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The *lux* autoinducer regulates the production of exoenzyme virulence determinants in *Erwinia carotovora* and *Pseudomonas aeruginosa*

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***Erwinia carotovora* and *Pseudomonas aeruginosa* secrete exoenzymes that contribute to the pathogenesis of plant and mammalian infections respectively. *E.carotovora* mutants defective in synthesis of the pectinase, cellulase and protease exoenzymes were isolated and classified into two groups. Group 2 mutants were found to be defective in the production of a small freely diffusible molecule, *N*-3-(oxohexanoyl)-L-homoserine lactone (HSL), and were avirulent. Addition of exogenous HSL to these group 2 mutants restores synthesis of the exoenzymes and virulence *in planta*. Of the exoenzymes of *P.aeruginosa* the metalloprotease, elastase, is an established virulence determinant. Mutants of *P.aeruginosa* that are defective in elastase production have been isolated and were again found to fall into two groups. Analogous to the group 2 mutants of *E.carotovora*, group 2 mutants of *P. aeruginosa* are defective in the synthesis of HSL and exogenous HSL restores elastase production. HSL has now been linked to the control of bioluminescence in *Vibrio fischeri*, carbapenem antibiotic production of *E.carotovora* and the above exoenzyme virulence determinants. This information significantly enhances our understanding of the extent and nature of pheromone mediated gene expression control in prokaryotes.**

Key words: Erwinia/exoenzymes/*N*-(3-oxohexanoyl)-L-homoserine lactone/*Pseudomonas*/virulence

Introduction

To gain an insight into the biosynthesis and regulatory mechanisms involved in the production of the β-lactam antibiotic 1 carbapen-2-em 3 carboxylic acid by *Erwinia carotovora*, we previously obtained blocked mutants with a carbapenem non-producing phenotype (Car^-) (Bainton *et al.*, 1992a,b). These mutants fell into two distinct groups: group 1 mutants secrete a low molecular mass diffusible factor which restores carbapenem biosynthesis in group 2 mutants but not vice versa. The factor was subsequently isolated and purified and shown to be *N*-(3-oxohexanoyl)-L-homoserine lactone (HSL; Bainton *et al.*, 1992a,b). Group 2 mutants can be genetically complemented by the *luxI* gene from *Vibrio fischeri* (Williams *et al.*, 1992) and studies on the regulation of carbapenem production indicate that it is a density dependent process analogous to bioluminescence in *V.fischeri* (Williams *et al.*, 1992; S.Swift *et al.*, submitted).

When *V.fischeri* cells are diluted into fresh medium, bioluminescence is not observed until the cells reach the mid-logarithmic phase of growth. This density dependent induction is the result of accumulation in the medium of a sufficient level of HSL to trigger autoinduction and is a characteristic feature of all known bacterial responses to HSL, which has been termed a bacterial pheromone or autoinducer. The *luxI* gene product is believed to be responsible for autoinducer synthesis since luminescence can be restored to $luxI^-$ cells by addition of exogenous HSL (Meighen, 1991). A positive regulatory element in *V.fischeri* is required along with HSL to activate transcription of the *lux* regulon. This regulatory element is a 28 kDa protein encoded by *luxR*, a gene located adjacent to the other *lux* genes but transcribed in the opposite direction (for review see Choi and Greenberg, 1992). It is now clear that the primary level of control of the *lux* regulon is a positive feedback loop in which basal transcription of *luxI* leads to the accumulation of a low level of HSL. A LuxR-HSL complex stimulates transcription of the rightward operon. Since *luxI* is the first gene in this operon this leads to increased levels of LuxI and hence HSL, further activating LuxR. Because HSL is freely diffusible, the induction of one cell leads directly to the induction of others, creating a positive feedback circuit that can generate a large and rapid response to a small initial stimulus providing a co-ordinated response from a population of cells (Stewart and Williams, 1992).

We have extended the carbapenem studies on *E.carotovora* by demonstrating density dependent HSL production in several enteric bacteria and HSL production by three species of *Pseudomonas*. A *luxI* analogue from *Enterobacter agglomerans* has been cloned and, from the translated sequence, a region with homology to LuxI identified (Bainton *et al.*, 1992a; S.Swift *et al.*, submitted). With the exception of bioluminescence and carbapenem production, however, a role for HSL in these diverse organisms has yet to be established.

E.carotovora is a phytopathogen that produces plant cell wall degrading exoenzymes that act as virulence determinants for soft rot diseases of various plants including the potato (Kotoujansky, 1987). Mutants affected in the co-ordinate production of these exoenzymes exhibit significantly reduced virulence *in planta* (Hinton *et al.*, 1989; Murata *et al.*, 1991; Pirhonen *et al.*, 1991). The main phenotypic class of such mutants has been referred to as Aep (activation of enzyme production), Exp (exoenzyme production) and Rex (regulation of exoenzymes) by various workers (Murata *et al.*, 1991; Pirhonen *et al.*, 1991; this study). To date, the mechanism regulating synthesis of these exo-

enzyme is unknown although several genes that complement regulatory mutants have been cloned from gene libraries of different *E.carotovora* strains (Murata *et al.*, 1991; Pirhonen *et al.*, 1991).

Pseudomonas aeruginosa is a common environmental organism which is frequently responsible for opportunistic infections in neutropenic hosts and in cystic fibrosis patients. Although it is the concerted action of many toxic extracellular proteins which contributes to the pathogenesis of a *P. aeruginosa* infection, there is extensive evidence to suggest that the metalloprotease elastase, the product of the *lasB* gene, is required for maximal virulence (Brumlik and Storey, 1992). Gambello and Iglewski (1991) have identified a gene termed *lasR* which is involved in regulating expression of *lasB*. LasR shares significant homology with LuxR and, recently, analysis of downstream sequences has revealed a gene product termed LasI which shares significant homology with LuxI (Cook and Iglewski, 1992). When coupled with previous descriptions of the growth phase dependency of elastase production (Galloway, 1991) and our discovery that *P.aeruginosa* produces HSL, there is strong circumstantial evidence for the involvement of HSL or structurally related compounds in regulating elastase synthesis.

In the present study, we present data to establish that expression of both the *E.carotovora rex* and the *P.aeruginosa lasB* gene are regulated by HSL. Consequently, exoenzyme virulence determinants of two major pathogens are demonstrably regulated by this small diffusible molecule previously considered unique to the marine bacterium *V.fischeri*.

Results

Some Car⁻ mutants of E.carotovora are also phenotypically Rex⁻

E.carotovora SCRI193 is a naturally occurring strain that does not make detectable levels of carbapenem antibiotic. This strain does, however, cross-feed the group 2 Car⁻ mutants, restoring carbapenem production, indicating that SCRI193 makes HSL (data not shown). This result encouraged us to look for other physiological phenotypes that might be regulated by HSL. One obvious phenotype to examine was the regulation of exoenzyme synthesis, particularly as these are important factors in the virulence of this plant pathogen. Examination of previously isolated Car⁻ mutants (Bainton *et al.*, 1992a,b) indicated that those in group 1 are wild-type for exoenzyme synthesis but the group 2 mutants are simultaneously down-regulated for pectinase (Pel), cellulase (Cel) and protease (Prt) production (i.e. they are phenotypically Rex⁻). Since the addition of exogenous HSL restores the Car⁺ phenotype to these group 2 mutants (Bainton *et al.*, 1992a,b), we examined their response to HSL in respect of their Rex phenotype. Group 2 Car⁻ mutants were fully restored for Pel, Cel and Prt synthesis by exogenous HSL (see Figure 1).

Rex mutants of SCRI193 constitute two distinct groups

Chemical mutagenesis with ethylmethane sulfonate (EMS) was used to produce Rex⁻ mutants of *E.carotovora* SCRI193 which are down-regulated for the production of pectinases, cellulases and proteases. mRNA transcript analysis (Figure 2) from two of the *pel* genes, *pelC* and *pelD* (Hinton *et al.*, 1989; Plastow *et al.*, 1986) and a *celV* gene

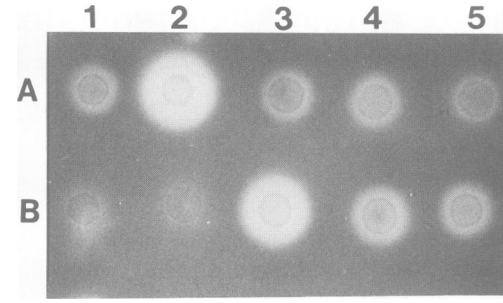

Fig. 1. Influence of HSL on protease production in the Rex⁻ mutants of *E.carotovora*. Samples of overnight cultures of bacteria grown in the absence (A1, A3, A5, B2 and B4) or presence (A2, A4, B1, B3 and B5) of 10 μg/ml HSL were spotted onto protease assay plates and incubated overnight. A1 and A2 represent the group 2 Car⁻ mutant PNP22, A3 and A4, A5 and B1, B4 and B5 represent the group 1 Rex⁻ mutants RJP111, RJP123 and RJP114 respectively. B2 and B3 represent the group 2 Rex⁻ mutant RJP113. Haloes around the zones of bacterial growth are due to protease activity. The same results were obtained in the pectinase and cellulase assays (data not shown).

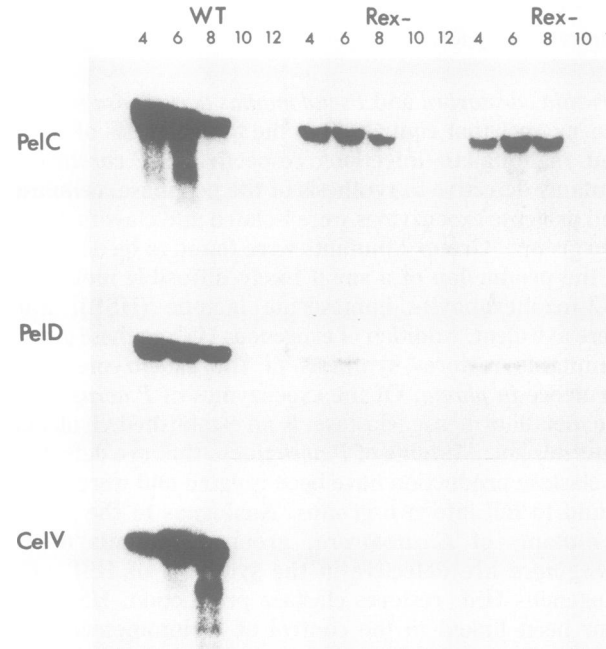

Fig. 2. Exoenzyme gene mRNA transcript assay from *E.carotovora* SCRI193 and two Rex⁻ mutants. Accumulation of mRNA was followed during the growth of the bacteria by Northern blot analysis of samples collected at varying times (4–12 h) after the onset of stationary phase. mRNA from the wild-type SCRI193 and two Rex⁻ mutants hybridizing to *pelC*, *pelD* and *celV* genes is shown.

(V.J.Cooper and G.P.C.Salmond, submitted) were examined both in the wild-type and in group 2 Rex⁻ mutants at varying times throughout the later stages of the growth cycle. Figure 2 shows that in the Rex⁻ mutants the block in exoenzyme production is at the level of transcription of the exoenzyme structural genes. In addition, although the *pelD* and *celV* transcripts were considerably down-regulated in the Rex⁻ mutants, down-regulation from *pelC* was much less dramatic. Furthermore, by late stationary phase (Figure 2, see time points 10–12 h) detection of transcripts from each of the exoenzyme genes examined was poor even in the wild-type strain.

Since we have established that SCRI193 cross-feeds the

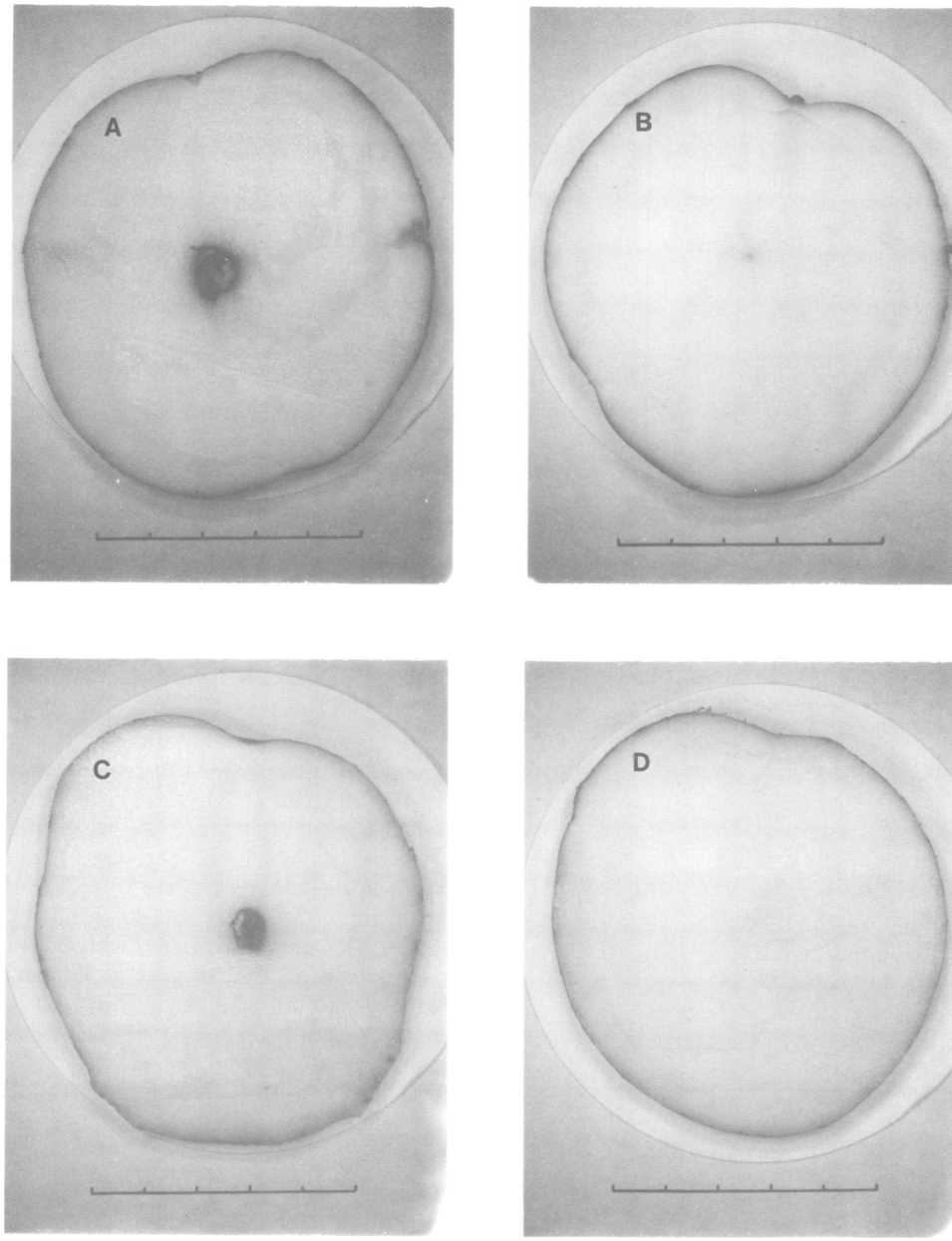

Fig. 3. Effect of HSL on the virulence of a Rex^- mutant of *E.carotovora*. Potato tubers were injected with 10 μl stationary phase broth cultures of wild-type (**A**), a group 2 Rex^- mutant (**B**), the same Rex^- mutant co-injected with 10 μg/ml HSL (**C**) or broth supplemented with 10 μg/ml HSL and incubated at 22°C for 7 days. The bar represents 5 cm.

group 2 Car^- mutants we examined the Rex^- mutants of SCRI193 for their ability to cross-feed. Co-cultivation experiments revealed that the Rex^- mutants could be assigned to two groups; when mutants in group 1 and group 2 were co-cultivated, the Rex^+ phenotype $(Pel, Cel, Prt)^+$ was restored. Group 2 but not group 1 mutants can also be restored to a Rex^+ phenotype by addition of exogenous HSL, illustrated for protease production in Figure 1. Of 20 Rex^- mutants isolated, four were phenotypically group 2.

Restoration of virulence in an E.carotovora group 2 Rex mutant by exogenous HSL

To determine whether HSL plays a role in regulating virulence determinants during growth *in planta*, we inoculated wild-type and Rex^- mutants into potato tubers with and without 10 μg/ml exogenous HSL. Figure 3 demonstrates that whilst the wild-type strain produces the characteristic tissue necrosis indicative of extensive exoenzyme-mediated tissue damage, no necrosis is observed following inoculation of a group 2 Rex^- mutant. The addition of HSL, however, restores such mutants to wild-type activity (Figure 3C). HSL in culture medium alone has no effect on the potato tuber (Figure 3D). These data provide potent evidence in support of the proposal that *E.carotovora* employs HSL as a regulatory molecule during growth *in planta*.

Elastase negative mutants of P.aeruginosa constitute two distinct groups

Following mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine, *P.aeruginosa* PAO1 was screened on elastin–nutrient agar plates for elastase negative (Las^-) mutants.

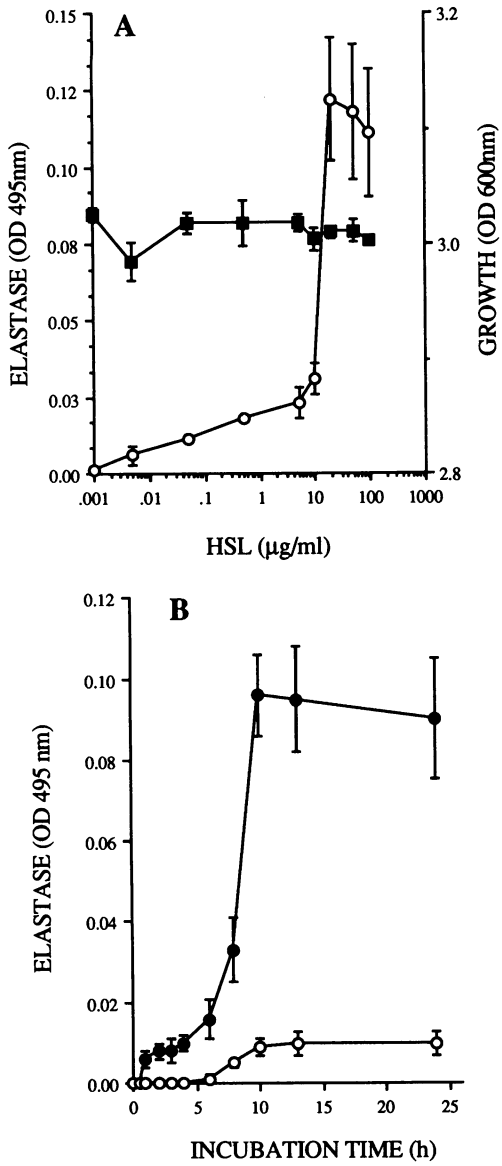

Fig. 4. Induction of elastase in *P.aeruginosa* PAN067 by HSL. (**A**) Influence of HSL concentration on induction of elastase. A range of concentrations of HSL were added to the growth medium immediately prior to inoculation and the elastase activity (○) in spent culture supernatants determined after 12 h using the elastin–Congo red assay. (■) indicates cell density (OD_{600}) observed after 12 h. (**B**) Effect of incubation time on the induction of elastase in PAN067 in the absence (○) or presence (●) of 20 μg/ml HSL.

From ~60 000 colonies, from 10 independent experiments, 25 Las^- mutants were obtained. These mutants could be divided into two classes, those which could be restored to a Las^+ phenotype by exogenously added HSL (6/25) and those which could not. One of these HSL-responsive mutants (PAN067) was chosen for further examination.

Restoration of elastase activity in PAN067

The effect of increasing concentrations of HSL on the induction of elastase in PAN067 is shown in Figure 4A. An approximately 4-fold increase in elastase activity was observed following the addition of ≥20 μg/ml of HSL, levels which appeared to have no effect on final cell density. At 20 μg/ml HSL, maximal elastase production was observed after 10 h (Figure 4B). Given that the elastin–

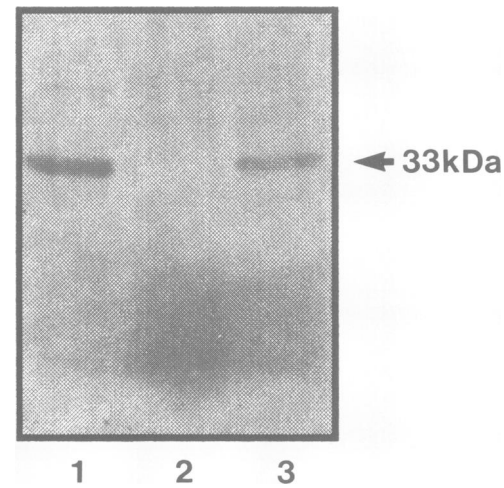

Fig. 5. SDS–PAGE of elastase purified from spent culture supernatants of the parent *P.aeruginosa* strain PAO1 (lane 1) and from PAN067 grown in the absence (lane 2) or presence (lane 3) of 20 μg/ml HSL, added exogenously at the time of inoculation. Lanes were loaded with 10 μl samples prepared from the same volume (3 l) of culture for both PAO1 and PAN067 which had been grown to the same OD_{600}. Molecular mass was calculated from protein standards run concurrently.

Congo red assay cannot distinguish between the products of the *lasA* and *lasB* genes (Peters *et al.*, 1992), both of which have elastolytic activity, we purified the 33 kDa *lasB* gene product by DEAE–Sepharose column chromatography from spent culture supernatants of PAO1 and PAN067 (grown with or without 20 μg/ml HSL). The SDS–polyacrylamide gel shown in Figure 5 confirms that PAN067 is negative for the *lasB* gene product unless supplied with exogenous HSL. Although HSL clearly restores elastase production in PAN067, both the level of protein (Figure 5, compare lanes 1 and 3) and the enzymatic activity are about one-third that of the wild-type PAO1 strain.

Discussion

We have now established that in *E.carotovora* HSL regulates both carbapenem production and exoenzyme synthesis. This is the first indication that HSL can function as a global regulator of multiple operons in a single organism. Given that both carbapenem production and HSL production in *E.carotovora* are cell density dependent (Williams *et al.*, 1992; S.Swift *et al.*, submitted), the genetic mechanism for HSL mediated regulation is likely to follow the well characterized density dependent system controlling bioluminescence in *V.fischeri* (Meighen, 1991). Thus *E.carotovora* is likely to possess genes analogous to the *V.fischeri lux* regulatory genes, *luxI* and *luxR*.

The *luxI* gene encodes a 22 kDa protein and this may function as an autoinducer synthetase, although the only evidence to support this assumption is that bioluminescence in $luxI^-$ mutants can be restored by addition of exogenous HSL (Meighen, 1991). Since HSL combines homoserine lactone (an intermediate in amino acid metabolism) and 3-oxohexanoic acid (related to intermediates in fatty acid metabolism), potential LuxI substrates have been suggested to be *S*-adenosylmethionine and 3-oxohexanoyl coenzyme A (Eberhard *et al.*, 1991). However, these experiments were based on cell free extracts and direct biochemical evidence

for the function of LuxI is still awaited. In *E.carotovora*, as well as *E.agglomerans* and *P.aeruginosa*, genes termed *expI* (Pirhonen *et al.*, 1993), *eagI* (Swift *et al.*, submitted) and *lasI* (Cook and Iglewski, 1992), respectively, have recently been cloned and sequenced and shown to possess open reading frames with homology to LuxI. These *luxI* homologues therefore probably constitute the genetic basis for HSL synthesis in these organisms, and, at least in the case of *Erwinia*, we have shown that they appear to be functionally equivalent in that *luxI* can complement the group 2 Car^- mutants leading to the restoration of carbapenem biosynthesis (Williams *et al.*, 1992). Pirhonen *et al.* (1993) have also reported that *luxI* can complement the exoenzyme-negative phenotype of *expI* mutants in *E.carotovora* and that *expI* can complement *lux*$^-$ mutations restoring the bioluminescent phenotype. Furthermore, from a cosmid library of the carbapenem producing *E.carotovora* strain ATCC39048, we have isolated a cosmid capable of complementing group 2 Car^- and Rex^- mutants. On Southern blots, internal DNA fragments from this cosmid hybridized with the *expI* gene cloned from *E.carotovora* strain SCC3193 of Pirhonen *et al.* (1991) (unpublished data). These data provide convincing evidence for HSL mediated regulation of both secondary metabolites and virulence determinants in *E.carotovora*.

Given the existence of a LuxI homologue in *Erwinia*, it follows that there is likely to be a protein equivalent to LuxR. Indeed, several proteins from different bacterial genera have been reported to share significant homology with the *V.fischeri* LuxR. These all belong to the superfamily of two-component (sensor−regulator) systems important in signal transduction which enable bacteria to respond to diverse intracellular and environmental stimuli. Examples include ComA, DegU, FixJ, UhpA, UvrC-23k, Uvrc-28k, BvgA, GerA, MalT,BrpA, GacA, SdiA and LasR (Henikoff *et al.*, 1990; Gambello and Iglewski, 1991; Wang *et al.*, 1991; Laville *et al.*, 1992). Of these, LasR is of particular interest as it functions as a transcriptional activator of the *P.aeruginosa* elastase structural gene, *lasB*. LasR and LasI therefore appear to function as homologues of LuxR and LuxI. This information, together with the biochemical data presented in this study, provides convincing evidence for a functional role for HSL in the control of elastase expression in *P.aeruginosa*.

We have now presented evidence for the existence of an HSL-based gene regulatory system in diverse bacterial genera including *Serratia*, *Citrobacter*, *Enterobacter*, *Proteus*, *Hafnia* and *Rahnella* as well as *Pseudomonas* and *Erwinia* (Bainton *et al.*, 1992a,b; Stewart and Williams, 1992; Williams *et al.*, 1992; Chhabra *et al.*, 1993; S.Swift *et al.*, submitted). Thus the LuxI−autoinducer−LuxR type regulatory control circuit appears to be much more widespread than at first thought. Furthermore, we have preliminary evidence that the LuxR homologue controlling carbapenem production in *E.carotovora* is different from that regulating the Rex phenotype. A family of hierarchical LuxR homologues in the same bacterial cell may therefore be co-ordinately regulating diverse physiological processes in a growth phase and cell density dependent manner. It must be remembered, however, that HSL constitutes only one member of what may eventually comprise an extensive family of autoregulators. For example, density dependent bioluminescence induction in the marine bacterium *V.harveyi* is regulated in a manner entirely analogous to that of *V.fischeri* except that the autoinducer in this instance is *N*-(3-hydroxybutyryl)homoserine lactone (Cao and Meighen, 1989). It may therefore be expected that, as for HSL, other bacteria will be identified which utilize this autoinducer in preference to HSL. Given that the genetic loci regulated by HSL in *E.carotovora* and *P.aeruginosa* are intimately associated with bacterial pathogenesis and virulence, the importance of rapidly assessing the role of small molecule mediated gene regulation in prokaryotes is self evident. It must be expected that the significance of this mode of gene regulation may prove equal to that of the well characterized histidine protein kinase mediated two-component response regulator systems. Perhaps the point of major divergence between these systems will be the relationship to cell density dependent regulation which appears to be a central feature of the HSL response. One crucial feature of almost all bacterial infections is the need for the invading pathogen to reach a critical cell population density sufficient to overwhelm host defences and thus establish the infection. The evolution of an elegant, density dependent genetic switch which, as we have shown, is common to both plant and animal pathogens may thus be a critical component in the aetiology of infection.

Materials and methods

Bacterial strains and growth conditions

E.carotovora subspecies *carotovora* strains SCRI193, GS101 (a restrictionless, modificationless derivative of ATCC39048) and the group 2 Car^- *E.carotovora* mutant PNP22 have been described before (Ellard *et al.*, 1989; Bainton *et al.*, 1992b). Unless otherwise indicated, *Erwinia* strains were grown at 28°C in nutrient broth (Oxoid). *P.aeruginosa* strain PAO1 (ATCC15692, Holloway *et al.*, 1979) is a Las^+ strain which was routinely grown overnight at 37°C in peptone trypticase soy broth medium (PTSB, Ohman *et al.*, 1989). For some experiments, growth media were supplemented with a range of concentrations of HSL (0−100 μg/ml). HSL was synthesized as described by Bainton *et al.* (1992b). Where indicated, growth was monitored by measurement of optical density at 600 nm (OD_{600}).

Exoenzyme assays

Pectinase and cellulase activities were detected using plate assays as described by Andro *et al.* (1984). Protease activity was determined using the plate assay of Hankin and Anagnostakis (1975). 20 μl samples of culture supernatants from *E.carotovora* strains grown in the absence or presence of 1 μg/ml HSL were spotted onto the appropriate enzyme assay plate and incubated at 28°C for 48 h. *Pseudomonas* elastase activity was examined on nutrient agar plates supplemented with 0.3% elastin (Sigma; Gambello and Iglewski, 1991). Following inoculation, plates were incubated at 37°C for 48 h. Elastase activity in liquid media was determined essentially as described by Ohman *et al.* (1980). Briefly, 1.0 ml of spent culture supernatant was added to a tube containing 20 mg elastin−Congo red (Sigma) and 2 ml reaction buffer (0.1 M Tris−maleate buffer, pH 7.0, containing 1 mM $CaCl_2$) and incubated with shaking for 3 h at 37°C. The reaction was terminated by placing the tube in an ice bath and adding 2 ml 0.7 M sodium phosphate buffer (pH 6.0). The suspension was filtered through a 0.45 μm membrane filter to remove insoluble elastin−Congo red and the OD_{495} determined.

Chemical mutagenesis

Chemical mutagenesis of *E.carotovora* SCRI193 was carried out using EMS *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) as described before (Bainton *et al.*, 1992a,b). Pleiotropic exoenzyme deficient mutants of *Erwinia* were identified on enzyme bioassay plates as described above. *P.aeruginosa* PAO1 was also subjected to MNNG mutagenesis and Las^- strains were selected by screening on elastin−nutrient agar plates using the method of Ohman *et al.* (1980).

Assay of mRNA transcripts from exoenzyme genes

Assay of mRNA transcripts of *E.carotovora* wild-type and Rex^- mutants were based on *pelC* and *pelD* which encode the major secreted pectate lyase

isoenzymes (Plastow *et al.*, 1986; Hinton *et al.*, 1989) and *celV* which encodes the major secreted cellulase (Cooper, 1992) of SCRI193. Methods for the extraction of bacterial mRNA, radiolabelling and hybridization analysis of mRNA transcripts from *E.carotovora* exoenzyme genes were essentially as described in Ausubel *et al.* (1990). Each *Erwinia* strain examined was grown overnight to stationary phase in L broth containing 0.5% polygalacturonate, diluted 1 in 100 into fresh medium at 28°C and the mRNA extracted from cells at 2 h intervals following the onset of stationary phase. An internal [^{32}P]dATP-labelled (Multiprime Kit, Amersham International plc, UK) restriction fragment of each gene (*celV*; 893 bp *Eco*RI–*Eco*RV fragment; *pelC*; 600 bp *Hin*cII–*Pst*I fragment; and *pelD*, 800 bp *Stu*I–*Bgl*II fragment) was used as the hybridization probe.

Virulence assay

The virulence of *E.carotovora* strains was evaluated by local inoculation into intact potato tubers. 10 μl of a stationary phase bacterial suspension (containing ~10^9 cells/ml) was injected into the centre of the potato. The potato was incubated at 22°C for 7 days after which it was cut in half and examined for the presence of necrotic tissue. For some experiments, HSL (10 μg/ml) was added to the bacterial suspension prior to injection into the potato. Control experiments in which HSL alone or HSL plus nutrient broth were injected into potato were also carried out.

Purification of elastase

Elastase was purified by ammonium sulphate precipitation of 3 l culture supernatants of *P.aeruginosa* strains PAO1 and PAN067 grown in PTSB medium, in the absence or presence of 20 μg/ml HSL, followed by DEAE–cellulose column chromatography as described by Morihara *et al.* (1965). Elastolytic activity was monitored using the elastin–Congo red assay and active fractions were pooled and concentrated by precipitation with acetone. The presence of the 33 kDa elastase was confirmed by electrophoresis of the samples on 15% SDS–polyacrylamide gels, which, following electrophoresis, were fixed and stained with Coomassie Brilliant blue.

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The *Agrobacterium tumefaciens* conjugation factor which enhances the conjugal transfer efficiency of Ti plasmids has recently been identified as *N*-3-(oxooctanoyl)-L-homoserine lactone a compound closely related to HSL [L.Zhang, P.J.Murphy, A.Kerr and M.E.Tate (1993) *Nature*, **362**, 446–448]. Furthermore conjugation is regulated by a transcriptional activator, TraR, which is a LuxR homologue, and requires *N*-3-(oxo-octanoyl)-L-homoserine lactone as a co-activator to induce *tra* gene expression [K.R.Piper, S.Beck von Bodman and S.K.Farrand (1992) *Nature*, **362**, 448–450].