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# Cell-to-Cell Signaling in Intestinal Pathogens

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## Abstract

**In the conventional view of prokaryotic life, bacteria live a unicellular existence, with responses to external stimuli limited to the detection of chemical and physical signals of environmental origin. This view of bacteriology is now recognized as overly simplistic, because bacteria communicate with each other through small "hormone-like" organic compounds referred to as autoinducers (AIs). These bacterial cell-to-cell signaling systems were initially described as mechanisms through which bacteria regulate gene expression via cell density, and, therefore, they have been named quorum sensing. When the AIs reach a threshold concentration, they interact with regulatory proteins, thereby driving bacterial gene expression. Bacterial intercellular communication provides a mechanism for the regulation of gene expression resulting in coordinated population behavior. The functions controlled by quorum sensing are varied and reflect the needs of a particular species of bacteria inhabiting a given niche. Quorum sensing-controlled processes include bioluminescence, virulence factor expression, biofilm development, and conjugation among others. Enteric pathogens use quorum sensing to regulate genes involved in virulence, such as motility, and type III secretion. Quorum sensing is utilized to sense the presence of the normal intestinal flora and to warrant successful colonization of the host.**

## Quorum sensing

Quorum sensing was first described in the regulation of bioluminescence in *Vibrio* species (Nealson *et al.*, 1970; Nealson and Hastings, 1979). The luciferase operon in *Vibrio fischeri* is regulated by two proteins, LuxI, which is responsible for the synthesis of the acyl-homoserine-lactone (AHL) autoinducer, and LuxR, which is activated by this autoinducer to increase transcription of the luciferase operon (Engebrecht *et al.*, 1983; Nealson and Hastings, 1979). Since this initial description, homologues of LuxR-LuxI have been identified in other bacteria. In all of these LuxR-LuxI systems, the bacteria produce an acyl-homoserine lactone (AHL) autoinducer, which binds to the LuxR protein and regulates the transcription of several genes involved in a variety of phenotypes. These include

the production of antibiotics in *Erwinia*, motility in *Yersinia pseudotuberculosis*, pathogenesis and biofilm formation in *Pseudomonas aeruginosa*, etc... (Davies *et al.*, 1998; de Kievit and Iglewski, 2000; Parsek and Greenberg, 2000). The LuxR-LuxI systems are generally used for intra-species communication. *E. coli* and *Salmonella* have a LuxR homologue, SdiA (Michael *et al.*, 2001; Wang *et al.*, 1991), but do not have a *luxI* gene, and do not produce AHLs (Michael *et al.*, 2001; Swift *et al.*, 1999b). The *E. coli* *sdiA* initially was isolated as a regulator of the cell division genes *ftsQAZ* (Wang *et al.*, 1991). However, the precise role of SdiA in quorum sensing was elusive for several years until Michael *et al.* (Michael *et al.*, 2001) reported that SdiA is not sensing an autoinducer produced by *Salmonella* itself, but rather AHLs produced by other bacterial species.

One of the most common quorum sensing systems is the *luxS*/AI-2 system, first described in *Vibrio harveyi* (Surette and Bassler, 1998). AI-2 (autoinducer-2) is a furanosyl-borate-diester (Chen *et al.*, 2002) and the enzyme responsible for synthesizing it is encoded by the *luxS* gene (Surette *et al.*, 1999). LuxS is an enzyme involved in the metabolism of S-adenosyl-methionine (SAM); it converts ribose-homocysteine into homocysteine and 4,5-dihydroxy-2,3-pentanedione, which is the precursor of AI-2 (Schauder *et al.*, 2001; Winzer *et al.*, 2002). The *luxS*/AI-2 quorum sensing system is present in both gram-positive and gram-negative bacteria and is hypothesized to be one of the most ancient bacterial cell-to-cell signaling systems (Schauder and Bassler, 2001). The presence of a LuxS enzyme is necessary for the production of yet another autoinducer, AI-3, whose biochemical structure is still unknown (Sperandio *et al.*, 2003).

## Quorum sensing in diarrheagenic *E. coli* (EHEC)

There are six different classes of diarrheagenic *E. coli*: enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffuse adhering *E. coli* (DAEC), enterohemorrhagic *E. coli* (EHEC), and enteropathogenic *E. coli* (EPEC) (Nataro and Kaper, 1998). Quorum sensing has been implicated in the pathogenesis of both EPEC and EHEC (Sperandio *et al.*, 1999).

## Quorum sensing in EHEC

EHEC is infamous for causing extensive outbreaks throughout the world of bloody diarrhea and hemolytic uremic syndrome. In North America, the United Kingdom, and Japan, the main serotype associated with these outbreaks is O157:H7 (Bell *et al.*, 1994; Izumiya and Watanabe, 1997; Thomas *et al.*, 1993). EHEC O157:H7 and many other Shiga toxin-producing *E. coli* (STEC) are part of a group of enteric pathogens that include enteropathogenic *Escherichia coli* (EPEC), rabbit enteropathogenic *E. coli* (REPEC), and *Citrobacter rodentium*, all of which are able to cause a lesion on intestinal epithelial cells termed

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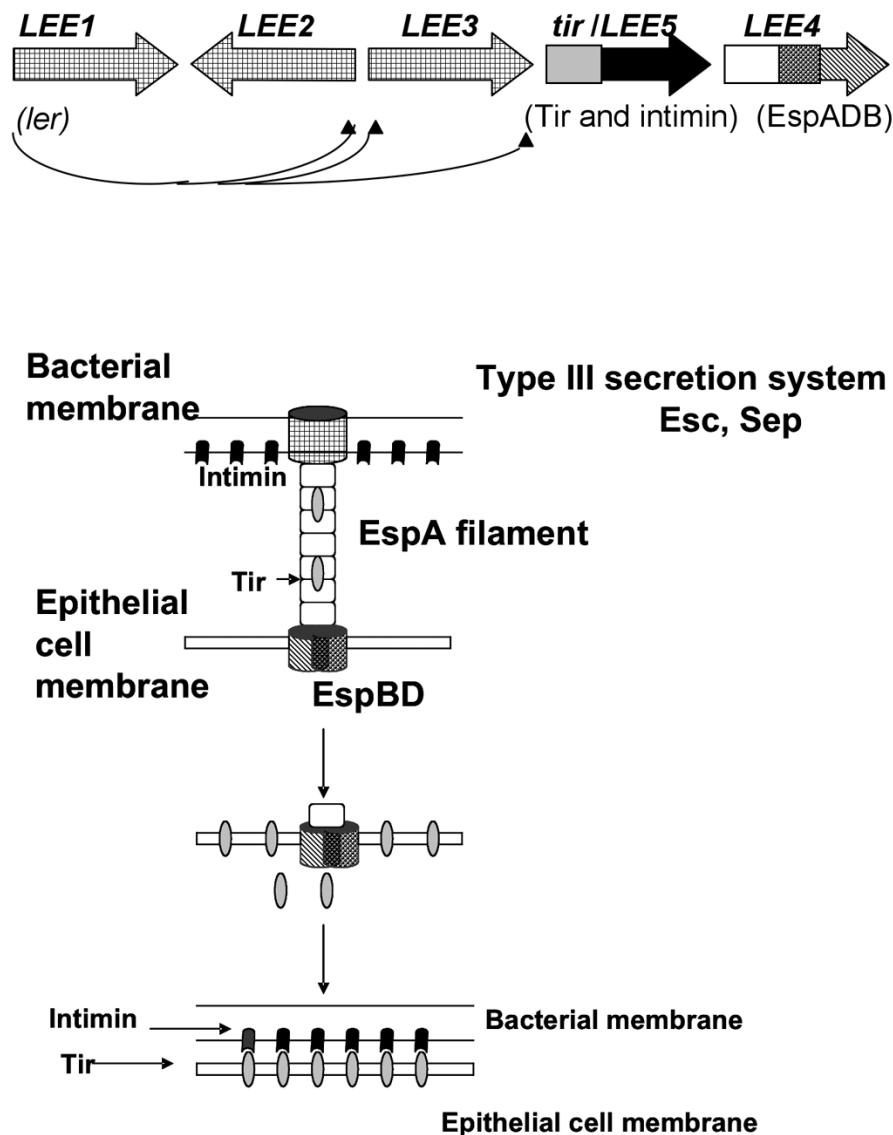


Figure 1. Operons *LEE1*, *LEE2* and *LEE3* encode for the type III secretion system (TTSS). *LEE4* encodes EscF which forms the needle complex of the TTSS; EspA which forms a sheath that involves EscF forming a pilus like structure; EspB and EspD which form a pore in the epithelial cell membrane. *LEE5* or *tir*, encodes Tir, which is translocated through the TTSS, inserts itself in the epithelial cell membrane where it serves as the receptor for the bacterial adhesin intimin (also encoded within *LEE5*). The first gene in the *LEE1* operon encodes Ler, which is the transcriptional activator of all other genes within the LEE region.

attaching and effacing (AE). This lesion is characterized by the destruction of the microvilli and the rearrangement of the cytoskeleton to form a pedestal-like structure, which cups the bacteria individually (Knutton *et al.*, 1987; Moon *et al.*, 1983; Tzipori *et al.*, 1986).

The genes involved in the formation of the AE lesion are contained on a pathogenicity island named the Locus of Enterocyte Effacement (LEE) (McDaniel *et al.*, 1995). This region contains: 1) genes encoding a type III secretion system (*sep* and *esc*) (Jarvis *et al.*, 1995); 2) the *eae* gene encoding the intimin adhesin, responsible for the intimate attachment of the bacterium to the epithelial cell (Jerse and Kaper, 1991); 3) *tir*, which encodes the translocated intimin receptor, translocated to the epithelial cells by the

bacterial type III secretion system (Kenny *et al.*, 1997); 4) the *espABDF* and *G* genes that encode proteins secreted by the type III secretion system (Donnenberg *et al.*, 1993; Kenny *et al.*, 1996; Lai *et al.*, 1997); 5) the *cesD* and *cesT* genes which encode chaperones for EspD/B and Tir, respectively (Wainwright and Kaper, 1998); and 6) *ler* (LEE Encoded Regulator) which encodes a protein that shares 24% identity and 44% similarity to HN-S of *E. coli* and activates transcription of the LEE genes (Bustamante *et al.*, 2001; Elliott *et al.*, 2000; Friedberg *et al.*, 1999; Mellies *et al.*, 1999; Sperandio *et al.*, 2000) (Figure 1).

The sequence of the entire LEE region has been reported for EPEC O127:H6 (Elliott *et al.*, 1998), EHEC O157:H7 (Perna *et al.*, 1998), REPEC (Zhu *et al.*, 2001),

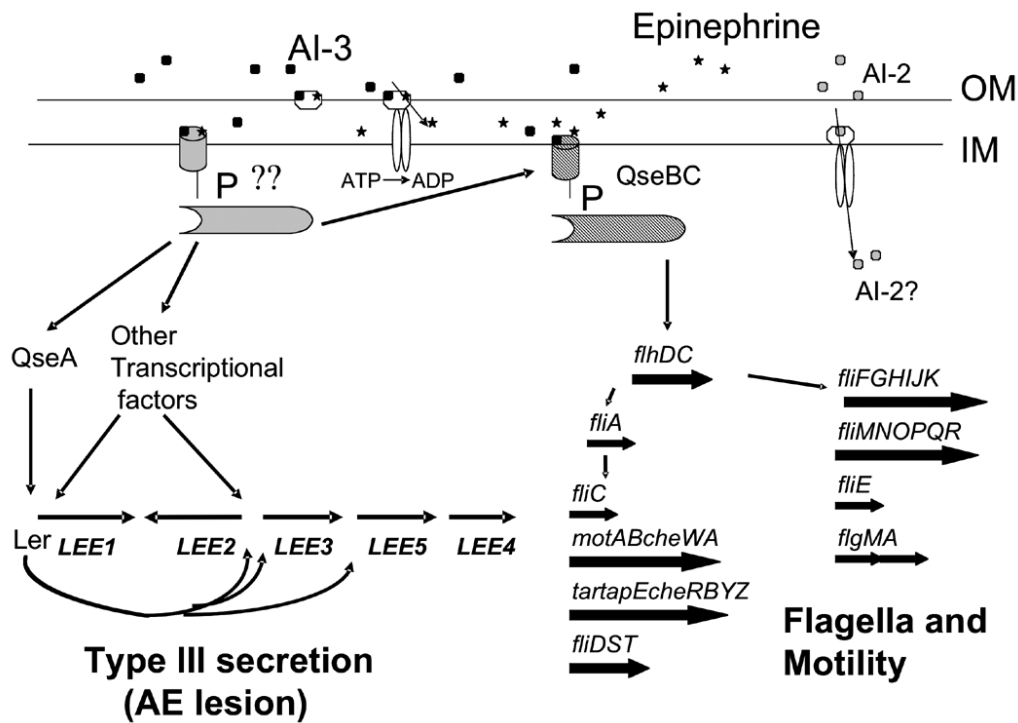


Figure 2. Model of virulence gene regulation in EHEC through AI-3 and epinephrine. Both AI-3 and epinephrine seem to be recognized by the same receptor, which is probably in the outer membrane of the bacteria, due to the non-polar nature of both signals. These signals might be imported to the periplasmic space where they interact with either one major sensor kinase that directs the transcription of other sensor kinases or with more than one sensor kinase. In the latter hypothesis QseC might be the sensor kinase transducing these signals towards activation of the flagella regulon, whether other yet unidentified sensor kinase transduces these signals to activate transcription of the LEE genes. QseA is one of the transcriptional factors involved in the regulation of *ler* (*LEE1*) transcription. Then, in a cascade fashion, *Ler* activates transcription of the other LEE genes. EHEC possess an *Isr* operon involved in recognition and uptake of AI-2, however the role of AI-2 signaling in EHEC remains to be addressed.

and *Citrobacter rodentium* (Deng *et al.*, 2001). The LEE regions in EPEC, REPEC and EHEC are very similar and each contains 41 open reading frames. The LEE regions from EPEC and EHEC contain five major operons: *LEE1*, *LEE2*, *LEE3*, *tir/LEE5* and *LEE4* (Elliott *et al.*, 2000; Mellies *et al.*, 1999; Sanchez-SanMartin *et al.*, 2001; Zhu *et al.*, 2001).

EHEC responds to both a bacterial quorum sensing signaling system and a mammalian signaling system to “fine tune” transcription of virulence genes. EHEC recognizes the bacterial autoinducer AI-3, which is produced by the resident intestinal flora, and the human hormones epinephrine and norepinephrine to recognize that it is in the large intestine (Sperandio *et al.*, 2003). Given the widespread nature of the *luxS* system in bacteria, an interesting extrapolation is that the *luxS*/AI-3 quorum sensing system might have evolved to mediate microflora-host interaction, but ended up being exploited by EHEC to activate its virulence genes. Furthermore, both AI-3 and epinephrine activate the LEE-encoded type III secretion system and the flagella regulon in EHEC, and supposedly are recognized through the same signaling cascade. The proposed model by which these signals might cross-talk suggests that both AI-3 and epinephrine are recognized by the same receptor, which is probably in the outer membrane of the bacteria due to the non-polar nature of both signals.

These signals might be imported to the periplasmic space where they interact with either one major sensor kinase that directs the transcription of other sensor kinases or with more than one sensor kinase. The interaction of AI-3 and epinephrine with more than one sensor kinase would give some “timing” to this system, which is a desirable feature, given that it would be inefficient for EHEC to produce both the LEE type III secretion system and flagella at the same time (Figure 2) (Sperandio *et al.*, 2003).

Using gene arrays, it was observed that about 10 % of the common genome between EHEC and *E. coli* K-12 (EHEC has 1.3 Mb of DNA absent in K-12, and K-12 has 0.53 Mb of DNA that is absent in EHEC; (Perna *et al.*, 2001)) is differentially regulated between wild-type EHEC and an isogenic *luxS* mutant (Sperandio *et al.*, 2001). As mentioned above, *LuxS* is not devoted to AI-2 production; it is in fact an enzyme involved in the metabolism of SAM. Consequently, altered gene expression due to a *luxS* mutation will comprise both genes affected by quorum sensing *per se*, and genes differentially expressed because of the interruption of this metabolic pathway. Furthermore, a knockout of *luxS* seems to affect the synthesis of two autoinducers, AI-2 and AI-3 (Sperandio *et al.*, 2003), suggesting that different sets of these genes may be regulated by either one or both of these signals. DeLisa and colleagues (DeLisa *et al.*, 2001) also reported, using gene

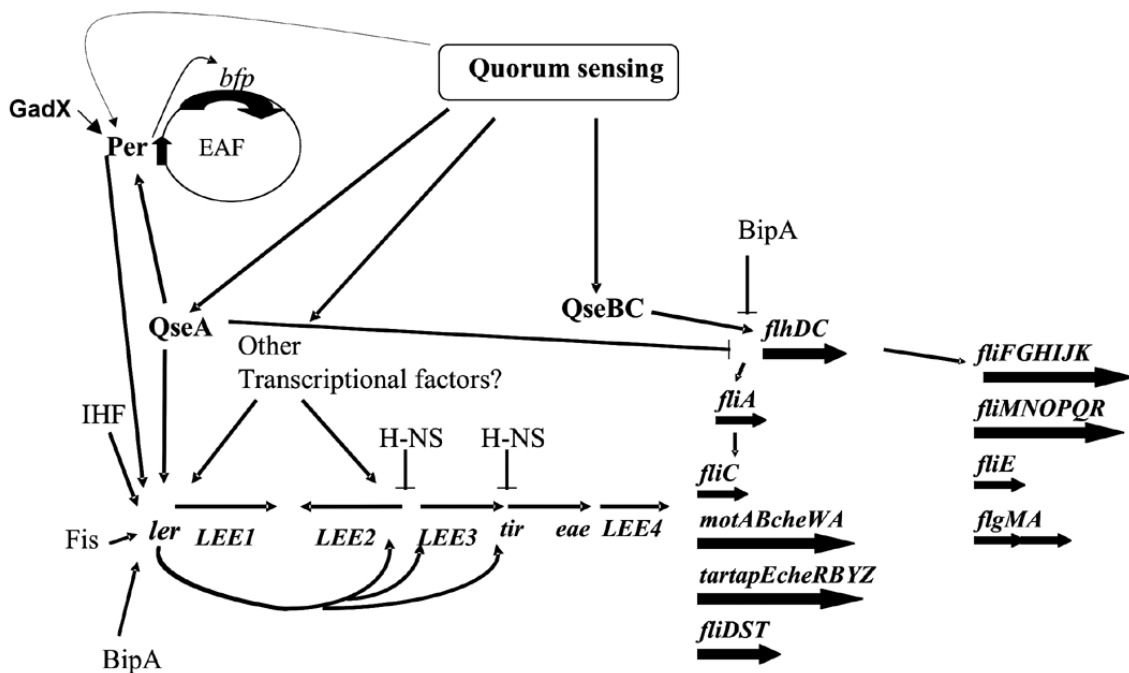


Figure 3. Schematic representation of virulence gene regulation in EPEC. *ler* activates transcription of the LEE genes by antagonizing H-NS repression. Transcription of *ler* is activated by Per, IHF, BipA, Fis, and by quorum sensing through QseA and other yet unidentified transcriptional factors. Transcription of *bfp* is activated by Per and transcription of *per* is auto-activated by Per and positively modulated by quorum sensing. Quorum sensing regulation of the flagella regulon is activated by QseBC and repressed by QseA.

arrays, that about 5.6 % of the K-12 genome was regulated by the *luxS* quorum sensing system. The difference in the amount of genes regulated by this quorum sensing system found in both reports may be due to differences in methodology (growth temperature: Sperandio *et al.* (2001) used 37°C while DeLisa *et al.* (2001) used 30°C; nutrient availability: Sperandio *et al.* (2001) grew these strains in DMEM, which was previously shown to give better expression of *luxS*-controlled genes (Sperandio *et al.*, 2001), while DeLisa *et al.* (2001) used LB broth) and in strains utilized (Sperandio *et al.* (2001) used an EHEC strain, while DeLisa *et al.* (2001) used a K-12 strain). Among the differentially regulated genes between the wild-type EHEC and the *luxS* mutant were the genes encoding Stx2 (which is the only Stx produced by the prototype EHEC strain 86-24 used in this study), flagella, and motility (which may also be involved in pathogenesis) (Sperandio *et al.*, 2001).

Very little is known regarding the details of the EHEC quorum sensing signaling cascade. Thus far, only two novel regulatory systems involved in this cascade have been described. These transcriptional factors were named quorum sensing *E. coli* regulator A (QseA) and quorum sensing *E. coli* regulators B and C (QseBC) (Sperandio *et al.*, 2002a; Sperandio *et al.*, 2002b). QseA is a regulator of the LysR family that shares homology with *V. cholerae*'s AphB and *Pseudomonas aeruginosa*'s PtXR. QseA activates transcription of the LEE genes and an EHEC *qseA* mutant had a striking reduction in type III secretion activity; secretion activity was restored when *qseA* was complemented *in trans* (Sperandio *et al.*, 2002a). In contrast

with a *luxS* mutant, in which type III secretion is abrogated "in vitro" (Sperandio *et al.*, 2003). The differential pattern of gene expression observed between a *luxS* and a *qseA* mutant suggests that there are additional transcriptional factors involved in this regulation. Thus this regulation is multifactorial. QseA is one of several transcriptional factors involved in the fine-tuning of LEE gene expression, and we have preliminary data suggesting the involvement of at least two other novel transcriptional factors in this regulation (Sperandio *et al.*, unpublished). The concerted action of several transcriptional factors is what one observes in a *luxS* mutant.

QseBC is a two-component system that shares homology with *Salmonella typhimurium* PmrAB. QseBC is another component of the quorum sensing regulatory cascade that is involved in activation of flagella and motility genes (Sperandio *et al.*, 2002b). QseC seems to be the sensor for AI-3 and epinephrine for the flagella regulon, while the sensor for the LEE genes remains unknown (Figure 2). This signaling cascade is conserved amongst enteric bacteria, given that both QseA and QseBC are present in: EHEC, EPEC, uropathogenic *E. coli* (UPEC), *E. coli* K-12, *Shigella flexneri*, *Salmonella typhimurium*, *Salmonella typhi*, and *Yersinia enterocolytica*. The role of this quorum sensing cascade has been established in EHEC and EPEC but remains to be unraveled in these other organisms.

The EHEC signaling cascade described above is responsive to AI-3 and epinephrine, the role of AI-2 in EHEC remains to be established (Sperandio *et al.*, 2003). EHEC does harbor the homologues of the *lsr* genes involved in

the uptake of AI-2 in *Salmonella typhimurium* (Taga *et al.*, 2001). *E. coli* also produces other AIs, besides AI-2 and AI-3, Wang *et al.* (Wang *et al.*, 2001) described that indole can be used as a quorum sensing signal by *E. coli*. Finally, EHEC harbors the SdiA transcriptional factor, responsive to AHLs produced by other species of bacteria (Michael *et al.*, 2001), and there is one report in the literature (Kanamaru *et al.*, 2000) suggesting that SdiA may be involved in downregulation of expression of intimin. The interactions between the circuits sensing all these signals are still in its infancy. Given that treatment of EHEC infections with conventional antibiotics is controversial due to the biology of the Stx phage, quorum sensing opens a different avenue in drug discovery. Specifically, AI-3 and epinephrine use the same signaling pathway to activate attaching and effacing lesion formation, inhibitors of these signals may constitute an alternative for treatment and prevention on potentially infected individuals. This alternative is especially promising given that the  $\beta$ -adrenergic antagonist propranolol completely abrogates the ability of EHEC to form attaching and effacing lesions in cultured epithelial cells (Sperandio *et al.*, 2003). Inhibition of the quorum sensing signaling pathway renders EHEC "blind" to the resident flora and the host signals that it uses to sense its location, therefore preventing it from activating its virulence traits.

### Quorum sensing in EPEC

EPEC colonizes the proximal small intestine and causes profuse and persistent watery diarrhea lasting up to 120 days (Fagundes-Neto, 1996) (Rothbaum *et al.*, 1982). EPEC pathogenesis has several steps, first the bacteria adheres to the intestinal epithelial cells, probably through the EspA filament, secreted by the LEE-encoded TTSS (Hicks *et al.*, 1998; Knutton *et al.*, 1998). Then Tir is translocated through the LEE-encoded type III secretion system and inserts itself into the mammalian cell membrane, where it serves as the intimin receptor allowing the intimate attachment characteristic of AE lesion formation (Kenny *et al.*, 1997). Other EPEC cells then interact with each other forming large microcolonies (Hicks *et al.*, 1998). The successful formation of these microcolonies requires the bundle forming pili (BFP) and flagella (Giron *et al.*, 1991; Giron *et al.*, 2002). The present knowledge about EPEC pathogenesis suggests that expression of EPEC virulence genes is dependent upon the concerted action of several regulatory factors (Figure 3).

EPEC contains a large plasmid, referred to as EPEC adherence factor (EAF) plasmid. The EAF plasmid encodes a regulator of virulence genes called Per (Plasmid-Encoded Regulator) consisting of three orfs: *perA*, *perB* and *perC*. PerA is an AraC homologue (Gomez-Duarte and Kaper, 1995) and activates the expression of the *bfp* operon encoding the Bundle Forming Pilus (Tobe *et al.*, 1996). The *per* loci also activate the expression of *ler*, which activates expression of the *LEE2*, *LEE3*, *LEE5* and *LEE4* operons in EPEC in a regulatory cascade (Mellies *et al.*, 1999). Transcription of *ler* is also regulated by IHF (Friedberg *et al.*, 1999), Fis (Goldberg *et al.*, 2001) and BipA (Grant *et al.*, 2003). Transcription of *per* is auto-activated by Per (Martinez-Laguna *et al.*, 1999) and downregulated by GadX (Shin *et al.*, 2001).

Quorum sensing regulation is different between EPEC and EHEC. EPEC colonizes the proximal small intestine which is thought to have very little or no resident flora. Therefore, while quorum sensing is primarily an inter-species signaling system during EHEC infection, it seems to be used for intra-species signaling during EPEC infection. In contrast to EHEC, type III secretion in EPEC is diminished, but never abrogated in a *luxS* mutant (Sperandio *et al.*, 1999; Sperandio *et al.*, 2003). This differential regulation can be explained by the additional control of the LEE genes through Per, which is absent in EHEC (Gomez-Duarte and Kaper, 1995; Mellies *et al.*, 1999). In EPEC, GadX also represses transcription of the LEE genes through Per in acid pH (possibly when EPEC is crossing the stomach) and activates their transcription in alkaline pH (possibly when EPEC reaches the small intestine) (Shin *et al.*, 2001). EPEC has to coordinate transcription of the LEE genes with microcolony formation. This is when quorum sensing regulation may play an active role. The length of the EspA filament is altered in a *luxS* mutant. EspD has been proposed to be involved in the regulation of the length of the EspA filament (Daniell *et al.*, 2001; Knutton *et al.*, 1998), and differential expression of EspD in the *luxS* mutant may be an explanation for this phenotype. Furthermore, flagellation and motility are also altered in both *luxS* and *gseA* mutants. Disruption of quorum sensing signaling affects expression of the LEE genes, BFP and the flagella regulon, thereby interfering with microcolony formation and adherence to epithelial cells (Sircili *et al.*, 2003). In EPEC, quorum sensing is probably involved in the spatial-temporal regulation of virulence genes, allowing successful colonization of host.

### Quorum sensing in *Salmonella*

*Salmonella enterica* serovar Typhimurium is a pathogenic enteric bacterium that causes an acute gastroenteritis. Regulation of essential pathogenic genes and virulence factors are of key interest, including the mechanisms by which the bacteria communicate in the environment to establish effective infection. *Salmonella* possess a *luxS* gene and produces AI-2 (Schauder *et al.*, 2001; Surette and Bassler, 1998, 1999; Surette *et al.*, 1999). A key feature of *S. typhimurium* quorum sensing is the fact that the regulation of AI-2 is monitored by environmental carbohydrates, in particular, glucose (Surette and Bassler, 1999). The only LuxS regulated genes reported in *Salmonella typhimurium* constitute the *lsr* (LuxS-regulated) operon, which encodes for a sugar ABC transporter. It was predicted that this ABC transporter, which resembles the Ribose ABC-transporter, is involved in the uptake of AI-2. Once inside the cell, AI-2 is modified and no longer used as a signaling compound. It was reported that the LsrR DNA-binding protein is required for the appropriate regulation of the *lsr* operon (Taga *et al.*, 2001). *Salmonella* also harbors the SdiA LuxR homologue, and it regulates the *rck* gene that confers resistance to human complement (Michael *et al.*, 2001). The signaling cascade for AI-3 and epinephrine is also present in *Salmonella*, and *Salmonella* does produce AI-3 (Sperandio *et al.*, unpublished). However, the role of these signaling systems in *Salmonella* pathogenesis has not yet been addressed.

### Quorum sensing in *Yersinia*

The genus *Yersinia* is comprised of 11 species, including four pathogenic species: *Yersinia enterocolitica*, *Yersinia pestis*, *Yersinia pseudotuberculosis*, and *Yersinia ruckeri*. The other seven species (*Y. aldovae*, *Y. berovieri*, *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. molaretti* and *Y. rohdei*) are considered to be non-pathogenic. Of the pathogenic species, *Y. ruckeri* is an economically important fish pathogen, which causes Red Mouth disease in many fish, particularly trout. *Y. enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis* are pathogenic for many mammals, including humans, and are currently the most well studied *Yersinia* species. *Y. enterocolitica* and *Y. pseudotuberculosis* are enteric pathogens that mainly cause a disease known as yersiniosis. The most virulent species, *Y. pestis*, causes the plague (Salyers and Whitt, 2002).

The first *Yersinia* species shown to possess a quorum sensing system was *Yersinia enterocolitica* (Throup *et al.*, 1995). *Y. enterocolitica* has been shown to produce compounds capable of transcriptionally activating the *Vibrio fischeri* bioluminescence *lux* operon. The compounds were identified as two signal molecules and characterized as N-hexanoyl-L-homoserine lactone (HHL) and N-(3-oxohexanoyl)-L-homoserine lactone (OHHL). The *yenI* gene of *Y. enterocolitica*, which is homologous to the *luxI* of *V. fischeri*, was isolated and found to direct the synthesis of both HHL and OHHL (Throup *et al.*, 1995). A second open reading frame downstream of *yenI* was described as *yenR* and found to encode a luxR homologue. An insertion mutation of *yenI* was shown to abolish acyl-HSL production (Throup *et al.*, 1995). Transcript analysis of the *yenI* mutated gene product in addition to the absence of a characteristic lux box has led to the conclusion that *yenI* expression is not subject to autoinduction. Furthermore, analysis of the secreted Yop proteins is indistinguishable between the wild type and *yenI* mutants. However, proteomic studies have indicated that a functional *yenI* is required for the expression of a number of unidentified proteins (Throup *et al.*, 1995). No other loss of phenotype was observed.

*Y. pestis* and *Y. pseudotuberculosis* genes were also described that produce LuxR/I homologues. These genes were named *ypeR* and *ypel* in *Y. pestis* and *ypsR* and *ypsl* in *Y. pseudotuberculosis* (Atkinson *et al.*, 1999; Swift *et al.*, 1999a; Yates *et al.*, 2002).

The role of quorum sensing in *Y. pestis* has not been shown, although a *ypel* and *ypeR* were identified and the strain produces signaling molecules (OHHL and HHL). No significant difference in whole cell protein profiles or expression of important virulence genes was found in studies with a *ypeR* mutant (Swift *et al.*, 1999a). In the study mentioned above, bacteria were cultured at either 28°C or 37°C. The "in vivo" assay alone showed the *yenR* mutant to be less virulent than the wild type as an increase in time to death was observed in mice challenged with the mutant.

*Yersinia pseudotuberculosis* is currently the only *Yersinia* species where the role of quorum-sensing system regulation of virulence gene expression has been addressed (Atkinson *et al.*, 1999; Yates *et al.*, 2002). In addition to the

synthesis of OHHL and HHL, this species also synthesizes a third signaling molecule classified as OHL (N-octanoyl-L-homoserine lactone). The regulation of virulence genes in *Y. pseudotuberculosis* by quorum-sensing is complex and subject to the action of at least two *luxR/I* homologues described as *ypsR/I* and *ytbR/I* (Atkinson *et al.*, 1999). It was observed that a *ypsl* mutant failed to produce OHHL (N-3-oxohexanoyl-L-homoserine-lactone) at 28°C, but at 37 °C and 22 °C the acyl-HSL production was identical to that of the wild type. The examination of liquid cultures of *ypsR* and *yenI* mutant grown at both 28 °C and 37 °C presented a number of different phenotypes in comparison to the wild type (Atkinson *et al.*, 1999). Clumping (*ypsR* mutant), over expression of a major flagellin subunit (*ypsR* mutant) and increased motility (both *ypsR* and *ypsl* mutants) were observed in the mutants. Furthermore, temperature appears to affect cellular aggregation and motility phenotypes (Atkinson *et al.*, 1999). The fact that temperature affects the behavior of these mutants involved in quorum sensing regulation is very interesting once the expression of a number *Yersinia* virulence genes is subject to changes in temperature (Badger and Miller, 1998; Mikulskis *et al.*, 1994).

It is worth to mention more about the flagellin subunit produced by the *ypsR* mutant. N-terminal analysis of the product showed this protein to be identical to *fleA* of *Y. enterocolitica* encoding the flagellin subunit. These findings support a possible role of quorum-sensing regulation of flagella production in *Y. pseudotuberculosis*. Another very interesting finding is that there may be hierarchical regulation of the *ytbR/I* by the YpsR protein. This was concluded by the observation that a *ypsR* mutant results in the loss of OHL, a product of *ytbI* gene expression.

The role of quorum sensing in non-pathogenic *Yersinia*, as well as, its role in the pathogenicity of *Y. ruckeri*, has not been examined much at this point. It is known that the non-pathogenic species as *Y. frederiksenii* and *Y. intermedia* produce acyl-HSLs (Throup *et al.*, 1995). Quorum sensing regulation of *Yersinia* genes seems to be a vast field yet to be more explored.

### References

- Atkinson, S., Throup, J.P., Stewart, G.S., and Williams, P. 1999. A hierarchical quorum-sensing system in *Yersinia pseudotuberculosis* is involved in the regulation of motility and clumping. *Mol Microbiol* 33: 1267-1277.
- Badger, J.L., and Miller, V.L. 1998. Expression of invasins and motility are coordinately regulated in *Yersinia enterocolitica*. *J Bacteriol* 180: 793-800.
- Bell, B.P., Goldoft, M., Griffin, P.M., Davis, M.A., Gordon, D.C., Tarr, P.I., Bartleson, C.A., Lewis, J.H., Barrett, T.J., Wells, J.G., and et al. 1994. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. *Jama* 272: 1349-1353.
- Bustamante, V.H., Santana, F.J., Calva, E., and Puente, J.L. 2001. Transcriptional regulation of type III secretion genes in enteropathogenic *Escherichia coli*: Ler antagonizes H-NS-dependent repression. *Mol Microbiol* 39: 664-678.

- Chen, X., Schauder, S., Potier, N., Van Dorssealaer, A., Pelczar, I., Bassler, B.L., and Hughson, F.M. 2002. Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* 415: 545-549.
- Daniell, S.J., Delahay, R.M., Shaw, R.K., Hartland, E.L., Pallen, M.J., Booy, F., Ebel, F., Knutton, S., and Frankel, G. 2001. Coiled-coil domain of enteropathogenic *Escherichia coli* type III secreted protein EspD is involved in EspA filament-mediated cell attachment and hemolysis. *Infection & Immunity* 69: 4055-4064.
- Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W., and Greenberg, E.P. 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 280: 295-298.
- de Kievit, T.R., and Iglewski, B.H. 2000. Bacterial quorum sensing in pathogenic relationships. *Infect Immun* 68: 4839-4849.
- DeLisa, M.P., Wu, C.F., Wang, L., Valdes, J.J., and Bentley, W.E. 2001. DNA microarray-based identification of genes controlled by autoinducer 2-stimulated quorum sensing in *Escherichia coli*. *J Bacteriol* 183: 5239-5247.
- Deng, W., Li, Y., Vallance, B.A., and Finlay, B.B. 2001. Locus of enterocyte effacement from *Citrobacter rodentium*: sequence analysis and evidence for horizontal transfer among attaching and effacing pathogens. *Infect Immun* 69: 6323-6335.
- Donnenberg, M.S., Yu, J., and Kaper, J.B. 1993. A second chromosomal gene necessary for intimate attachment of enteropathogenic *Escherichia coli* to epithelial cells. *J Bacteriol* 175: 4670-4680.
- Elliott, S.J., Wainwright, L.A., McDaniel, T.K., Jarvis, K.G., Deng, Y.K., Lai, L.C., McNamara, B.P., Donnenberg, M.S., and Kaper, J.B. 1998. The complete sequence of the locus of enterocyte effacement (LEE) from enteropathogenic *Escherichia coli* E2348/69. *Mol Microbiol* 28: 1-4.
- Elliott, S.J., Sperandio, V., Giron, J.A., Shin, S., Mellies, J.L., Wainwright, L., Hutcheson, S.W., McDaniel, T.K., and Kaper, J.B. 2000. The locus of enterocyte effacement (LEE)-encoded regulator controls expression of both LEE- and non-LEE-encoded virulence factors in enteropathogenic and enterohemorrhagic *Escherichia coli*. *Infect Immun* 68: 6115-6126.
- Engbrecht, J., Nealson, K., and Silverman, M. 1983. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* 32: 773-781.
- Fagundes-Neto, U. 1996. Enteropathogenic *Escherichia coli* infection in infants: clinical aspects and small bowel morphological alterations. *Rev. Microbiol.* 27: 117-119.
- Friedberg, D., Umanski, T., Fang, Y., and Rosenshine, I. 1999. Hierarchy in the expression of the locus of enterocyte effacement genes of enteropathogenic *Escherichia coli*. *Mol Microbiol* 34: 941-952.
- Giron, J.A., Ho, A.S., and Schoolnik, G.K. 1991. An inducible bundle-forming pilus of enteropathogenic *Escherichia coli*. *Science* 254: 710-713.
- Giron, J.A., Torres, A.G., Freer, E., and Kaper, J.B. 2002. The flagella of enteropathogenic *Escherichia coli* mediate adherence to epithelial cells. *Mol Microbiol* 44: 361-379.
- Goldberg, M.D., Johnson, M., Hinton, J.C., and Williams, P.H. 2001. Role of the nucleoid-associated protein Fis in the regulation of virulence properties of enteropathogenic *Escherichia coli*. *Mol Microbiol* 41: 549-559.
- Gomez-Duarte, O.G., and Kaper, J.B. 1995. A plasmid-encoded regulatory region activates chromosomal *eaeA* expression in enteropathogenic *Escherichia coli*. *Infect Immun* 63: 1767-1776.
- Grant, A.J., Farris, M., Alefounder, P., Williams, P.H., Woodward, M.J., and O'Connor, C.D. 2003. Coordination of pathogenicity island expression by the BipA GTPase in enteropathogenic *Escherichia coli* (EPEC). *Mol. Microbiol.* 48: 507-521.
- Hicks, S., Frankel, G., Kaper, J.B., Dougan, G., and Phillips, A.D. 1998. Role of intimin and bundle-forming pili in enteropathogenic *Escherichia coli* adhesion to pediatric intestinal tissue in vitro. *Infect Immun* 66: 1570-1578.
- Izumiya, H., and Watanabe, H. 1997. Genes involved in the virulence of enterohemorrhagic *Escherichia coli*. *Nippon Rinsho* 55: 641-645.
- Jarvis, K.G., Giron, J.A., Jerse, A.E., McDaniel, T.K., Donnenberg, M.S., and Kaper, J.B. 1995. Enteropathogenic *Escherichia coli* contains a putative type III secretion system necessary for the export of proteins involved in attaching and effacing lesion formation. *Proc Natl Acad Sci U S A* 92: 7996-8000.
- Jerse, A.E., and Kaper, J.B. 1991. The *eae* gene of enteropathogenic *Escherichia coli* encodes a 94-kilodalton membrane protein, the expression of which is influenced by the EAF plasmid. *Infect Immun* 59: 4302-4309.
- Kanamaru, K., Tatsuno, I., Tobe, T., and Sasakawa, C. 2000. SdiA, an *Escherichia coli* homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohaemorrhagic *Escherichia coli* O157:H7. *Mol Microbiol* 38: 805-816.
- Kenny, B., Lai, L.C., Finlay, B.B., and Donnenberg, M.S. 1996. EspA, a protein secreted by enteropathogenic *Escherichia coli*, is required to induce signals in epithelial cells. *Mol Microbiol* 20: 313-323.
- Kenny, B., DeVinney, R., Stein, M., Reinscheid, D.J., Frey, E.A., and Finlay, B.B. 1997. Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell* 91: 511-520.
- Knutton, S., Lloyd, D.R., and McNeish, A.S. 1987. Adhesion of enteropathogenic *Escherichia coli* to human intestinal enterocytes and cultured human intestinal mucosa. *Infect Immun* 55: 69-77.
- Knutton, S., Rosenshine, I., Pallen, M.J., Nisan, I., Neves, B.C., Bain, C., Wolff, C., Dougan, G., and Frankel, G. 1998. A novel EspA-associated surface organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells. *Embo J* 17: 2166-2176.
- Lai, L.C., Wainwright, L.A., Stone, K.D., and Donnenberg, M.S. 1997. A third secreted protein that is encoded by the enteropathogenic *Escherichia coli* pathogenicity island is required for transduction of signals and for attaching and effacing activities in host cells. *Infect Immun* 65: 2211-2217.
- Martinez-Laguna, Y., Calva, E., and Puente, J.L. 1999. Autoactivation and environmental regulation of *bfpT* expression, the gene coding for the transcriptional activator of *bfpA* in enteropathogenic *Escherichia coli*.

- Mol Microbiol 33: 153-166.
- McDaniel, T.K., Jarvis, K.G., Donnenberg, M.S., and Kaper, J.B. 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proc Natl Acad Sci U S A* 92: 1664-1668.
- Mellies, J.L., Elliott, S.J., Sperandio, V., Donnenberg, M.S., and Kaper, J.B. 1999. The Per regulon of enteropathogenic *Escherichia coli*: identification of a regulatory cascade and a novel transcriptional activator, the locus of enterocyte effacement (LEE)-encoded regulator (Ler). *Mol Microbiol* 33: 296-306.
- Michael, B., Smith, J.N., Swift, S., Heffron, F., and Ahmer, B.M. 2001. SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities. *J Bacteriol* 183: 5733-5742.
- Mikulskis, A.V., Delor, I., Thi, V.H., and Cornelis, G.R. 1994. Regulation of the *Yersinia enterocolitica* enterotoxin Yst gene. Influence of growth phase, temperature, osmolarity, pH and bacterial host factors. *Mol Microbiol* 14: 905-915.
- Moon, H.W., Whipp, S.C., Argenzio, R.A., Levine, M.M., and Giannella, R.A. 1983. Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. *Infect Immun* 41: 1340-1351.
- Nataro, J.P., and Kaper, J.B. 1998. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 11: 142-201.
- Nealson, K.H., Platt, T., and Hastings, J.W. 1970. Cellular control of the synthesis and activity of the bacterial luminescent system. *J Bacteriol* 104: 313-322.
- Nealson, K.H., and Hastings, J.W. 1979. Bacterial bioluminescence: its control and ecological significance. *Microbiol Rev* 43: 496-518.
- Parsek, M.R., and Greenberg, E.P. 2000. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proc Natl Acad Sci U S A* 97: 8789-8793.
- Perna, N.T., Mayhew, G.F., Posfai, G., Elliott, S., Donnenberg, M.S., Kaper, J.B., and Blattner, F.R. 1998. Molecular evolution of a pathogenicity island from enterohemorrhagic *Escherichia coli* O157:H7. *Infect Immun* 66: 3810-3817.
- Perna, N.T., Plunkett, G., 3rd, Burland, V., Mau, B., Glasner, J.D., Rose, D.J., Mayhew, G.F., Evans, P.S., Gregor, J., Kirkpatrick, H.A., Posfai, G., Hackett, J., Klink, S., Boutin, A., Shao, Y., Miller, L., Grotbeck, E.J., Davis, N.W., Lim, A., Dimalanta, E.T., Potamouisis, K.D., Apodaca, J., Anantharaman, T.S., Lin, J., Yen, G., Schwartz, D.C., Welch, R.A., and Blattner, F.R. 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature* 409: 529-533.
- Rothbaum, R., McAdams, A.J., Giannella, R., and Partin, J.C. 1982. A clinicopathologic study of enterocyte-adherent *Escherichia coli*: a cause of protracted diarrhea in infants. *Gastroenterology* 83: 441-454.
- Salyers, A.A., and Whitt, D.D. 2002. *Yersinia pestis*, the cause of Plague, and its relatives. In *Bacterial pathogenesis: a molecular approach*. Salyers, A.A. and Whitt, D.D. (eds). Washington DC: ASM Press.
- Sanchez-SanMartin, C., Bustamante, V.H., Calva, E., and Puente, J.L. 2001. Transcriptional regulation of the *orf19* gene and the *tir-cesT-eae* operon of enteropathogenic *Escherichia coli*. *J Bacteriol* 183: 2823-2833.
- Schauder, S., and Bassler, B.L. 2001. The languages of bacteria. *Genes Dev* 15: 1468-1480.
- Schauder, S., Shokat, K., Surette, M.G., and Bassler, B.L. 2001. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Mol Microbiol* 41: 463-476.
- Shin, S., Castanie-Cornet, M.P., Foster, J.W., Crawford, J.A., Brinkley, C., and Kaper, J.B. 2001. An activator of glutamate decarboxylase genes regulates the expression of enteropathogenic *Escherichia coli* virulence genes through control of the plasmid-encoded regulator, Per. *Mol Microbiol* 41: 1133-1150.
- Sircili, M.P., Walters, M., Trabulsi, L., and Sperandio, V. 2003. Modulation of enteropathogenic *E. coli* (EPEC) virulence by quorum sensing. Submitted.
- Sperandio, V., Mellies, J.L., Nguyen, W., Shin, S., and Kaper, J.B. 1999. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc Natl Acad Sci U S A* 96: 15196-15201.
- Sperandio, V., Mellies, J.L., Delahay, R.M., Frankel, G., Crawford, J.A., Nguyen, W., and Kaper, J.B. 2000. Activation of enteropathogenic *Escherichia coli* (EPEC) *LEE2* and *LEE3* operons by Ler. *Mol Microbiol* 38: 781-793.
- Sperandio, V., Torres, A.G., Giron, J.A., and Kaper, J.B. 2001. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. *J Bacteriol* 183: 5187-5197.
- Sperandio, V., Li, C.C., and Kaper, J.B. 2002a. Quorum-sensing *Escherichia coli* regulator A (QseA): a regulator of the LysR family involved in the regulation of the LEE pathogenicity island in enterohemorrhagic *Escherichia coli*. *Infect. Immun.* 70: 3085-3093.
- Sperandio, V., Torres, A.G., and Kaper, J.B. 2002b. Quorum sensing *Escherichia coli* regulators B and C (QseBC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in *E. coli*. *Mol. Microbiol.* 43: 809-821.
- Sperandio, V., Torres, A.G., Jarvis, B., Nataro, J.P., and Kaper, J.B. 2003. Bacteria-host communication: the language of hormones. *Proc. Natl. Acad. Sci. USA* 100: 8951-8956.
- Surette, M.G., and Bassler, B.L. 1998. Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. *Proc Natl Acad Sci U S A* 95: 7046-7050.
- Surette, M.G., and Bassler, B.L. 1999. Regulation of autoinducer production in *Salmonella typhimurium*. *Mol Microbiol* 31: 585-595.
- Surette, M.G., Miller, M.B., and Bassler, B.L. 1999. Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proc Natl Acad Sci U S A* 96: 1639-1644.
- Swift, S., Isherwood, K.E., Atkinson, S., Oyston, P.C., and Stewart, G.S. 1999a. Quorum sensing in *Aeromonas* and *Yersinia*. In *Microbial signaling and communication*. England, R., Hobbs, G., Bainton, N. and Roberts, D. (eds). Cambridge: Cambridge University Press.
- Swift, S., Lynch, M.J., Fish, L., Kirke, D.F., Tomas, J.M., Stewart, G.S., and Williams, P. 1999b. Quorum sensing-

- dependent regulation and blockade of exoprotease production in *Aeromonas hydrophila*. *Infect Immun* 67: 5192-5199.
- Taga, M.E., Semmelhack, J.L., and Bassler, B.L. 2001. The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. *Mol Microbiol* 42: 777-793.
- Thomas, A., Chart, H., Cheasty, T., Smith, H.R., Frost, J.A., and Rowe, B. 1993. Vero cytotoxin-producing *Escherichia coli*, particularly serogroup O 157, associated with human infections in the United Kingdom: 1989-91. *Epidemiol Infect* 110: 591-600.
- Throup, J.P., Camara, M., Briggs, G.S., Winson, M.K., Chhabra, S.R., Bycroft, B.W., Williams, P., and Stewart, G.S. 1995. Characterisation of the *yenI/yenR* locus from *Yersinia enterocolitica* mediating the synthesis of two N-acylhomoserine lactone signal molecules. *Mol Microbiol* 17: 345-356.
- Tobe, T., Schoolnik, G.K., Sohel, I., Bustamante, V.H., and Puente, J.L. 1996. Cloning and characterization of *bfpTVW*, genes required for the transcriptional activation of *bfpA* in enteropathogenic *Escherichia coli*. *Mol Microbiol* 21: 963-975.
- Tzipori, S., Wachsmuth, I.K., Chapman, C., Birden, R., Brittingham, J., Jackson, C., and Hogg, J. 1986. The pathogenesis of hemorrhagic colitis caused by *Escherichia coli* O157:H7 in gnotobiotic piglets. *J Infect Dis* 154: 712-716.
- Wainwright, L.A., and Kaper, J.B. 1998. EspB and EspD require a specific chaperone for proper secretion from enteropathogenic *Escherichia coli*. *Mol Microbiol* 27: 1247-1260.
- Wang, D., Ding, X., and Rather, P.N. 2001. Indole can act as an extracellular signal in *Escherichia coli*. *J Bacteriol* 183: 4210-4216.
- Wang, X.D., de Boer, P.A., and Rothfield, L.I. 1991. A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*. *Embo J* 10: 3363-3372.
- Winzer, K., Hardie, K.R., Burgess, N., Doherty, N., Kirke, D., Holden, M.T., Linforth, R., Cornell, K.A., Taylor, A.J., Hill, P.J., and Williams, P. 2002. LuxS: its role in central metabolism and the in vitro synthesis of 4-hydroxy-5-methyl-3(2H)-furanone. *Microbiology* 148: 909-922.
- Yates, E.A., Philipp, B., Buckley, C., Atkinson, S., Chhabra, S.R., Sockett, R.E., Goldner, M., Dessaux, Y., Camara, M., Smith, H., and Williams, P. 2002. N-acylhomoserine lactones undergo lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. *Infect Immun* 70: 5635-5646.
- Zhu, C., Agin, T.S., Elliott, S.J., Johnson, L.A., Thate, T.E., Kaper, J.B., and Boedeker, E.C. 2001. Complete nucleotide sequence and analysis of the locus of enterocyte Effacement from rabbit diarrheagenic *Escherichia coli* RDEC-1. *Infect Immun* 69: 2107-2115.

