bacillus* sp. 240B1 (Dong et al. 2001). These *aiiA*-plants, expressing lactonase activity directed at N-AHSL, were always more resistant to *P. carotovorum* infection than the parental, wild-type plants.

The biocontrol approach

In consideration of the debate that exists in Europe on the use and release of GM plants (e.g. Hodgson 2001; Williams 2002; Wisniewski et al. 2002), a more acceptable biocontrol approach was developed by various researchers. Several studies aimed at isolating bacteria able to inactivate the N-AHSL signals produced by *Pectobacterium.* These studies have been facilitated by the occurrence of N-AHSL-degrading bacteria in soil and plant environments. This community represents 2–5 and up to 10% of the culturable bacteria (Dong et al. 2000; Steidle et al. 2001; Morello et al. 2004; D'Angelo-Picard et al. 2004, 2005), a feature that translates into a demonstrable N-AHSL-degradation potential for soils (Wang and Leadbetter 2005).

Bacterial populations from bare and rhizospheric soil could be screened for N-AHSL degraders by randomly assaying the N-AHSL-inactivation capability of individual isolates in vitro. Using this strategy, *Bacillus* strains exhibiting the AiiA-borne lactonase activity were identified (Dong et al. 2000). A similar experimental design led to the identification of a *Ralstonia* strain from a complex biofilm population (Lin et al. 2003). A mass screen of bacteria isolated from the root system of wild-type plants and plants producing N-AHSL permitted the identification of additional *Bacillus* strains, *Agrobacterium* spp., *Sphingopyxis witflariensis* and *Bosea thiooxi-*

dans isolates inactivating N-AHSL (D'Angelo-Picard et al. 2005). However, none of these isolates have been used against *Pectobacterium* strains in biocontrol experiments. The strategy proved to be valuable, however, as it allowed the isolation of an *Acinetobacter* strain which degraded N-AHSL and was capable of attenuating soft-rot symptoms caused by *P. carotovorum* in potato tuber slice assays (Kang et al. 2004).

Another strategy, close to but distinct from the one described above, aimed at isolating bacteria with N-AHSL-dissimilating ability by selection on minimal media supplemented with N-AHSL as the sole carbon source. The prototypic experiment led to the identification of the first degrader, a *Variovorax paradoxus* strain (Leadbetter and Greenberg 2000), and later to the demonstration of the ability of the PAO1 strain of *Pseudomonas aeruginosa* to dissimilate long chain N-AHSLs such as 3O,C12-HSL (Huang et al. 2003). Valuable biocontrol strains (also termed quenchers) directed against pathogenic *Pectobacterium* were also isolated using this technique; examples include *Comamonas* spp., *Ochrobactrum*, and *Rhodococcus* strains (Uroz et al. 2003, 2005, 2006; Jaffra et al. 2006; Park et al. 2006). The remarkable ability of several *Rhodococcus* strains to quench pathogenicity in *Pectobacterium* (complete disappearance of disease symptoms at 1 to 1 and 1 to 10 ratios), though variable as a function of the origin of the strain, possibly is due to the occurrence, in these bacteria, of a triple inactivation pathway consisting of an acyclase/amidohydrolase, a lactonase and an oxidoreductase (Uroz et al. 2005; Park et al. 2006).

Perspectives in QQ

Targeting the QS-regulated virulence functions of *Pectobacterium* should not cause the disappearance of the pathogen from the plant environment, since these functions are not vital to *Pectobacterium*. This circumstance could lead to the appearance of healthy, contaminated plants, from which disease may spread in the absence of the quencher treatment. Also, QQ strategies being non-selective for the time being, they may also prevent bacterial functions possibly beneficial to plants (such as antifungal synthesis) (Molina et al. 2003). Whether these points constitute major drawbacks in the development of QQ strategies aimed at *Pectobacterium* remains to be evaluated.

At this time, none of the isolated quenchers have been assayed for their biocontrol ability outside the laboratory, a step which remains crucial (and often time-consuming) to evaluate the potential value of a biocontrol agent under agricultural conditions (Mc Intyre and Press 1991). However, the commercial use of transgenic plants expressing N-AHSL-degrading enzymes essentially depends on the legal authorization given by each State.

In addition to lactonases of the AiiA family and amidohydrolases of the AiiD family, other genes could be used if they confer an increased N-AHSL-degradation ability upon the host plants. Known genes such as those encoding paraoxonase (Yang et al. 2005) may be candidates, along with genes originating from unculturable bacteria that represent the vast majority of bacterial soil inhabitants (e.g. Felkse et al. 1999; for review see: Saleh-Lakha et al. 2005). Such genes have been detected in soil bacteria via a metagenomic approach (Williamson et al. 2005; our laboratory, unpublished) but the mechanism that led to N-AHSL degradation or inhibition of N-AHSL detection remains unknown. Finally, the increased number of QQ chemicals offers a third strategy to neutralize QS pathogens. The biocontrol, transgenic and chemical approaches are not exclusive, however, and represent complementary ways to fight QS-regulated virulence in *Pectobacterium* and related plant pathogens.

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