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REVIEW

QseBC, a two-component bacterial adrenergic receptor and global regulator of virulence in *Enterobacteriaceae* and *Pasteurellaceae*

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SUMMARY

The QseBC two-component system (TCS) is associated with quorum sensing and functions as a global regulator of virulence. Based on sequence similarity within the sensor domain and conservation of an acidic motif essential for signal recognition, QseBC is primarily distributed in the *Enterobacteriaceae* and *Pasteurellaceae*. In *Escherichia coli*, QseC responds to autoinducer-3 and/or epinephrine/norepinephrine. Binding of epinephrine/norepinephrine is inhibited by adrenergic antagonists; hence QseC functions as a bacterial adrenergic receptor. *Aggregatibacter actinomycetemcomitans* QseC is activated by a combination of epinephrine/norepinephrine and iron, whereas only iron activates the *Haemophilus influenzae* sensor. QseC phosphorylates QseB but there is growing evidence that QseB is activated by non-cognate sensors and regulated by dephosphorylation via QseC. Interestingly, the QseBC signaling cascades and regulons differ significantly. In enterohemorrhagic *E. coli*, QseC induces expression of a second adrenergic TCS and phosphorylates two non-cognate response regulators, each of which induces specific sets of virulence genes. This signaling pathway integrates with other regulatory mechanisms mediated by transcriptional regulators QseA and QseD and a fucose-sensing TCS and likely controls the level and timing of virulence gene expression. In contrast, *A. actinomycetemcomitans* QseC signals through QseB to regulate genes involved in anaerobic metabolism and energy production, which may prime cellular metabolism for growth in an anaerobic host niche. QseC represents a novel target for therapeutic intervention and small molecule inhibitors already show promise as broad-spectrum antimicrobials. Further characterization of QseBC signaling may identify additional differences in QseBC function and inform further development of new therapeutics to control microbial infections.

INTRODUCTION

The ability to quickly respond and adapt to environmental flux is central for microbial colonization and persistence in a given niche or host organism. Bacteria sense and respond to changes in their environment using a variety of mechanisms such as two-component sensing and quorum sensing. In two-component systems (TCS), an environmental 'signal' is detected by a sensor histidine kinase, which initiates a signal transduction cascade that in many cases results in transcriptional regulation of target genes. Although TCS are broadly distributed in bacteria, for many TCS the specific signal that activates the sensor kinase and the

Molecular Oral Microbiology **31** (2016) 379–397

target genes that are regulated upon its activation have not been identified. The QseBC TCS is conserved in a broad spectrum of bacterial species (Clarke *et al*., 2006) and is closely associated with quorum-sensing mechanisms (Sperandio *et al*., 2002; Novak *et al*., 2010) and generally functions as a global regulator of virulence. As shown in Fig. 1, the QseC histidine kinase is comprised of a periplasmic sensor domain, two membrane-spanning domains, and a cytoplasmic kinase domain. The QseB response regulator is comprised of a receiver domain and a helix-turn-helix (HTH) DNA binding domain. QseBC is one of the few TCS where the signal that activates the sensor has been identified for several organisms and the regulon that is controlled by the system has been characterized. This review focuses on the distribution of the QseBC TCS, the mechanisms of activation of the sensor kinase and response regulator, the signal transduction pathways that are initiated upon activation of the system, and the regulons and functional outcomes of QseBC. It will also highlight the similarities and differences of this system in pathogens that colonize mucosal surfaces in the human oral cavity and in the gastrointestinal and urogenital tracts.

DISTRIBUTION AND STRUCTURE OF THE QSEBC TCS

The National Center for Biotechnology Information (NCBI) Protein Database contains over 12,000 proteins that exhibit similarity to the Conserved Protein Domain Family PRK10337, which is designated as sensor protein QseC. However, PRK10337 encompasses the cytoplasmic kinase and ATPase domains that are highly conserved across a wide range of sensor kinase polypeptides. Hence, it is likely that many of these 12,000 proteins are not paralogs of QseC but instead represent sensor kinases of other TCS. In contrast, a search of the NCBI Gene Database for genes that are annotated as *qseC* yielded a more limited set of only 164 genes. Of these, 159 are encoded by α-, β- and γ-proteobacteria and the remaining five are contained in eukaryotic genomes. Consistent with this, a protein BLAST search carried out by Clarke *et al.* (2006) using only the periplasmic sensor domain of *Escherichia coli* QseC identified related sequences in the genera *Shigella*, *Salmonella*, *Erwinia*, *Haemophilus*, *Pasteurella*, *Actinobacillus*, *Chromobacterium*, *Rubrivivax*, *Thiobacillus*, *Ralstonia*, *Psychrobacter* and *Aspergillus*. Similar results were obtained using the periplasmic sensor domain of *Aggregatibacter actinomycetemcomitans* QseC (residues 37–169) as a probe. This search identified QseC-like sequences primarily in the α-, β- and γ-proteobacteria (see Table 1). As shown in Fig. 1, a further comparison of the periplasmic sensor domains of QseC-like sequences encoded by organisms in the *Enterobacteriaceae* and *Pasteurellaceae* families showed that the EYRDD motif (boxed in Fig. 2) which was previously shown to be essential for QseC signal

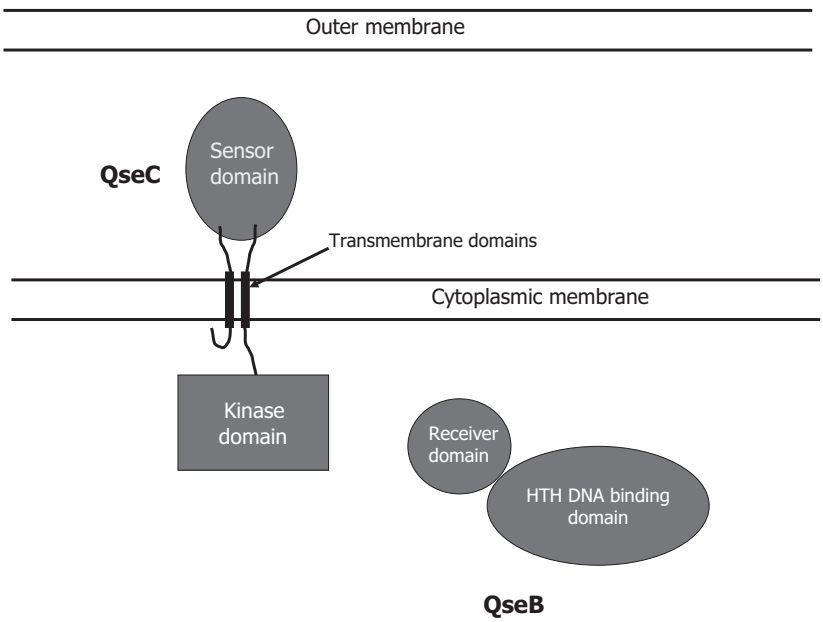

Figure 1 Schematic illustrating the structural domains of the QseC histidine kinase and the QseB response regulator.

Table 1 Comparison of QseC-like proteins with the periplasmic sensor domain of *Aggregatibacter actinomycetemcomitans* QseC

			Acidic motifs[2]		
	% identity	% coverage[1]	EDD	EDDDE	EYRDD
γ-Proteobacteria					
Enterobacteriaceae	45–52	97–100	+[3]	+	+
Pasteurellaceae	45–100	96–100	+	+	+
Pseudomonadales	24–38	19–96	±	–	±
Alteromonadales	22–38	25–99	–	–	±
β-Proteobacteria					
Burkholderiaceae	25–32	42–100	–	–	–
Alcaligenaceae[4]	27–54	54–100	±	±	±
Comamonadaceae	26–48	15–79	–	–	–
Neisseriaceae	27–48	23–97	±	±	±
α-Proteobacteria					
Rhizobiales	26–47	36–99	–	–	–
Rhodobacterales	25–45	32–	–	–	–
Eukaryotes					
Animals	43[5]	61	+	+	+
Fungi	48–56	13–16	–	–	–
Green plants	44	19	–	–	–

[1]Coverage represents the portion (in per cent) of the probe sequence that exhibits similarity to the target sequence.

[2]The acidic motifs present in the periplasmic signal domain of *A. actinomycetemcomitans* QseC are highlighted in red in Fig. 1.

[3]'+' indicates that the motif was conserved in all of the sequences that were examined; '±' indicates that the motif was conserved in some of the sequences; '–' indicates that the motif was not conserved in the sequences that were examined.

[4]Only one organism in this family, *Basilea psittacipulmonis*, exhibited 54% sequence identity with the periplasmic signal domain of *A. actinomycetemcomitans* QseC and contained all three acidic motifs.

[5]The Mediterranean fruit fly, *Ceratitis capitata*, exhibited 43% sequence identity across 80 residues in the periplasmic domain and contains the three acidic motifs present in QseC of Enterobacteria and Pasteurellales.

recognition in *A. actinomycetemcomitans* (Weigel *et al*., 2015) and *Haemophilus influenzae* (Steele *et al*., 2012) was conserved in all sequences. A similar acidic motif is also present in multiple copies in the PmrB sensor of *Salmonella enterica*, which is closely related to QseC. Further examination of the QseC-like sequences in Fig. 2 identified two additional acidic motifs that are highly conserved (shown in red).

Only two QseC-like sequences outside the *Enterobacteriaceae* and *Pasteurellaceae* families showed similar high levels of sequence identity of the periplasmic sensor domain and conservation of the acidic motifs described above; QseC of *Basilea psittacipulmonis* in the family *Alcaligenaceae* and, surprisingly, QseC encoded by the Mediterranean Fruit Fly, *Ceratitis capitata*. Both of these proteins also possess the cytoplasmic kinase, dimer interface and ATPase domains that are present in the other QseC polypeptides. As shown in Table 1, the QseC-like proteins encoded by the remaining families and orders of organisms either exhibited lower overall sequence identity (typically $< 40\%$), exhibited similarity to only a portion of the *A. actinomycetemcomitans* sensor domain or both. These QseC-like proteins also lacked the conserved acidic motif(s) that is essential for QseC signal recognition. As a representative example, the QseC-like sequence from *Laribacter hongkongensis* (family *Neisseriaceae*) is included in Fig. 2. This sequence exhibits only 30% identity to the sensor domain of *A. actinomycetemcomitans* QseC and lacks the EYRDD motif involved in signal recognition.

Together, the comparisons described above suggest that QseC is structurally and functionally conserved mainly in the *Enterobacteriaceae* and *Pasteurellaceae* and in a limited number of organisms outside of these groups. Furthermore, the observation that the EYRDD motif involved in QseC signal recognition is not conserved in the QseC-like sequences in the other organisms suggests that these sensors may respond to different stimuli. However, it is also possi-

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Aa      -QVRKEVNQVFDAQQILFAQRLASSDLRTI-LIGHQRGKP--SHKRHGFK-R-PDYEDDA
Ec      -QTTDNVDELFDTQLMLFAKRLSTLDLNEI---NAADRMA--QTPNRLKH---GHVDDDA
Se      RKTTDNVDELFDTQLMLFARRLSTLDLNEI---NAPQRMA--HTPKKLKH---GHIDDDA
Sf      KQTTDNVDELFDTQLMLFAKRLSTLDLNEI---NAADRMA--QTPNKLKH---GHVDDDA
Pa      -QTRHNINELFDTQQMLFAKRLATMNPDEL--QIQSTSLP--KTKSLVHK-NRGKQDDDA
Ecl     -QTTHKLDKLFDTQQMLFAKRLLTMDLDEI---RAPERMR--EIPKKVKH---GRLDDDA
Pan     -KTRDTVNELFDTQQMLLAKRLLTLDFTTL----NGTSLP--KTKKLLDH-NRGEQDDDA
Sm      --SRNTINEVFDTQQMLFAKRLATANLGDLLADESARSLP--KTKKLVHHGKRGEQDDDA
Kp      QQTSKKLDKLFDTQQLLFARRLSVMHFDEL---RAPPALL--GEKKKVRH---GHIDDDA
Ap      -KFREEMDKQFDTQQVLFAERLASSNIMQ----GFHEIRP--RHRRHFYQ---KHVDDDA
Hi      -QVRHDVNKVFDAQQVLFAERLANSDLSTI-LLESSTTLN--KNPQSVLK-K--SYDDDA
Pm      -QVRKEVNDVFDAQQILLAQRLASANLHNM-LIARA---P--HDVNKQLK-KVRHYDDDA
Bp      -RASEEVNELFDAQQILFAQRLASSNLHEL-LNASEPRKRLIQRQKRKYR-K-INIEDDA
Cc      ----------------------------------------------------MDDDA
Lh      -RIQHEMKDLMEGELERSALILIHTEADD-------------RTLQELRK----YEDEHT

Aa      LA--FAIFAPDGDILLSDGENGENFIFA-PARGFSKSRLREDDDE------WRIFWLPVG
Ec      LT--FAIFTHDGRMVLNDGDNGEDIPYSYQREGFADGQLVGEDDP------WRFVWMTSP
Se      LA--FAIFSADGKMLLHDGDNGQDIPYRYRREGFDNGYLKDDNDL------WRFLWLNSA
Sf      LT--FAIFTHDGRMVLNDGDNGEDIPYSYQREGFADGQLVGEDDP------WRFVWMTSP
Pa      LA--FAIFTRNGKMVLNDGDNGKDFIFDSTRNGFTDGKLRDDNDA------WRIVWLTTE
Ecl     LA--FAIYATDGTMILNDGENGRDIPYHYRRDGFDDGRLKDDNDD------WRFLWLSSP
Pan     LA--FAVFDREGKQVLNDGDNGKDLPFNADHQGFQDGQLNGDDDL------WRFVWLTTP
Sm      LA--FAIFDRDGKMLLNDGENGADFLFDGEREGFTDGERKGDDDS------WRLVWLTSP
Kp      LA--FAIFTRDGKMVLNDGENGEDIQWNSQREGFSDGYLRDDDDE------WRFLWLTTA
Ap      LA--FAVFTEQGDPIFNDGRDGQFIEFA-PHRGFKNVRLIEHDDEEDEVDTWRIFWLKHR
Hi      LA--FAIFSKTGKLLFSDGRNGKDFIFN-NKMGFYNSHTYDDDDN------WRIFWRMAA
Pm      LA--FAIFNHRGDLLLSDGNNGENFIFA-PHNGFSVSAIREDDDR------WRIFWLPVN
Bp      LA--FAVFSRDGNLLLSDGRNGDDFYFV-NKRGFSIDRIKDDDEK------WRIFWLPSA
Cc      LT--FAIFTVDGRMVINDGENGPDIPYAYTQDGFFDGPMYNDEDE------WRYLWITTP
Lh      LGTVFSIYDNKGRLLASSSDHPLPLIGD-------DDTIRYRGKP------WLAHVLTGH

Aa      DGPLMIAVGQEQ EYRDD LVNEMVFGQ---
Ec      DGKYRIVVGQEW EYRED MALAIVAGQ---
Se      DGKYRIVVGQEW DYRED MALAIVAAQLTP
Sf      DGKYRIVVGQEW EYRED MALAIVAGQLIP
Pa      DNRYVIAVGQEW EYRQD MTLDIVKTNLMP
Ecl     DGKYRVVVGQEW EYRQE MALDVVSSQLTP
Pan     DQRYRVVVGQEW EYRDD MAKDLVDASMLP
Sm      DGRYRIVVGQEW DYRRD MALGMVTGQLVP
Kp      DGRYRIAVGQEW DYRRE MAMDIVTSQLTP
Ap      D--LYIAVGQEI DYRNK IINKVMASK---
Hi      NGELVIAVGQEL DYRED LIEEMIFGQ---
Pm      QGKWIIAVGQEM DYRED LINQMVFGQ---
Bp      NGKLMIAVGQEV DYRQS LIRKIVFAQ---
Cc      DGKYHIVVGQEK EYREE MAMKIITRQ---
Lh      DRTIVLAMPESI QL--D MAREVLKK----
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Figure 2 Alignment of sequences derived from the QseC periplasmic sensor domains of the following organisms: Aa, *Aggregatibacter actinomycetemcomitans*; Ec, *Escherichia coli*; Se, *Salmonella enterica*; Sf, *Shigella flexneri*; Pa, *Pectobacterium atrosepticum*; Ecl, *Enterobacter cloacae*; Pan, *Pantoea ananatis*; Sm, *Serratia marsescens*; Kp, *Klebsiella pneumonia*; Ap, *Actinobacillus pleuropneumoniae*; Hi, *Haemophilus influenzae*; Pm, *Pasteurella multocida*; Bp, *Basilea psittacipulmonis*; Cc, *Ceratitis capitatta*; and Lh, *Laribacter hongkongensis*. Three conserved acidic motifs are shown in red text and the motif that is essential for signal binding is boxed.

ble that sensor proteins exist that are functionally related to QseC but without significant sequence similarity in the sensory domain. For example, the QseC-like sensor of *Francisella tularensis* is reported to be functionally interchangeable with QseC of *E. coli* (Rasko *et al.*, 2008) but the periplasmic domain of this protein exhibits no homology with the sensor domain of the *E. coli* or *A. actinomycetemcomitans* QseC proteins. This review will focus primarily on the properties and activities of QseC in the *Enterobacteriaceae* and *Pasteurellaceae*.

In many organisms, the *qseBC* locus is associated with another gene, designated *ygiW*, that encodes a putative periplasmic protein in the OB fold family (Ginalski *et al.*, 2004), but transcription and genetic organization of *ygiW* relative to the *qseBC* operon varies. As shown in Fig. 3A, *ygiW* resides upstream from and is co-transcribed with *qseBC* in many *Pasteurellaceae*. Steele *et al.* (2012) and Juarez-Rodriguez *et al.* (2013) have shown that an attenuator stem loop exists in the intergenic region between *ygiW* and *qseBC* in *H. influenzae* and *A. actinomycetemcomitans*, respectively. Hence, primary transcripts encoding *ygiW* alone and *ygiW-qseBC* are produced by these organisms and the overall expression of *qseBC* is reduced relative to *ygiW* (Steele

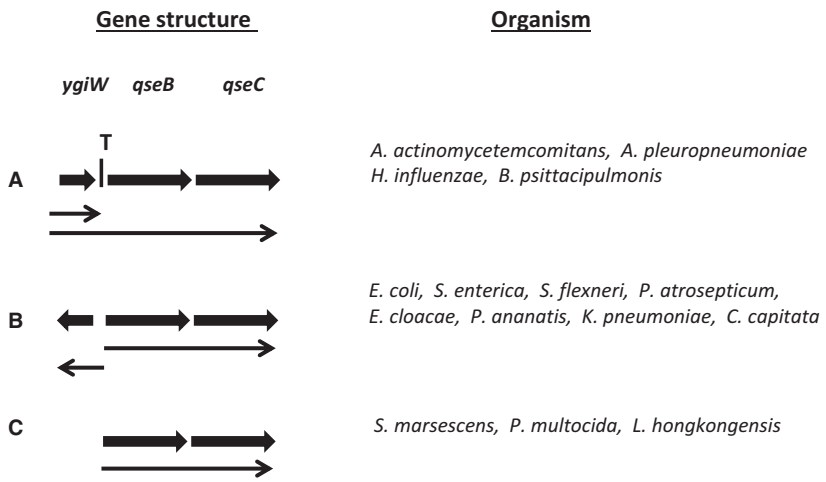

Figure 3 Comparison of the organization of the *qseBC* locus. In many organisms in the *Enterbacteriaceae* and *Pasteurellaceae* families, *ygiW* is adjacent to *qseBC* and is either co-transcribed with *qseBC* (A) or transcribed from the opposite strand as *qseBC* (B). For other organisms, *ygiW* is not adjacent to *qseBC* (C) and is either located elsewhere in the genome or is not present at all. The transcriptional attenuator located between ygiW and qseBC is indicated by the letter 'T'. Arrows indicate the direction of transcription.

et al., 2012). A similar attenuation of *qseBC* transcription probably occurs in *Actinobacillus pleuropneumoniae* and *B. psittacipulmonis* as both of these operons also contain an inverted repeat in the *ygiW-qseB* intergenic region that may form a stem loop resembling a rho-independent terminator. *ygiW* also resides upstream of *qseBC* in many of the available *Enterobacteriaceae* genome sequences, but in these organisms it is transcribed from the opposite strand (see Fig. 3B). A similar gene configuration is present in the fruit fly, *C. capitata*. Finally, in organisms such as *Serratia marsescens*, *Pasteurella multocida* and *L. hongkongensis*, *ygiW* is not adjacent to the *qseBC* locus (see Fig. 3C) but in some cases is present elsewhere in the genome.

Transcription of *ygiW* has been shown to be regulated by the QseB response regulator *S. enterica* (Merighi *et al*., 2009), *A. actinomycetemcomitans* (Juarez-Rodriguez *et al*., 2013) and *H. influenzae* (Steele *et al*., 2012), and presumably in the other organisms shown in Fig. 3A,B. In *S. enterica*, *ygiW* has been associated with virulence through several mechanisms. Moreira *et al.* (2013) showed that *visP* (*ygiW*) encodes a protein that binds to the sugar moiety of peptidoglycan and inhibits Fe^{2+}/α-ketoglutarate-dependent dioxygenase (LpxO). This results in decreased LpxO-dependent modification of lipopolysaccharide and increased resistance to stressors within the vacuole during intramacrophage replication. However, VisP was also shown to function independently of LpxO in a murine colitis model and conferred resistance to cationic antimicrobial peptides (Moreira *et al*., 2013). Similarly in *E. coli*, YgiW has been suggested to be part of a stress response circuit that confers resistance to hydrogen peroxide, cadmium and acid stress (Lee *et al*., 2010). The function of YgiW in other organisms has not been determined.

ACTIVATION OF THE QSEC SENSOR

Sperandio *et al.* (1999) showed that expression of the Locus of Enterocyte Effacement (LEE) operon encoding the type III secretion system (TTSS) of enterohaemorrhagic *E. coli* (EHEC) serotype O157:H7 was induced by autoinducer-2 (AI-2)-dependent quorum sensing and to identify other potential quorum-sensing-regulated virulence genes and regulatory components, a gene array was hybridized with cDNA derived from *E. coli* O157:H7 or an isogenic *luxS* mutant. The quorum-sensing *E. coli* regulator B and C (QseBC) was identified in these studies as a TCS that was regulated by AI-2-dependent quorum sensing (Sperandio *et al*., 2002). This study also showed that the motility of a *luxS*, but not *qseC*, mutant could be restored by exogenous AI-2, suggesting that QseBC is necessary to respond to AI-2. Consistent with these early observations, Gonzalez Barrios *et al.* (2006) subsequently showed that AI-2 increased biofilm growth of wild-type *E. coli* MG1655 but not an isogenic *qseBC* mutant. In addition, Novak *et al.* (2010) showed that *qseBC* expression in *A. actinomycetemcomitans* was induced by exogenous AI-2 and that induction required the putative AI-2 receptors, LsrB and RbsB. Athough each of these studies suggested that QseBC may represent a part of the AI-2 signaling circuit, they did not directly demonstrate that AI-2 functions as a signal that activates the QseC sensor. Indeed, AI-2 that was partially

purified by chromatography on a C-18 Sep Pack column was shown to induce bioluminescence of a *Vibrio harveyi* reporter strain, but this preparation failed to induce expression of the LEE1 operon, restore type III secretion, or induce *qseBC* expression in EHEC. In contrast, a fraction that was eluted with methanol increased transcription of LEE1 and *qseBC* (Sperandio *et al*., 2003) but did not induce *V. harveyi* bioluminescence. The structure of this compound, designated autoinducer-3 (AI-3) has not yet been determined but electrospray mass spectroscopy demonstrated a major peak of 213.1 Da and several minor peaks between 109.1 Da and 222.9 Da. Gonzalez Barrios *et al.* (2006) also suggested that AI-2 does not directly activate the QseC sensor and showed that stimulation of *E. coli* biofilm growth by AI-2 requires the protein encoded by open reading frame b3022, which in turn regulates *qseBC*. B3022 was designated MqsR (motility quorum-sensing regulator) and is now known to be a CGU-specific mRNA interferase (Yamaguchi & Inouye, 2009). This signaling mechanism may also function in *A. actinomycetemcomitans*, which encodes a homolog of MqsR (Novak *et al*., 2010), but not in *E. coli* O157:H7 which lacks this gene. Hence, AI-2 is not the signal that activates QseC and it is possible that the link between AI-2 quorum sensing and QseBC function may exist only in organisms that also express MqsR. In contrast, AI-3 represents an autoinducer that directly signals through QseC but its initial identification was confounded by the fact that AI-3 production is reduced as a result of the metabolic deficiency that is caused by the inactivation of the AI-2 synthase *luxS* (Walters *et al*., 2006). Walters *et al.* (2006) also showed that AI-3 is produced by other enterobacteria such as *Shigella* sp., *Salmonella* sp., *Klebsiella pneumonia* and *Enterobacter cloacae*. However, it is not known if AI-3 is more widely produced by other groups of organisms, as is AI-2. For example, we have so far been unable to identify AI-3 in *A. actinomycetemcomitans* (Weigel *et al*., 2015).

Sperandio *et al.* (2003) also showed that the ability of the EHEC *luxS* mutant (deficient in AI-3 production) to generate attaching and effacing (A/E) lesions on HeLa cells was indistinguishable from the parent strain. This was surprising because in the absence of AI-3, the expression of the LEE operon encoding the TTSS required for the development of A/E lesions should be significantly reduced. This result suggested that HeLa cells may produce a factor that complements AI-3 deficiency. Consistent with this, type III secretion of the *luxS* mutant was restored when bacteria were incubated with conditioned medium from HeLa cells that were size fractionated for compounds > 1 kDa. It was subsequently shown that purified epinephrine (Ep) or norepinephrine (Ne) at physiologic concentrations (i.e. 50 μM) increased expression of LEE1 and activated type III secretion in the *luxS* mutant. Furthermore, complementation of AI-3 deficiency by these hormones was blocked by α- and β-adrenergic antagonists (Sperandio *et al*., 2003). In addition, a *qseC* mutant was not complemented by exogenous AI-3 or Ep whereas each compound complemented the EHEC *luxS* mutant, suggesting that both AI-3 and Ep may signal via the QseC sensor. This was subsequently demonstrated directly by Clarke *et al.* (2006), who showed that Ne and AI-3 bound to the periplasmic domain of QseC and induced autophosphorylation of the sensor. Binding and autophosphorylation of QseC were significantly reduced in the presence of an α-adrenergic receptor antagonist. Hence, the *E. coli* QseC sensor mediates both interspecies and interkingdom signaling and is activated by signals that are produced by bacteria (AI-3) and the eukaryotic host (Ep, Ne). In addition, Moreira & Sperandio (2012) suggest that the QseC paralog in *S. enterica* (PreB) functions as an adrenergic receptor whereas Merighi *et al.* (2009) report that the PreB does not respond to catecholamine hormones.

Although biochemical identification of the signals that activate many of the other QseC paralogs shown in Fig. 2 has not been reported, some surprising differences exist for those that have been characterized. Weigel *et al.* (2015) showed that *A. actinomycetemcomitans* QseC is activated by a combination of catecholamine hormones and iron, but not by either compound individually. This suggests that a catecholamine–iron complex may be the signal that is recognized by the *A. actinomycetemcomitans* sensor, or alternatively that catecholamines and iron interact individually with the sensor and activation of QseC occurs only when both are bound. In addition, as production of AI-3 by *A. actinomycetemcomitans* has not yet been demonstrated (Weigel *et al*., 2015), catecholamine hormones and iron may be the only signals that activate QseC in this organism. Interestingly, *qseBC* expression in *E. coli* is also

induced by elevated levels of Fe^{3+} but QseC is not directly activated by iron. Instead, iron activates the PmrB sensor, which in turn phosphorylates response regulator PmrA. Activated PmrA then binds to the *qseBC* promoter and induces expression of the operon. Furthermore, Guckes *et al.* (2013) showed that the PmrB sensor phosphorylates the non-cognate QseB response regulator, which autoregulates the *qseBC* operon (see below). The *pmrAB* genes are not present in the *A. actinomycetemcomitans* genome but the QseB binding site in the *ygiW-qseBC* promoter is identical to the consensus PmrA binding sequence in the *pmrAB* operon of *E. coli* (Juarez-Rodriguez *et al.*, 2014). Hence, it is possible that in the absence of PmrAB, the *A. actinomycetemcomitans* QseC sensor may have evolved to integrate the iron and catecholamine sensory functions of the PmrAB and QseBC TCSs of *E. coli*.

In contrast with QseC from *E. coli* and *A. actinomycetemcomitans*, the QseC paralog of *H. influenzae* is activated only by ferrous iron or zinc, and does not appear to respond to Ep or Ne. Hence this TCS was designated as a ferrous-iron-responsive system (FirS) (Steele *et al.*, 2012). Iron activation of the FirS also differed from iron activation of *A. actinomycetemcomitans* QseC in that both ferrous and ferric iron activates *A. actinomycetemcomitans* QseC in the presence of catecholamines (Weigel *et al.*, 2015) whereas only ferrous iron activates *H. influenzae* FirS (Steele *et al.*, 2012). This is interesting because a DYRED motif in the periplasmic domain of FirS is conserved in *A. actinomycetemcomitans* QseC (EYRDD, see Fig. 2) and both sequences have been shown to be essential for activation of the respective sensors (Steele *et al.*, 2012; Weigel *et al.*, 2015). Hence, as summarized in Table 2, a variety of signals including AI-3, catecholamine hormones, iron and zinc, have been shown to activate QseC and the responsiveness of QseC to each of these signals can differ among organisms. Furthermore, there is increasing evidence that other signaling mechanisms that are independent of QseC may mediate the response of some organisms to catecholamine hormones (Karavolos *et al.*, 2013) and it is possible that *H. influenzae* responds to catecholamines via one of these pathways rather than QseC (FirS). Finally, *qseBC* expression in *Actinobacillus pleuropneumoniae* is also induced by Ep and Ne (Li *et al.*, 2012), suggesting that catecholamine hormones may function to activate the QseC sensor in this organism, but this has not yet been directly demonstrated.

Table 2 Signals that activate the QseC sensor

Organism	Signal	Reference
Escherichia coli	Ep, Ne; AI-3	Clarke *et al.* (2006)
Aggregatibacter actinomycetemcomitans	Ep,Ne/Fe^{2+} or Fe^{3+} [1]	Weigel *et al.* (2015)
Haemophilus influenzae	Fe^{2+}, Zn	Steele *et al.* (2012)
Salmonella enterica	Ep, Ne	Moreira & Sperandio (2012), Merighi *et al.* (2009)[2]

[1]Activation of QseC occurs only in the presence of both the catecholamine and iron.

[2]Merighi *et al.* reported that *S. enterica* PreAB (QseBC) did not respond to catecholamines.

ACTIVATION OF QSEB AND TRANSCRIPTIONAL REGULATION OF *QSEBC*

Similar to most other TCS, the activation of QseC leads to phosphorylation of the QseB response regulator and several studies have confirmed this either by directly demonstrating phosphate transfer (Clarke *et al.*, 2006) or by showing that site-specific mutation of the conserved Asp^{51} inhibits QseB function (Kostakioti *et al.*, 2009; Juarez-Rodriguez *et al.*, 2014). In addition, using a mobility shift assay, Clarke & Sperandio (2005a,b) demonstrated that phosphorylation of QseB is required for it to bind to QseB-regulated promoters *in vitro*. One of the initial outcomes resulting from activation of QseB is the auto-induction of the *qseBC* operon and *ygiW* and the induction of *ygiW* expression occurs regardless of the architecture of the *ygiW-qseBC* locus (see Fig. 3). In *A. actinomycetemcomitans*, the promoter that drives *ygiW-qseBC* expression resides completely within a fragment of 138 bp upstream from the *ygiW* start codon (Juarez-Rodriguez *et al.*, 2013) and two transcriptional start sites at nucleotides -15 and -53 have been mapped in this region (Juarez-Rodriguez *et al.*, 2014). The distal initiation site at nucleotide -53 is the main transcriptional start site and although both sites are associated with putative -10 and -35 elements, only the upstream promoter is regulated by QseBC. Within this promoter, QseB binds to the direct repeat sequence CTTAA-N_6-CTTAA where the CTTAA repeats flank the -35 element. Similar to *A. actinomycetemcomitans*, the *qseBC* promoter of EHEC also

contains two transcriptional initiation sites at nucleotides -27 and -77 relative to the *qseB* start codon; each is associated with putative -10 and -35 elements and the distal promoter is regulated by QseBC. However, using DNAse footprinting, two QseB binding sites were mapped in this promoter, a high-affinity site that appears to overlap the distal transcriptional start site and a low-affinity site located upstream between nucleotides -409 and -423 (Clarke & Sperandio, 2005b). Comparison of these sites with the promoter sequence of the QseB-regulated *flhDC* operon generated a QseB binding consensus sequence, CAATTACGAATTA, where the underlined residues are most highly conserved (Clarke & Sperandio, 2005a). However, a direct repeat CTTAA-N_6-CTTAA identical to the QseB binding site in *A. actinomycetemcomitans* overlaps the -10 element of the QseB-regulated distal promoter and this site was suggested by Guckes *et al.* (2013) to be the site in the *qseBC* promoter that is bound by the PmrA response regulator. Given the proximity of this repeat sequence to the protected region identified by DNAse footprinting (Clarke & Sperandio, 2005b), it is possible that this direct repeat also represents the QseB binding site in EHEC.

There is also growing evidence that activation of QseB may be modulated by several additional mechanisms. Juarez-Rodriguez *et al.* (2014) showed that *lacZ* expression from the *ygiW-qseBC* promoter was significantly reduced in a Δ*qseBC* strain of *A. actinomycetemcomitans* and that expression was complemented to wild-type levels by a single-copy chromosomal insertion of *qseBC*. Interestingly, partial complementation of *lacZ* expression was obtained by a single copy insertion of *qseB* but not by *qseB*-$D^{51}A$. This suggests that QseB can be phosphorylated and partially activated in the absence of QseC. A similar reaction occurs in *E. coli* (Kostakioti *et al.*, 2009). In *E. coli*, the absence of QseC results in constitutively high *qseB* transcription that arises from bidirectional cross-regulation between the structurally related QseBC and PmrAB TCS (Guckes *et al.*, 2013). Without QseC, the non-cognate PmrB sensor phosphorylates QseB and this reaction exhibits kinetics similar to phosphorylation of QseB by its cognate sensor (Guckes *et al.*, 2013). As a result, transcription of *qseBC* can be activated by PmrA resulting in constitutive expression of *qseB*. In contrast, transcription of *ygiW-qseBC* is not constitutively high in the absence of QseC in *A. actinomycetemcomitans*. One explanation for this is that *A. actinomycetemcomitans* genome does not encode the PmrAB TCS. Partial activation of QseB in the absence of QseC in *A. actinomycetemcomitans* may instead result from inefficient phosphorylation by other non-cognate sensors, or alternatively by the transfer of a phosphate from the small phosphate donor acetyl-phosphate (Wolfe, 2005). Consistent with the latter possibility, the genes that encode enzymes required for the production of acetyl phosphate are present in *A. actinomycetemcomitans* genome.

QseB activation can also be modulated by dephosphorylation mediated by either QseC or PmrB (Kostakioti *et al.*, 2009; Guckes *et al.*, 2013). Incubation of phospho-QseB with vesicles containing QseC resulted in the rapid loss of phosphate from the response regulator and a concomitant increase in phospho-QseC, indicating that QseC can reverse phosphate flow via dephosphorylation of QseB. A similar reaction occurs with PmrB, but although the kinetics of PmrB-mediated activation of QseB are similar to QseC, the kinetics of PmrB-mediated dephosphorylation are significantly slower than QseC. Together, these results suggest that QseB activation can occur through interaction with either cognate or non-cognate sensors but is modulated primarily by the phosphatase activity of the cognate QseC. In addition, Hughes *et al.* (2009) suggested that unphosphorylated QseB may play an active role in regulating *E. coli* motility by repressing *flhDC* expression. Overexpression of QseB in a Δ*qseC* strain resulted in a decrease in cell motility and a five-fold reduction in *lacZ* activity from a reporter construct containing the *flhDC* promoter, presumably arising from an overabundance of unphosphorylated QseB. Unphosphorylated QseB was also shown by motility shift experiments to bind to the *flhDC* promoter at a site between the high- and low-affinity QseB binding sites that are bound by phospho-QseB (Hughes *et al.*, 2009). Together, this suggests that QseC controls transcription of *flhDC* via QseB and that QseB plays a dual role in its phosphorylated and unphosphorylated forms to fine tune this process. These results also suggest the possibility that QseB may play a broader regulatory role in gene expression by controlling the transcription of other genes in its unphosphorylated form.

Reading *et al.* (2009) also suggest that the QseC sensor is capable of activating several non-cognate

response regulators. By screening a panel of 31 purified *E. coli* response regulators, QseC was shown to phosphorylate two additional non-cognate proteins, QseF and KdpE. QseF is a response regulator that is also activated by its cognate sensor, the QseE adrenergic receptor (Reading *et al.*, 2009), indicating that cross talk occurs between the two catecholamine responsive TCS of *E. coli*. KdpE is also activated by its cognate sensor KdpD, which responds to changes in osmolarity and various metabolites (Hughes *et al.*, 2009). Of the 344 genes induced by QseC that have been identified (see below), 336 are present in the regulons of these three response regulators that are activated by phosphorylation via QseC.

Finally, two recent studies suggest that the transcription of the *qseBC* operon is also regulated by nucleoid-associated proteins. Sharma & Casey (2014) demonstrated that deletion of *hha* resulted in a decrease in motility of EHEC and compared the contribution and hierarchy of Hha and QseBC in controlling EHEC motility. Using single and double gene deletion mutants, they showed that transcription of *qseC* was significantly reduced in a *hha*-deficient background relative to wild-type or a complemented strain and that *hha* was hierarchically superior to *qseBC* in regulating motility. This suggests that Hha functions as a net positive regulator of *qseBC* expression. In addition, Juarez-Rodriguez *et al.* (2014) identified three integration host factor (IHF) binding sites in the *ygiW-qseBC* promoter region of *A. actinomycetemcomitans*. One of these sites was located just upstream from the QseB binding direct repeat sequence and deletion of this region reduced *ygiW-qseBC* expression by approximately 2.5-fold, suggesting that binding of IHF to this site positively regulates transcription of *ygiW-qseBC.* The other IHF binding sites mapped to the region between the -10 and -35 elements of the proximal promoter and a sequence near the 5′-end of the *ygiW* open reading frame. Presumably, one or both of these sites function as negative regulators of expression since deleting *ihfA* or *ihfB* in *A. actinomycetemcomitans* results in a net two-fold increase in *ygiW-qseBC* expression.

FUNCTIONAL OUTCOMES OF QSEBC ACTIVATION: BIOFILMS AND VIRULENCE

In many organisms for which QseBC has been characterized, this TCS functions as a global regulator that controls complex phenotypes such as biofilm formation and virulence. For example, Novak *et al.* (2010) showed that inactivation of *qseC* in *A. actinomycetemcomitans* reduced total biofilm biomass and average biofilm depth by greater than 90% and complementation of the mutant with a plasmid-borne copy of *qseC* restored biomass and biofilm depth to levels even greater than the wild-type, presumably due to the presence of *qseC* in multicopy. Juarez-Rodriguez *et al.* (2014) also obtained a similar biofilm phenotype using a strain of *A. actinomycetemcomitans* that expressed QseC with an in-frame deletion of the periplasmic sensor domain. This suggests that the interaction of catecholamines and iron with the sensor domain of QseC is required for stimulation of biofilm growth and consistent with this, an increase in biofilm biomass occurs when *A. actinomycetemcomitans* is cultured in the presence of Ep or Ne and iron (W.A. Weigel and D.R. Demuth, unpublished). Similarly, Yang *et al.* (2014) showed that Ep and Ne increased *E. coli* K-12 biofilm formation by approximately 50% over control cultures that were grown without the hormone. Furthermore, an isogenic Δ*qseC* mutant exhibited a reduction in biofilm growth of approximately 80% in control medium and exhibited little increase in biofilm biomass when cultured in the presence of catecholamines. Unal *et al.* (2012) also showed that a *qseC*-deficient strain of *H. influenzae* exhibited a significant decrease in biofilm formation relative to wild-type when cultured under static, semi-static or open flow conditions. Hence, for these organisms, the activation of QseBC clearly promotes sessile growth. Catecholamine hormones have also been shown to stimulate the planktonic growth of other organisms such as *Bordetella*, *E. coli*, and *S. enterica* (Freestone *et al.*, 2000, 2008), suggesting that activation of QseBC by catecholamines may promote sessile growth of these organisms as well. However, it is also important to note that induction of *Actinobacillus pleuropneumoniae* growth by Ep and Ne has recently been demonstrated to occur independently of QseBC (Li *et al.*, 2015), suggesting that alternative mechanisms may exist to promote growth by catecholamines. Indeed, Karavolos *et al.* (2013) suggest that other sensor kinases such as QseE, BasS and CpxA may also play a role in catecholamine interkingdom communication.

Comparing wild-type and *qseBC* mutant strains using a variety of *in vitro* and *in vivo* model systems

has also clearly demonstrated the important role that this TCS plays in regulating virulence. QseBC has been shown to regulate the expression of genes associated with production of flagella and motility of EHEC (Sperandio *et al.*, 2002) and a *qseC* mutant exhibited attenuated virulence after intragastric inoculation in a rabbit model (Clarke *et al.*, 2006). The LEE pathogenicity island encoding a TTSS and various effector proteins are also under QseC-dependent regulation but this does not occur through direct activation by QseB (Hughes *et al.*, 2009). Instead, QseC activates a non-cognate response regulator, KdpE, which in turn induces the expression of Ler, the main activator of the LEE loci. Finally, QseC also regulates the expression of a second TCS, QseEF, which is essential for pedestal formation and the formation of attaching and effacing (A/E) lesions (Reading *et al.*, 2007). Indeed, as discussed in greater detail below, QseBC initiates a complex signaling cascade that integrates both positive and negative controls of EHEC virulence factors (Hughes *et al.*, 2009; Pifer & Sperandio, 2014). In uropathogenic *E. coli*, deletion of *qseC* resulted in reduced bladder titers and decreased formation of intracellular bacterial communities in a mouse acute infection model (Kostakioti *et al.*, 2009), and a decreased ability to establish chronic cystitis (Kostakioti *et al.*, 2012). This was partially explained by a reduction in the production of the type 1 pilus, which led to decreased adherence and invasion of bladder epithelial cells. Restoration of type 1 pilus production in the *qseC* mutant increased adherence, invasion, and the ability to establish a chronic infection. However, the complemented strain still exhibited a fitness disadvantage relative to wild-type (Kostakioti *et al.*, 2012), suggesting that a functional QseC sensor is necessary for maintaining chronic uropathogenic *E. coli* infections.

The role of QseBC in virulence of *Salmonella* is less clear. Several studies have shown that catecholamine hormones enhance *Salmonella* virulence (Williams *et al.*, 2006; Methner *et al.*, 2008) but there is some controversy over whether QseBC mediates this process. In *S. enterica* serovar Typhimurium, *qseBC* was shown to be required for invasion of HeLa cells and for intracellular replication in J774 macrophages (Moreira *et al.*, 2010; Moreira & Sperandio, 2012). In addition, a *qseC* mutant exhibited attenuated virulence in a mouse systemic infection model (Rasko *et al.*, 2008; Moreira *et al.*, 2010) and decreased colonization of the swine gastrointestinal tract (Bearson & Bearson, 2008). Similarly, a *qseC* mutant of *S. enterica* serovar Dublin was reported to be attenuated after oral infection of cattle (Pullinger *et al.*, 2010b). In contrast, virulence of a *qseC* mutant of *S. enterica* serovar Typhimurium did not differ from wild-type in a bovine ligated ileal loop model (Pullinger *et al.*, 2010a). This inconsistency has not yet been fully explained but may arise from differences in the strains, media, assay systems and/or constructs used in these studies.

There is also differing evidence to correlate the function of *qseBC* and virulence in oral and respiratory pathogens. Using a mouse model of periodontitis, Novak *et al.* (2010) showed that after oral infection, wild-type *A. actinomycetemcomitans* significantly induced the resorption of alveolar bone, one of the main clinical symptoms of periodontal disease in humans. In contrast, a *qseC* mutant was avirulent and bone loss in the group of mice infected with the mutant strain was indistinguishable from that in the sham-infected controls. Complementation of the *qseC* mutant increased alveolar bone resorption back to wild-type levels. Hence, these results clearly indicate that *qseBC* regulates *A. actinomycetemcomitans* virulence. Similar to *A. actinomycetemcomitans*, expression of the *qseBC* operon in *Actinobacillus pleuropneumoniae* is induced by both Ep and Ne (Li *et al.*, 2012) and catecholamines induce several other virulence factors as well, including *apxIA* (encoding a toxin in the RTX family), *pgaB* (production of extracellular matrix carbohydrate) and APL_0443 (autotransporter adhesion). Inactivation of the *qseBC* operon also resulted in reduced expression of 17 *Actinobacillus pleuropneumoniae* genes including *hugZ*, encoding a putative heme/iron utilization protein, and *pilM* encoding a Tfp pilus assembly protein (Liu *et al.*, 2015). PilM mediates adherence of *Actinobacillus pleuropneumoniae* to porcine lung cells and QseB was shown to directly bind to the *pilM* promoter (Liu *et al.*, 2015). However, following intratracheal challenge, virulence of the Δ*qseBC* mutant did not significantly differ from wild-type in a porcine pneumonia model (Liu *et al.*, 2015). Interestingly, although *pilM* expression is reduced in the Δ*qseBC* strain, deletion of *pilM* resulted in a significant attenuation of virulence. Hence, *qseBC* regulates an essential virulence factor of *Actinobacillus pleuropneumoniae* but deletion of *qseBC* does not generate a detectable

phenotype. One explanation for this may be that *Actinobacillus pleuropneumoniae* possesses redundant mechanisms that respond to catecholamine hormones and the loss of *qseBC* is complemented by other pathways. Alternatively, *pilM* expression may be reduced but not completely eliminated in the Δ*qseBC* mutant and it is possible that the basal level of *pilM* expression is sufficient to still allow Tfp pilus assembly to occur.

The QseBC TCS of the fish pathogen *Eduardsiella tarda* also responds to catecholamine hormones and is phylogenetically related to QseBC of EHEC and *S. enterica.* This TCS exhibits functional similarity to QseBC as well (Wang *et al*., 2011) and *Eduardsiella tarda* causes intramuscular infections exhibiting hemorrhagic necrotic lesions and suppurative abscesses, suggesting that the organism is capable of cell invasion and systemic spreading. Similar to EHEC, deletion of *qseC* or *qseB* significantly reduced the expression of flagellar genes *fldH*, *fliA* and *motA* and the mutated strain exhibited impaired motility. The *qseC* mutant also showed reduced intracellular survival in J774 macrophages. Replication of *Eduardsiella tarda* in macrophages is dependent on a TTSS and consistent with this, expression of the TTSS genes *esaC* and *eseB* were significantly downregulated in the mutated strain. Furthermore, the *qseB* and *qseC* mutants were attenuated in virulence by 8- to 16-fold in a zebra fish infection model and were out-competed by the wild-type strain (Wang *et al*., 2011). Together, these results clearly indicate that the QseBC two TCS of *Eduardsiella tarda* also functions as a global regulator of virulence. Finally, the association of QseBC and virulence is not limited to organisms that infect animal hosts, but plant hosts as well. *Pectobacterium carotovorum* causes soft rot in a variety of crop plants including cabbage, onion, radish, and potato and a screen of a transposon library for mutations exhibiting reduced virulence in Chinese cabbage identified 14 different loci, including *qseC* (Lee *et al*., 2013). Subsequent characterization of the mutant showed that in addition to reduced virulence, the loss of QseC function resulted in an 80% reduction in biofilm growth. In addition, although motility is important for *Pectobacterium carotovorum* virulence (Cui *et al*., 2008), *qseC* did not regulate the expression of flagellar genes as in EHEC and the *qseC* mutant exhibited motility similar to the wild-type strain.

QSEBC REGULONS AND SIGNAL CASCADES

Given the range of organisms that express *qseBC*, the broad scope of host organisms that these bacteria infect, and the different virulence strategies that are used by these pathogens, it is likely that the QseBC regulon and signaling cascade may be species-specific. At present, the QseBC regulons for many of the organisms listed in Fig. 2 have not been thoroughly characterized. However, QseC-regulated genes and its signaling cascade have been characterized in EHEC (see Fig. 4) and in the oral pathogen *A. actinomycetemcomitans* (see Fig. 5). This section will focus on comparing these systems as a model to highlight how the QseBC regulons and signaling cascades may differ.

Using a microarray that represented all of the genes in *E. coli* K-12 MG1655, EHEC strains EDL933 and Sakai, uropathogenic *E. coli* strain CFT073, and 700 probes representing intergenic regions, Hughes *et al.* (2009) showed that when EHEC was grown in Luria–Bertani broth, 708 genes were upregulated and 126 genes were downregulated in a *qseC* mutant relative to wild-type. Most of the differentially expressed genes represent genes that are in the core *E. coli* genome (Rasko *et al*., 2008) and are present in all pathovars. Many of these genes code for proteins with metabolic functions. However, 260 of the differentially expressed genes were specific for EHEC, indicating that the QseBC regulon differs among the *E. coli* pathovars. These results show that in Luria–Bertani medium, the majority of differentially expressed genes are repressed upon activation of QseC. In contrast, when EHEC was cultured under conditions that are conducive to virulence gene expression, e.g. in Dulbecco's modified Eagle's medium containing AI-3, Ep, or a combination of both, the overall pattern of differentially expressed genes changed dramatically. Under these growth conditions in the presence of AI-3, 106 genes were upregulated and 273 were downregulated, whereas in medium containing both AI-3 and Ep, 70 genes were upregulated and 311 were downregulated. Hence, in Dulbecco's modified Eagle's medium, the expression of the majority of QseC-regulated genes was induced. Included in the genes that were induced in a QseC-dependent manner are the *qseBC* operon itself, flagellar genes (*flhDC, fliA, fliC* and *motA*) and the LEE1 operon which encodes *ler*, the transcriptional activator

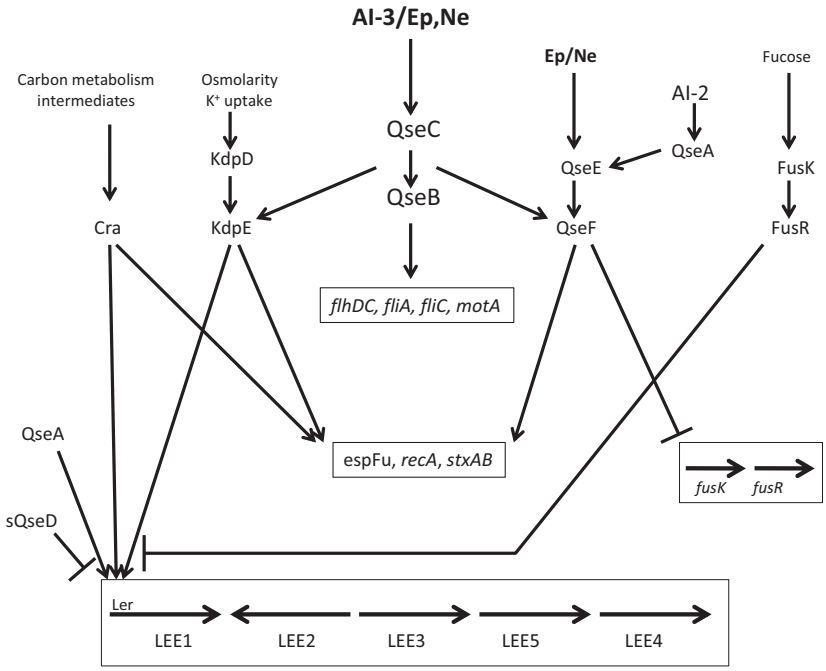

Figure 4 The QseC signaling cascade of enterohemorrhagic *Escherichia coli*. The QseC sensor is activated by autoinducer-3 (AI-3) and/or catecholamine hormones [epinephrine (Ep), norepinephrine (Ne)] and phosphorylates its cognate response regulator QseB and two non-cognate response regulators, QseF and KdpE. Each of the response regulators induces the expression of specific sets of genes shown in boxes. The non-cognate regulators QseF and KdpE can also be activated by their cognate sensors, QseE and KdpD, respectively. Like QseC, QseE is activated by Ep,Ne but it does not interact with AI-3, and KdpD senses osmolarity and potassium. In addition, two quorum sensing-regulated LysR-type transcriptional regulatory proteins, QseA and sQseD, contribute to the regulation of the LEE locus and QseA also induces the expression of QseEF. FusKR senses fucose and functions to downregulate LEE expression and the expression of the *fusKR* operon itself can be downregulated by the QseF response regulator.

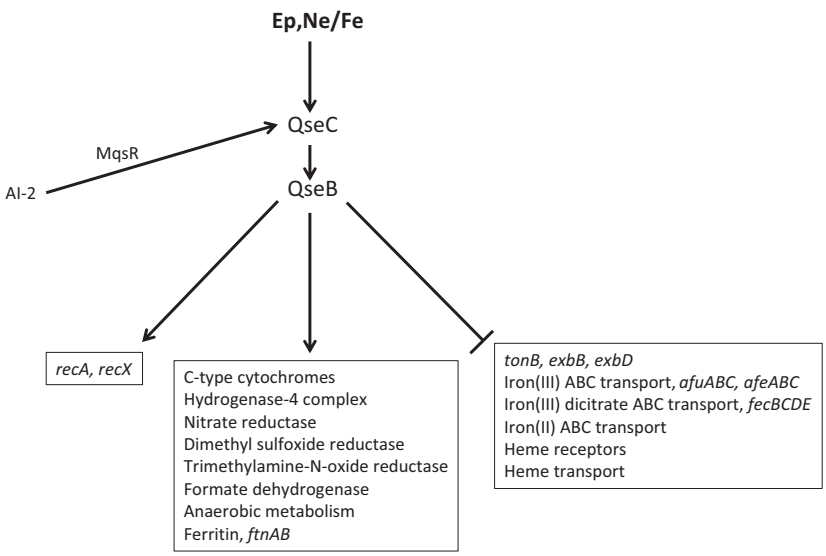

Figure 5 The QseC signaling cascade of *Aggregatibacter actinomycetemcomitans*. The QseC sensor is activated by both epinephrine (Ep) and norepinephrine (Ne), and iron and primarily signals through its cognate response regulator QseB to induce genes associated with anaerobic metabolism and energy production and downregulate genes encoding high-affinity iron acquisition proteins (shown in boxes). Expression of *qseBC* is induced by autoinducer-2 (AI-2) and may involve the MqsR regulator, similar to *Escherichia coli* K-12 MG1655.

of the other operons in the LEE pathogenicity island. In addition, many genes encoding effectors that are translocated by the TTSS were induced by QseC as was *stxAB*, encoding Shiga toxin. The *stxAB* genes are late genes encoded by the λ-bacteriophage and expressed during the lytic phase only after induction

of the bacterial SOS response (Neely & Friedman, 1998). Consistent with this, QseC also induced *recA* expression. Initially, Hughes *et al.* (2009) presumed that the induction of the QseC-regulated genes was mediated by the QseB response regulator and indeed, QseB was known to bind to the promoters of *qseBC* and *flhDC*, the master regulator of the flagella regulon (Clarke & Sperandio, 2005a,b). However, although QseB interacts with the *flhDC* promoter, deletion of *qseB* had no effect on motility and the Δ*qseB* strain expressed flagella at the same level as wild-type. Hence, the phenotype exhibited by the *qseC* mutant differs from the strain in which its cognate response regulator was deleted. It has subsequently been demonstrated that in EHEC, QseB has dual regulatory functions that are dependent upon its state of phosphorylation. Non-phosphorylated QseB functions to repress *flhDC* transcription by interacting with a site in the *flhDC* promoter that resides between nucleotides -300 and -650 whereas phospho-QseB induces transcription by binding to sites located between nucleotides −650 to −950 and −300 and +50 (Hughes *et al.*, 2009). In the absence of QseB, basal levels of *flhDC* expression (i.e. QseC independent) allow for production of flagella and motility whereas in the absence of QseC, QseB remains unphosphorylated and represses *flhDC* transcription.

Deletion of *qseB* also had no effect on the expression of *ler* or *stxAB*, suggesting that QseC-dependent regulation of these genes may be indirect. Although it is generally believed that cross talk between a sensor kinase and a non-cognate response regulator is rare, Yamamoto *et al.* (2005) showed that trans-phosphorylation of non-cognate response regulators can occur *in vitro*. Using a similar approach, Hughes *et al.* (2009) showed that QseC was capable of activating two non-cognate response regulators, KdpE and QseF, and a functional role for these response regulators was demonstrated using gene deletion mutants. Deletion of *kdpE* reduced the expression of *ler* but had no effect on *stxAB* expression or motility and deletion of QseF reduced *stxAB* expression but had no effect on *ler* expression or motility. This suggests that QseC-dependent regulation of *ler* and *stxAB* occurs via QseC-mediated activation of the KdpE and QseF response regulators, respectively.

As shown in Fig. 4, activation of QseC by AI-3 and/or Ep results in the phosphorylation of the QseB, KdpE and QseF response regulators, each of which induces the expression of distinct sets of virulence genes. QseF is also activated by its cognate sensor QseE. Expression of *qseEF* is stimulated by Ep in a QseC-dependent manner but the QseE sensor itself is also activated by catecholamine hormones. However, QseE is not activated by AI-3 (Reading *et al.*, 2009). This central signaling cascade is also integrated with several additional signaling mechanisms. The expression of *ler* is repressed in a glycolytic environment and induced under gluconeogenic conditions and this regulation is mediated by the catabolite repressor/activator protein, Cra. KdpE and Cra each bind to distinct sites in the *ler* promoter and their binding is diminished under glycolytic conditions (Njoroge & Sperandio, 2012;). In addition, the interaction of KdpE and Cra facilitates binding to their respective sites in the *ler* promoter. Cra and KdpE also function coordinately to facilitate pedestal formation by EHEC and regulate the expression of espFu, an effector required for the formation of AE lesions (Njoroge *et al.*, 2013). Finally, Cra may also play a role in the post-transcriptional regulation of the LEE4 operon. Together, these results indicate that the sensing of carbon metabolites via Cra integrates with QseC signaling to control the expression of EHEC virulence genes. The functions of two additional LysR-type transcriptional regulators are also integrated with QseBC signaling. As shown in Fig. 4, QseA is induced by AI-2 and in turn it induces the expression of *qseEF* and also binds to the *ler* promoter to promote *ler* expression (Kendall *et al.*, 2010). In addition, a second LysR-type transcriptional regulator designated QseD is present in a truncated form in EHEC and functions to repress *ler* expression as deletion of this gene results in increased expression of all of the LEE operons (Habdas *et al.*, 2010). QseD expression is repressed in a QseBC-dependent manner. Finally, a fucose sensing system encoded by *fusKR* is also integrated with the QseBC cascade. Fucose is generated in the gut by cleavage from mucin by commensal organisms such as *Bacteroides thetaiotaomicron*. Activation of FusKR represses the expression of *ler* and other fucose utilization genes, which may allow EHEC to conserve energy by limiting virulence gene expression as it transits the mucus layer and also avoid competition with commensals for using fucose as a carbon source (Pacheco *et al.*, 2012; Pacheco & Sperandio, 2015). Conversely, under conditions where virulence gene expression is required and the

QseBC signaling cascade is activated, QseF functions to downregulate the expression of *fusKR*. Together, the QseBC cascade and the other signaling mechanisms that integrate with this pathway may function coordinately to allow EHECC to fine tune and optimize virulence gene expression.

As shown in Fig. 5, the QseBC regulon and signaling cascade of *A. actinomycetemcomitans* is significantly different from that of EHEC, which may reflect the different niches and virulence strategies that are used by these organisms. The *A. actinomycetemcomitans* genome does not code for a TTSS and the organism is non-motile and lacks the flagellar apparatus. Hence, many of the QseC-regulated virulence genes encoded by the LEE and flagellar loci of EHEC are not present in *A. actinomycetemcomitans.* Overall, 235 genes (> 11% of the genome) are differentially expressed when *A. actinomycetemcomitans* was grown in a chemically defined medium containing Ne and iron relative to cultures in the absence of signal (Weigel *et al*., 2015). Of these, 99 genes are induced and 136 are downregulated in a QseC-dependent manner. In contrast to EHEC, none of the genes encoding the well-characterized virulence factors of *A. actinomycetemcomitans*, e.g. the RTX leukotoxin, cytolethal distending toxin, tad fimbriae, autotransporter epithelial cell adhesins, EmaA, or the pga matrix biogenesis components were upregulated by QseBC. Instead, the majority of the induced genes encode proteins associated with anaerobic metabolism or respiration. This group includes electron transport components such as a hydrogenase complex and proteins involved in the reduction of nitrate, DMSO, trimethylamine-*N*-oxide, fumarate and formate. In addition, enzymes associated with the metabolism of aspartate, fumarate, malate, oxaloacetate, pyruvate, and formate were significantly induced upon activation of QseBC in *A. actinomycetemcomitans*. Novak *et al.* (2010) showed that *qseBC* was required for *A. actinomycetemcomitans* virulence but the results of Weigel *et al.* (2015) suggest that the shift in cellular metabolism and energy production that occurs upon activation of QseBC, rather than the direct regulation of virulence factors, may be the primary link between this TCS and *A. actinomycetemcomitans* virulence. Hence, the function of QseBC in *A. actinomycetemcomitans* may be to prime the organism to persist in an anaerobic host environment. Consistent with this, it is striking that many of the genes induced by the activation of QseBC were also identified by Jorth *et al.* (2013) as being induced during subcutaneous growth *in vivo* in a mouse abscess model relative to *A. actinomycetemcomitans* biofilm growth *in vitro*. Finally, the *recA* and *recX* genes are induced by QseBC, suggesting that the TCS may influence the SOS response in *A. actinomycetemcomitans* as well as EHEC.

The genes of the QseBC regulon that are downregulated encode a variety of metabolic functions but a significant number of these genes are associated with iron uptake (Weigel *et al*., 2015). In contrast, the expression of *ftnAB* encoding ferritin is strongly induced, indicating that activation of QseBC increases iron storage capacity and decreases high-affinity acquisition of iron. Recent studies suggest that activated neutrophils, polymorphonuclear cells and macrophages release catecholamines and lactoferrin in response to inflammatory stimuli (Brown *et al*., 2003; Flierl *et al*., 2007, 2008, 2009) and catecholamines can function as pseudosiderophores capable of extracting iron from transferrin and lactoferrin (Freestone *et al*., 2000; Anderson & Armstrong, 2008; Bearson & Bearson, 2008; Sandrini *et al*., 2010). Hence, the inflamed subgingival pocket may be an iron-replete environment and QseBC may play a dual role of priming cellular metabolism as well as allowing *A. actinomycetemcomitans* to detect and exploit the production of catecholamines by host cells to facilitate the acquisition of iron from lactoferrin or other host iron-binding proteins during infection.

The QseBC signal cascade of *A. actinomycetemcomitans* shown in Fig. 5 is significantly less complex than what occurs in EHEC. There is no current evidence to suggest that *A. actinomycetemcomitans* produces AI-3 (Weigel *et al*., 2015), so QseC may be activated only by a combination of catecholamines and iron. In *A. actinomycetemcomitans*, the expression of *qseBC* is also induced by AI-2, which may occur via the MqsR regulator as described for *E. coli* K-12 MG1655 by Gonzalez Barrios *et al.* (2006). In addition, *A. actinomycetemcomitans* does not possess genes encoding the *qseEF* or *kdpDE* TCS, so in contrast with EHEC, QseB is the only response regulator that is currently known to be phosphorylated by QseC. Similarly, *A. actinomycetemcomitans* also lacks genes encoding QseA, QseD and FusKR, indicating that the additional signaling pathways that integrate with the QseBC cascade in EHEC do not exist

in this organism. It is possible that some genes in the *A. actinomycetemcomitans* QseBC regulon are acted upon indirectly by transcriptional regulators other than QseB, but these additional putative regulatory proteins have not yet been identified.

QSEBC AS A POTENTIAL VACCINE OR THERAPEUTIC TARGET

The initial identification of catecholamines as the activating signal for QseC rapidly led to reports that existing α- and/or β-adrenergic antagonists functioned to inhibit QseC-mediated signaling and suggested that QseC may represent a novel therapeutic target. To identify more potent inhibitors, Rasko *et al.* (2008) screened a library of 150,000 small organic molecules for candidates that were capable of reducing *lacZ* expression of a LEE1::*lacZ* reporter. Ultimately, 75 compounds were identified and one, designated LED209 (*N*-phenyl-4-(3-phenylthioureido)benzenesulfonamide) was chosen for further study. LED209 at a concentration of 5 pM was shown to inhibit binding of Ne by QseC and to abolish A/E lesion formation on cultured epithelial cells. However, the compound did not influence ligand binding or the function of QseE (Curtis *et al*., 2014), indicating that it is specific for QseC. In addition, LED209 did not affect the growth of EHEC, which is a desirable trait because without survival pressure, it is less likely that EHEC will develop resistance to the compound. Although it failed to reduce EHEC intestinal colonization of infant rabbits, oral administration in mice before and subsequent to intraperitoneal injection of a lethal dose of *S. typhimurium* resulted in increased survival and reduced recovery of viable bacteria from the spleens and livers of treated animals. Similarly, LED209 was shown to reduce *F. tularensis* virulence in a variety of *in vitro* and *in vivo* model systems (Rasko *et al*., 2008). Interestingly, LED209 was subsequently shown by Curtis *et al.* (2014) to function as a prodrug and is cleaved within the bacterial cell to generate the active component, which labels cytoplasmic lysine residues 256 and 427 of QseC. Substituting Arg for Lys^{256} or Lys^{427} rendered QseC inactive, confirming their functional importance. Presumably, the modification of these cytoplasmic residues alters the conformation of the periplasmic domain to prevent binding of Ep or Ne, but this has not yet been demonstrated experimentally. In addition, Lys^{256} and Lys^{427} are not conserved in the QseC proteins expressed by organisms in the family *Pasteurellaceae*, or in *Serratia marsescens*, *Pantoea ananatis* or *B. psittacipulmonis*. Indeed, in many of these organisms, the residue at position 256 is Arg, suggesting that these QseC proteins may be functionally distinct from EHEC and that LED209 may not be active against these bacteria. LED209 has also recently been conjugated with poly (amidoamine) dendrimers (PAMAM) to successfully improve their selectivity against Gram-negative bacteria and reduce the cytotoxic activity of unconjugated PAMAM against mammalian cells (Xue *et al*., 2015).

Dean & van Hoek (2015) also conducted a small molecule screen to identify potential new therapeutics targeting QseC of *Francisella novicida*. A screen of 420 FDA-approved drugs was conducted and three drugs, toremifene, chlorpromazine and maprotiline, were identified that inhibited QseC-dependent formation of biofilms. Of these, toremifine and chlorpromazine had additional undesirable activities and were subsequently excluded and maprotiline was chosen for further study. Maprotiline significantly reduced the expression of virulence factor IglC encoded on the *Francisella* pathogenicity island and rescued wax worm larvae infected with *F. novicida*. Additional *in vivo* studies showed that treatment with maprotiline prolonged the time of disease onset and increased the overall survival of *F. novicida*-infected mice. These results suggest that the FDA-approved polycyclic antidepressant maprotiline may have additional use against *Francisella* infections by targeting the QseC sensor.

Plant secondary metabolites encompass a broad range of chemical scaffolds and are known to contribute to plant defense systems against bacterial, fungal and insect infections (Langenheim, 1994). Vikram *et al.* (2012) screened various limonoids derived from citrus species for anti-biofilm and TTSS-inhibitory activity of EHEC and identified five compounds that reduced EHEC biofilm formation, the most potent of which was isolimonic acid. This compound also reduced EHEC adhesion to Caco-2 cells by approximately three-fold without influencing cell viability. As the adherence of EHEC to epithelial cells requires a variety of QseC-regulated factors, Vikram *et al.* (2012) subsequently examined the effect of isolimonic acid on the expression of *flhC*, *ler* and several other genes encoded by the LEE1 and LEE2 operons and showed that treatment of bacteria resulted in a 5-fold to 12-fold

reduction of all virulence genes tested. Inhibition of gene expression and biofilm formation was subsequently shown to be dependent on QseBC signaling and to also require QseA; however, its mechanism of action remains to be determined. Nonetheless, isolimonic acid likely represents a lead compound for the development of additional, more potent agents that reduce EHEC virulence by targeting the QseBC signaling cascade. Finally, Chaudhari & Kariyawasam (2014) showed that treatment of avian macrophage-like cells with purified recombinant QseC stimulated the expression of interferon-γ, Toll-like receptor-4 and Toll-like receptor-15 and that conditioned medium from these cells reduced the expression of virulence genes of avian pathogenic *E. coli* O78. This suggests that QseC may induce host innate immune factors that downregulate the expression of important *E. coli* virulence factors. Hence, in addition to representing a viable therapeutic target, it is possible that QseC may also have utility as a potential subunit vaccine candidate. Additional functional characterization of QseBC signaling in *E. coli*, *A. actinomycetemcomitans* and other organisms may highlight additional similarities and differences in this TCS and should be informative to further develop new drug candidates and therapeutic approaches to circumvent the increase in antibiotic resistance and control microbial infections.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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