Journal of Bacteriology

Host-Microbial Interactions | Minireview

Regulation of bacterial virulence genes by PecS family transcription factors

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ABSTRACT Bacterial plant pathogens adjust their gene expression programs in response to environmental signals and host-derived compounds. This ensures that virulence genes or genes encoding proteins, which promote bacterial fitness in a host environment, are expressed only when needed. Such regulation is in the purview of transcription factors, many of which belong to the ubiquitous multiple antibiotic resistance regulator (MarR) protein family. PecS proteins constitute a subset of this large protein family. PecS has likely been distributed by horizontal gene transfer, along with the divergently encoded efflux pump PecM, suggesting its integration into existing gene regulatory networks. Here, we discuss the roles of PecS in the regulation of genes associated with virulence and fitness of bacterial plant pathogens. A comparison of phenotypes and differential gene expression associated with the disruption of *pecS* shows that functional consequences of PecS integration into existing transcriptional networks are highly variable, resulting in distinct PecS regulons. Although PecS universally binds to the *pecS-pecM* intergenic region to repress the expression of both genes, binding modes differ. A particularly relaxed sequence preference appears to apply for *Dickeya dadantii* PecS, perhaps to optimize its integration as a global regulator and regulate genes ancestral to the acquisition of *pecS-pecM*. Even inducing ligands for PecS are not universally conserved. It appears that PecS function has been optimized to match the unique regulatory needs of individual bacterial species and that its roles must be appreciated in the context of the regulatory networks into which it was recruited.

KEYWORDS bacterial fitness, MarR, PecM, plant pathogen, virulence

When a bacterial pathogen transitions to the host environment, it must adapt to the new ecological niche by modulating gene expression. The purpose of such adaptation is survival, and it may manifest in the production of factors that promote bacterial fitness or overcome host defenses. It may also cause damage to the host, generating the characteristic symptoms of the resulting disease (1).

Differential expression of genes associated with virulence or bacterial fitness relies on the detection of host-derived signals or other environmental cues by dedicated transcription factors. The pathogen may need to sense multiple environmental signals simultaneously to achieve an optimal response, such as changes in pH, oxygen tension, or the concentration of metal ions. In addition, host cells will deploy an array of defenses designed to kill the would-be invader. The ability to mount an effective counter-defense is therefore critical to survival of the bacterial pathogen. Accordingly, several transcription factors may participate to induce a coordinated response and promote virulence (1).

Regulatory transcription factors function as molecular switches—or rheostats in some cases (2). They bind cognate DNA sites to effect their regulatory function, be it repression or activation. The gene regulation is achieved when they sense a specific signal because of which DNA binding is modified, and gene expression is altered. One family of transcription factors, which is exquisitely suited as sensors of environmental

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The authors declare no conflict of interest.

See the funding table on p. 10.

Published 17 September 2024

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signals and regulators of virulence genes, is the multiple antibiotic resistance regulator (MarR) protein family (3, 4). MarR proteins are identified in InterPro as belonging to two homologous superfamilies, IPR036388 and IPR000835, described as winged-helix DNA-binding and MarR-type HTH, respectively. MarR proteins are ubiquitous in bacteria, and sub-families have been described, which are characterized by specific functions or molecular signatures. One such sub-family is the PecS protein family (5). PecS was first described in the plant pathogen *Dickeya dadantii* (at the time denoted *Erwinia chrysanthemi*) and named for its regulation of genes encoding pectinase (6). *D. dadantii* PecS was subsequently shown to be a master regulator of virulence genes (7), and PecS proteins have now been described in several other bacterial species (8–12). Here, we discuss how PecS has been integrated into existing transcriptional regulatory networks, resulting in distinct functions.

WHAT ARE THE CUES TO VIRULENCE GENE INDUCTION?

When a bacterium enters a potentially hostile host environment, the first requirement is to survive and grow. Proteins or secondary metabolites, which contribute to such survival, would be considered fitness factors. In addition, the bacteria may express virulence genes, which encode factors associated with a measurable increase in pathogenicity or virulence (13). Since the production of virulence factors is energetically costly, potentially even compromising fitness, pathogenic bacteria use environmental or host-derived signals to ensure that their virulence factors are produced only at the appropriate location and at the right time. Since multiple cues may need to be integrated to achieve a coordinated response, identifying such cues and defining their roles have proven challenging.

At each step of the infection process, the bacteria encounter distinct signals. For soil saprophytes, a common first step is bacterial chemotaxis toward plant exudates such as phenolic compounds or simple sugars, which are sensed by bacterial proteins and interpreted as proximity to a susceptible plant, resulting in the induction of virulence genes (14). The phenolic compound p-hydroxybenzoate, for instance, is among the more abundant metabolites in the plant rhizosphere, and it is a chemoattractant for bacteria such as *Agrobacterium fabrum* and *Pseudomonas putida* (15, 16). Flagellar motility brings the bacteria to the plant root where they may enter through wounds, root tips, or secondary root emerging points; from there, the bacteria can gain access to the vascular system.

Epiphytic bacteria, which survive on a plant surface, such as a leaf, are met with a distinct set of environmental challenges, including exposure to UV light and desiccation (17). They also utilize flagellar motility, in this case, to migrate toward stomata or wounds, from where they can enter the apoplast, a strategy that is for instance utilized by certain pathovars of the genus *Xanthomonas*, who initially keep their plant host alive. For biotrophic pathogens, which derive nutrients from live cells, and hemibiotrophs such as *Xanthomonas*, who ultimately kill their host, deployment of their type III secretion system (T3SS) is essential for the delivery of effectors into plant cells and for colonizing the host (18). Triggers of T3SS gene expression are not fully delineated but may include plant metabolites such as p-hydroxybenzoate, citrate, and aspartate, when encountered in the presence of simple sugars such as fructose (19). Other bacteria produce enzymes, which destroy the plant cell wall, a common tactic for necrotrophic pathogens that subsist on dead or dying cells such as *D. dadantii*, with such virulence factors secreted through a type II secretion system. In the case of *D. dadantii*, induction of genes that encode plant cell wall-degrading enzymes involves about a dozen different transcription factors, some of which respond to metabolites deriving from pectin degradation and cAMP (20, 21).

Once the bacteria colonize the plant apoplast, which comprises the intercellular space, the cell walls, and the xylem, they encounter a nutrient-limited environment where they must adjust their metabolism to exploit available energy sources. Plant responses to bacterial infection also manifest in this space, including events such as alkalinization, release of pathogenesis-related (PR) proteins, and an increase in the

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concentration of Ca^{2+}, all of which may be detected by the bacteria (22). Another early host response to the bacterial invasion is the oxidative burst in which reactive oxygen species (ROS) are produced, primarily by the action of plasma membrane-bound nicotinamide adenine dinucleotide phosphate(NADPH) oxidases. Many redox-sensitive transcription factors have been described that mediate the expression of genes associated with detoxification of the ROS as well as induction of virulence genes (23, 24).

MarR FAMILY TRANSCRIPTION FACTORS

MarR family proteins are represented in most bacteria, and they are particularly abundant in bacterial species that transition between free-living and parasitic or symbiotic stages (25). They are obligate homodimers, as reflected in the numerous structures of MarR proteins. Each monomer contributes a winged helix-turn-helix DNA-binding motif, and the DNA recognition helices are arranged such that they can interact with consecutive DNA major grooves while the wings contact the adjacent minor grooves (Fig. 1A). Accordingly, their cognate DNA sequences are palindromic. Since MarR proteins are typically autoregulatory, such palindromes may often be identified in their gene promoters. Most characterized MarR proteins are repressors, which interfere with transcription by covering RNA polymerase promoter elements or sometimes by altering the topology of promoter DNA or impeding elongation (3).

MarR proteins are one-component regulators, meaning that the DNA- and regulatory signal-binding regions reside within the same protein. In addition, MarR proteins comprise a single domain as opposed to featuring separate DNA- and ligand-binding domains connected by a linker. The regulatory signal is typically a small molecule ligand, a metal ion, or an oxidant. For ligand binding, a shared binding pocket located at the junction between the DNA-binding motif and the dimer interface has been identified in many proteins (Fig. 1B). Cysteine oxidation by different types of oxidants has also been reported for several MarR proteins, with outcomes ranging from intersubunit disulfide bond formation to tetramerization (28, 29). Ligand binding—or cysteine oxidation—brings about conformational changes, which are communicated to the DNA-binding motifs, often through the dynamic dimer interface (3). As sensors of environmental cues, different MarR proteins may control metabolic processes in response to nutrient availability, or they may regulate virulence genes when host-derived signals are perceived (3, 4, 25).

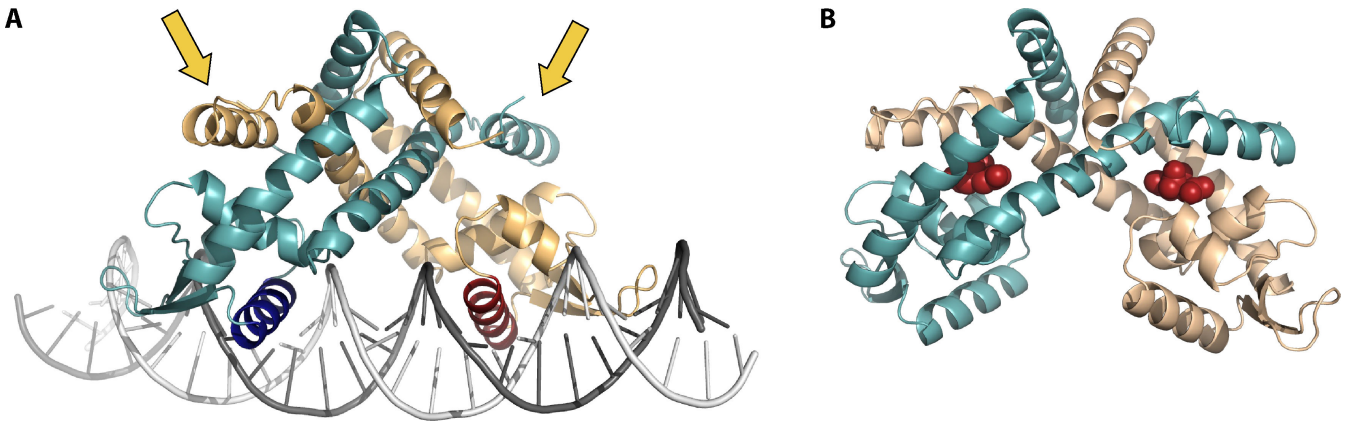

FIG 1 General mode of MarR interaction with DNA and ligand. (A) Model of *A. fabrum* PecS with cognate DNA generated with AlphaFold3 (26). The two subunits are in tan and teal, with the corresponding recognition helices in red and blue. Arrows identify N-terminal helical extensions not found in canonical MarR proteins. (B) Model of PecS with ligand. Citrate was used as the best available option in AlphaFold3, although citrate is not known to be a ligand for PecS. Citrate was chosen because it has a negative charge and because it is a ligand for the PecS homolog TamR from *S. coelicolor* (27). PecS subunits are colored in tan and teal, with citrate shown in red space-filling representation. PecS was modeled with very high confidence (pLDDT >90; pTM = 0.9; ipTM = 0.86 for the PecS-DNA complex) and the DNA and citrate with high confidence (90 > pLDDT > 70).

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THE PecS PROTEIN FAMILY

PecS proteins belong to the urate-responsive transcriptional regulator (UrtR) subfamily, a distinct group within the MarR superfamily (5). The founding member of this subfamily is *Deinococcus radiodurans* HucR, which binds the purine metabolite urate to control expression of a gene encoding the enzyme uricase, required for purine degradation (30). HucR features a helical N-terminal extension, which is not found in canonical MarR proteins (31), and UrtR family proteins were identified based on the presence of this N-terminal extension (Fig. 1A and 2) along with a set of amino acids shown to be important either for ligand binding or structural integrity (5, 8, 32, 33). The N-terminal extension of HucR participates in creating the binding site for urate (as shown for citrate binding to the model of *A. fabrum* PecS; Fig. 1B), and analysis of the structure of HucR with urate bound suggests that a conserved Trp in helix 1 (Fig. 2) is important for maintaining the proper fold for HucR to bind cognate DNA (34).

In *D. dadantii*, *pecS* is divergently oriented from *pecM*, which encodes an efflux pump belonging to the drug metabolite exporter (DME) family (6, 36, 37). Accordingly, PecS was distinguished from other UrtR family proteins by being encoded divergent to *pecM* (5). *D. dadantii* PecM exports a blue pigment called indigoidine, an antioxidant that contributes to virulence by protecting the bacteria against host-derived ROS (36, 38). The *pecS-pecM* locus is predominantly found in species belonging to Proteobacteria and Actinobacteria, particularly members of the γ-proteobacterial species *Dickeya, Pectobacterium, Klebsiella*, *Shewanella*, and *Vibrio*, the α-proteobacterial species *Agrobacterium* and *Rhizobium*, and the actinobacterial genus *Streptomyces* (5). This patchy occurrence

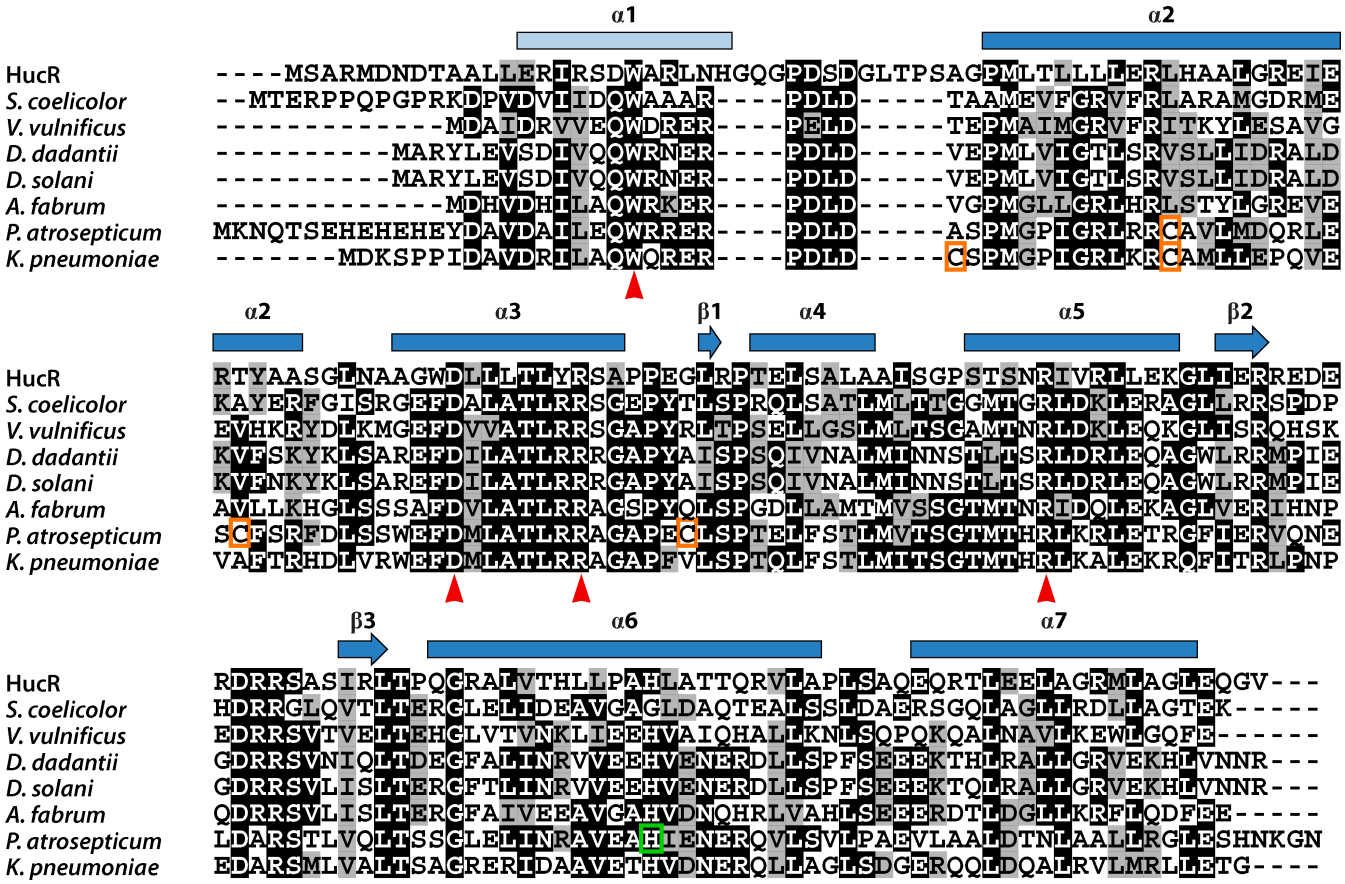

FIG 2 Alignment of PecS proteins. Sequences of experimentally characterized PecS proteins discussed were aligned with MUSCLE and shaded using boxshade, where white letters on a black background indicate ≥50% identity and gray shading indicates ≥50% conservation (35). Secondary structure elements are based on the structure of HucR [top line; accession 2fbk (31)]; α1 is unique to UrtR proteins; α4 is the DNA recognition helix. Residues diagnostic of UrtR proteins are marked with red arrowheads (5). Cys residues are framed in orange. The pH-sensing His in *Pectobacterium atrosepticum* PecS (in α6) is framed in green.

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reflects discrepancies between the evolutionary histories of the *pecS-pecM* sequences compared with that of their hosts and suggests dissemination through horizontal gene transfer (HGT), a common event inferred to occur in these bacterial groups (39). HGT provides a competitive advantage, allowing these bacteria to acquire new regulatory capabilities, thus enhancing their adaptability and fitness in diverse environments (39).

In all species in which PecS has been characterized, PecS binds the intergenic region between *pecS* and *pecM*, repressing expression of both genes (8–12, 40). Substrates for PecM have yet to be identified for bacterial species other than *D. dadantii*. Other *Dickeya* spp. such as *Dickeya solani* produce indigoidine, leading to the prediction that their PecM proteins likewise export this pigment (*D. solani* and *D. dadantii* PecM proteins share ~95% identity). Most other bacterial species, which harbor the *pecS-pecM* locus, do not encode the indigoidine biosynthetic genes, suggesting that their PecM proteins transport distinct substrates.

REGULATION OF VIRULENCE GENES

PecS from soft-rot *Pectobacteriaceae*

The genus *Dickeya*, a member of the family *Pectobacteriacea*e, currently comprises 12 recognized species, and it has been classified among the most significant plant pathogens because of its economic implications and impact on food security (41, 42). *D. dadantii* causes soft rot due to progressive tissue maceration, mainly driven by pectinases that are secreted through the type 2 secretion system named Out, and it has the widest host range among *Dickeya* species. PecS is a master regulator in *D. dadantii*, as evidenced by its control over numerous virulence factors, and it is involved in preventing the premature expression of virulence genes during infection (7). Early studies using microarray analyses of wild-type *D. dadantii* 3937 and the corresponding *pecS* disruption strain revealed a global regulatory role of PecS, identifying its direct or indirect involvement in the expression of 134 genes, including genes associated with virulence (43).

However, more recent *in planta* analyses have significantly expanded our understanding of PecS's regulatory reach. A comparison between the *pecS* disruption strain and the wild-type 3937 strain during infection of *Arabidopsis thaliana* revealed that PecS modulates the expression of a significant proportion of the genes that are differentially expressed during early colonization (44). During early epiphytic colonization of the leaf surface, 575 genes were differentially expressed in the *pecS* mutant, whereas 137 genes were differentially expressed during the later leaf-infection stage. During epiphytic colonization, regulated genes included traditional virulence genes such as genes encoding cell wall-degrading enzymes, secretion systems, and flagellar components, but they also encompassed a broad array of genes associated with metabolism, transport, and chemotaxis, as well as more than 40 regulatory genes (44). During leaf colonization, the transcriptomes of wild-type and *pecS* disruption strains were more similar; the most highly upregulated genes in the *pecS* disruption strain included the indigoidine biosynthetic genes and biosynthesis of the VFM (virulence factor modulating cluster) quorum sensing signal. Taken together, the conclusion was that PecS is important for appropriate timing of virulence gene expression, acting primarily in the very early stages of infection (44).

PecS does not act alone. In the regulatory network that controls virulence gene expression in *D. dadantii*, additional regulators such as KdgR and PecT independently act on the production of plant cell wall degrading enzymes. For example, KdgR responds to products of pectin degradation such as 2-keto-3-deoxygluconate, which elicits upregulation of virulence genes (45). DNA binding by the LysR-type transcriptional regulator PecT has been reported to respond to temperature due to a temperature-dependent change in DNA topology, with increased repression of target genes at higher temperatures (around 37°C) (46). The ligand to which PecS responds has yet to be reported. The combined activity of transcription factors that respond to distinct environmental cues has likely evolved to ensure optimal gene expression when all environmental parameters line up.

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D. solani has emerged as a serious pathogen of potato plants in Europe (47). Using four separate isolates of *D. solani* with different levels of virulence on potato, it was reported that their PecS proteins were 100% identical, and 95% identical to *D. dadantii* 3937 PecS. Not only was virulence distinct among the four original *D. solani* isolates, but disruption of their *pecS* genes resulted in different levels of indigoidine production as well as other phenotypic variations such as pectate lyase activity (47). This suggests that PecS functions similarly in *D. solani* and *D. dadantii* and that phenotypic variations among *D. solani* strains include contributions from other transcription factors.

The genus *Dickeya* is closely related to *Pectobacterium*, another soft-rot phytopathogen that is also a member of the family *Pectobacteriaceae*. Since species belonging to the genus *Pectobacterium* also harbor the *pecS-pecM* locus, it is tempting to speculate that PecS serves a comparable regulatory function in *Pectobacterium* spp. However, PecS proteins from *D. dadantii* 3937 and *P. atrosepticum* SCRI1043 are only 48% identical (Fig. 2; the corresponding PecM proteins share 43% identity), and as discussed below, they are likely to respond to distinct signals (only *P. atrosepticum* PecS responds to oxidant), a circumstance that may also presage distinct regulons. The genomic context in which the *pecS-pecM* gene pair resides is also different between *Dickeya* and *Pectobacterium* spp. For example, in *D. dadantii, pecM* is flanked by the indigoidine biosynthetic gene cluster *indABC*, which is repressed by PecS, and by a respiratory nitrate reductase gene in *Pectobacterium*. Although a number of virulence genes are shared between *Dickeya* and *Pectobacterium*, only *Dickeya* encodes the VFM quorum sensing system, which is under the control of PecS. Thus, the *Pectobacterium* PecS regulon is undoubtedly distinct from that of *D. dadantii* PecS.

PecS from other bacterial species

A. fabrum, originally known as *Agrobacterium tumefaciens* genomovar G8, harbors a tumor-inducing Ti plasmid. The delivery of the transfer-DNA (T-DNA) from the Ti plasmid to the host for integration into the host genome leads to the biosynthesis of amino acid-sugar conjugates named opines and the formation of crown gall tumors (48). *A. fabrum* encodes a PecS protein, which shares 45% identity with *D. dadantii* PecS (Fig. 2; 48% identity between the corresponding PecM proteins). Since *A. fabrum* is not pectinolytic, a logical inference is that the *A. fabrum* PecS regulon must likewise be distinct from that of *D. dadantii* PecS. Recent studies using a *pecS* disruption strain of *A. fabrum* demonstrated that PecS controls several phenotypes linked to bacterial fitness and survival, specifically during the transition from the rhizosphere to the plant host (49). *A. fabrum* PecS was shown to control processes related to chemotaxis, biofilm formation, oxidative stress response, and accumulation of acyl homoserine lactone (AHL). In addition, PecS appears to function as a regulator of antibiotic resistance. Notably, *A. fabrum* PecS is not directly involved in virulence. By comparison, PecS from the related α-proteobacterium *Sinorhizobium meliloti* accumulated when the bacteria were grown along with the alga *Chlamydomonas reinhardtii* compared with growth in monoculture, likewise suggesting induction of the PecS regulon prior to infection of a host (50). This characterization identifies PecS as a master regulator with a broad regulatory scope in *A. fabrum*, much like in *D. dadantii*, except that it appears to exert its function prior to infection.

Klebsiella pneumoniae is best known as an opportunistic human pathogen, but it resides in soil and water, and it can adhere to or colonize an assortment of plants, potentially resulting in food poisoning (51). Several *K. pneumoniae* strains were shown to share a genomic organization in which the *pecS-pecM* locus resides between the *fim* and *mrk* gene clusters, which encode the type 1 and type 3 fimbriae, respectively (11). In addition to the negative regulation of *pecS* and *pecM*, it was reported that PecS exerted a regulatory effect on the expression of type 1 fimbriae, which mediate attachment to surfaces and are a major determinant for urinary tract infections. Notably, *pecS* and *pecM* were also repressed by the two-component regulator CpxR, and PecS-mediated regulation was only detectable in the absence of CpxR. This is the first documented

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example of a separate transcription factor participating in regulation of *pecS* and *pecM*. Unlike *A. fabrum* PecS, *K. pneumoniae* PecS had no apparent effect on biofilm formation, nor did deletion of *pecS* affect oxidative stress responses (11). Furthermore, the effect of PecS on type 1 fimbriae expression appeared to be indirect and to result from differential expression of the global repressor H-NS (11). This demonstrates that PecS does not work in isolation but that it has become part of a broader regulatory network, exerting its effects on gene expression in concert with other regulatory proteins, thereby creating a multi-layered regulatory pathway. This is comparable to what was reported in *D. dadantii*, where PecS likewise interacts with several other regulatory proteins to control the expression of virulence genes (40).

Vibrios are commonly found in salt and brackish waters and may associate with shellfish as well as cause a range of diseases in humans (52). Vibrios are among the few bacteria harboring a *pecS-pecM* locus that are not associated with plants. In addition, Vibrios frequently harbor duplications of the *pecS-pecM* locus; however, the functional reasons for these duplications remain unexplored (9). *Vibrio vulnificus* PecS was found to regulate divergent genes encoding the nitric oxide-sensing transcription factor NsrR and a nitric oxide dioxygenase, which participates in detoxification of nitric oxide. This once again highlights the integration of PecS into existing regulatory systems.

MODE OF BINDING TO *pecS-pecM* INTERGENIC DNA

PecS has been experimentally shown to bind the *pecS-pecM* intergenic region and repress the expression of both *pecS* and *pecM*. However, binding modes differ among bacterial species, likely reflecting diverse regulatory requirements. Analysis of the intergenic regions between genes encoding UrtR proteins, including PecS, and the divergent gene revealed the occurrence of a conserved 18 bp palindromic sequence for which a consensus sequence of TATCTTNAN·NTNAAGATA was identified, where N is any nucleotide (5). In some cases, three such palindromes were detected in the intergenic region between the *urtR* gene and the divergent gene, in which case a single site was found in the *urtR* promoter, and two sites were identified in the promoter for the divergent gene (Fig. 3A). In some instances, the two adjacent sites overlapped by three base pairs, a configuration that would place the centers of the palindromes on opposite faces of the DNA duplex. It is conceivable that a distinct configuration of protein binding sites in the divergent gene promoters has evolved to facilitate differential gene regulation.

A. fabrum PecS falls in the latter category (8). DNase I footprinting showed protection of a single palindrome in the *pecS* promoter, whereas two overlapping palindromes were protected in the *pecM* promoter with protection extending into the *pecM* coding region (Fig. 3A, top panel). The presence of overlapping palindromes implies that two PecS dimers bind on opposite sides of the double helix, assuming normal *B*-form DNA. The *K. pneumoniae pecS-pecM* intergenic region likewise featured this organization of palindromic sequences, and three distinct PecS-DNA complexes were reported (11). By contrast, two non-overlapping palindromes were identified in the *P. atrosepticum pecS-pecM* intergenic region (Fig. 3A, bottom panel), with modestly preferred protection of the site in the *pecS* promoter, possibly allowing differential regulation of the two genes (10). The binding of PecS to either site would likely repress transcription of both genes, given that only 62 bp separate the start codons of *pecM* and *pecS*; the very short intergenic region also suggests that *pecS* and *pecM* cannot be transcribed simultaneously (10). Unlike *A. fabrum* PecS, *P. atrosepticum* PecS imposes distortions in the DNA as evidenced by the presence of very pronounced hypersensitive DNase I cleavage (8, 10, 53).

In *V. vulnificus*, the *pecS-pecM* intergenic region likewise features two palindromic sequences, and they were similarly protected by PecS (9). However, when PecS was in stoichiometric excess over the DNA, the protected region was expanded, indicating the accretion of additional PecS dimers following binding to the preferred site. *V. vulnificus* PecS did not induce DNA conformational changes. A clear palindrome was not identified

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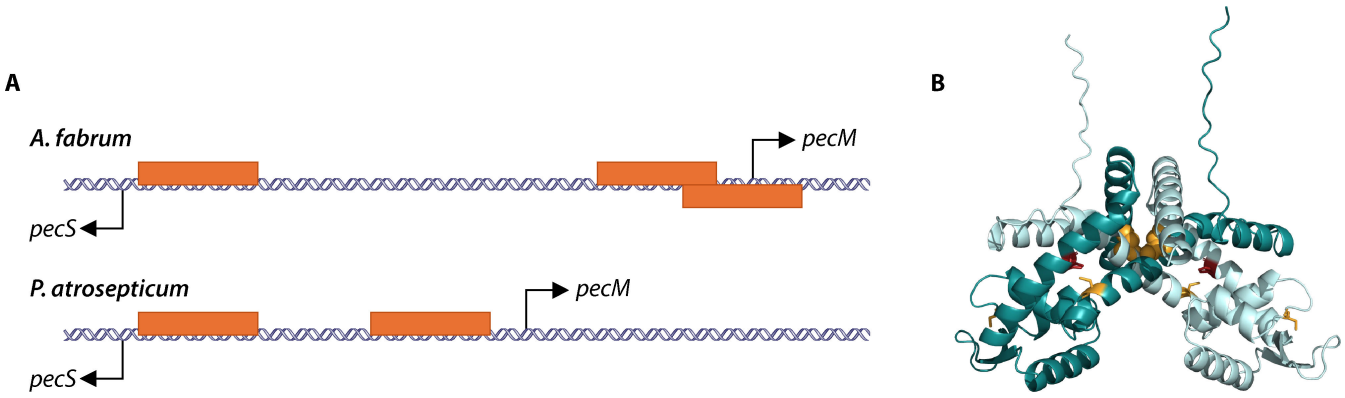

FIG 3 Positions of palindromes and regulatory amino acids. (A) Common arrangements of PecS binding sites. For *A. fabrum*, the *pecS-pecM* intergenic region features three 18 bp palindromic sequences (orange bars). Palindromes in the *pecM* promoter overlap by 3 bp, and one palindrome extends into the *pecM* coding region (8). The *P. atrosepticum pecS-pecM* intergenic region features two non-overlapping palindromes (10). Black arrows mark the annotated translation starts. (B) Model of *P. atrosepticum* PecS. The model was generated with AlphaFold3; pLDDT >90 (very high confidence) except for N-terminal extensions for which pLDDT was <50 (very low confidence); pTM = 0.86 and ipTM = 0.87 (26). The two subunits are in teal and light cyan. His142 is in red. Cysteine residues are in yellow, with Cys45 in space-filling representation.

in the *Streptomyces coelicolor pecS-pecM* intergenic region; however, titration of this DNA with increasing concentrations of PecS resulted in the formation of multiple specific complexes, likely reflecting accumulation of additional PecS dimers following formation of an initial PecS-DNA complex (12). *S. coelicolor* is not a pathogen; however, the *pecS-pecM* locus is conserved in pathogenic *Streptomyces* species such as *Streptomyces scabiei* and *Streptomyces ipomoeae* (12). The ability to recruit additional PecS dimers may ensure that repression of *pecS* and *pecM* is maintained when PecS is in large excess, even when the inducing ligand is present.

DNase I footprinting showed that *D. dadantii* PecS binds specifically to the *pecS–pecM* intergenic DNA, which also does not feature identifiable palindromes (40). At high PecS concentrations, a large, protected region was observed, whereas at low protein concentrations, two high-affinity PecS-binding sites were identified. A somewhat degenerate consensus *D. dadantii* PecS binding sequence was subsequently identified using a selection approach; however, this sequence did not resemble the palindrome preferred by PecS from *P. atrosepticum, A. fabrum, K. pneumoniae*, and *V. vulnificus* (54). This suggests that *D. dadantii* PecS has evolved to recognize distinct DNA sites, perhaps facilitating its integration into existing gene regulatory networks.

The presence of identifiable palindromes in the *pecS-pecM* intergenic DNA in some species prompted a survey of the corresponding genomes in search of similar sequences in other promoters. Such surveys have largely proven unsuccessful. For instance, a survey of the *A. fabrum* genome identified a few instances of sequences resembling the consensus PecS binding site, and one was verified in the *V. vulnificus* genome (49). We speculate that sequences more closely resembling the consensus result in greater affinity for PecS. As a consequence, induction of *pecS* and *pecM* may require a higher concentration of ligand compared to genes to which PecS binds with lower affinity.

Ligands for PecS

As noted above, PecS belongs to the UrtR subfamily of MarR proteins based on the conservation of sequence found to be important for binding of the ligand urate to *D. radiodurans* HucR (Fig. 2) (5). This sequence conservation inspired an assessment of the ability of *A. fabrum* PecS to bind the same ligand (8). The physiological relevance of urate binding to PecS is its accumulation during host responses to infection. Xanthine oxidoreductase functions in purine salvage to convert hypoxanthine to xanthine, and it initiates purine degradation by converting xanthine to urate. Under conditions of infection, the enzyme undergoes modifications, as a result of which it transfers electrons

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from its substrates to molecular oxygen to produce ROS, a key event during the oxidative burst initiated by infected host cells (55). In plants, mobilization of xanthine oxidoreductase has been shown to result in the accumulation of both urate and xanthine, with the antioxidant urate inferred to protect plant cells from ROS-mediated damage (56).

Urate has now been identified as a ligand for PecS from *A. fabrum*, *P. atrosepticum*, *V. vulnificus*, *K. pneumoniae*, and *S. coelicolor*. In the presence of urate, DNA binding of PecS to *pecS* and *pecM* promoter DNA is attenuated *in vitro*, and expression of the genes is increased (8–12). The ligand for *D. dadantii* PecS has not been reported. Since *D. dadantii* PecS acts during the early stages of infection (44), one possibility is that it responds to a component of plant exudates. *A. fabrum* PecS binds urate and xanthine with comparable affinity (Kd ~9 µM); however, although urate induces the expression of both *pecS* and *pecM*, xanthine is able to induce the expression of only *pecS* and not *pecM*, a differential induction perhaps related to the distinct PecS binding to the respective promoters, as discussed above (8, 49). Similarly, induction of *K. pneumoniae pecS* by urate was more efficient than that of *pecM* (11). Curiously, full repression of *K. pneumoniae* PecS target genes required the presence of PecM, a phenomenon previously reported for *D. dadantii* PecS and inferred to imply direct modification of PecS by PecM (40). An alternative interpretation would be that the PecS ligand is a substrate for PecM such that it accumulates in a *pecM* disruption strain, reducing repression of PecS target genes. Such a regulatory mechanism would ensure that the PecS ligand is depleted rapidly when *pecM* expression is induced, allowing gene expression to return to basal levels. For *V. vulnificus* PecS, xanthine was reported to be the preferred ligand (9).

Although *P. atrosepticum* PecS does respond to urate, it additionally senses both oxidants and a change in pH, resulting in altered DNA binding and differential expression of *pecS* and *pecM* (10, 53). *P. atrosepticum* PecS has three cysteines per monomer (Fig. 2 and 3B), of which Cys45 located at the dimer interface, in the middle of α2, was shown to be the redox sensor (53). Addition of oxidant was inferred to result in reversible intersubunit disulfide bond formation between Cys45 and Cys45' from the other subunit. Oxidation of Cys45 leads to derepression *in vivo*, perhaps reflecting differential expression of the PecS regulon in response to host-derived ROS. This cysteine is conserved in *K. pneumoniae* PecS, raising the possibility that *K. pneumoniae* PecS is likewise sensitive to cellular redox state. It is not conserved in other characterized PecS proteins, which do not respond to redox changes.

P. atrosepticum PecS also has a regulatory histidine, His142 in the middle of α6, located in the crevice between the dimer interface and the DNA-binding region (Fig. 3B); deprotonation of His142 at pH ~8 relieves repression compared with neutral pH, which would be relevant in the context of alkalinization of the plant apoplast in response to stress (10). Although this residue is conserved in other PecS proteins, it may not confer comparable regulation. This inference is based on the physiology of *A. fabrum*, for which acidic and not basic pH is an important environmental signal (48). *A. fabrum* PecS exhibits comparable protein stability at pH 7.0 and 8.0 but significantly reduced thermal stability at pH 6.0. At acidic pH, the affinity of *A. fabrum* PecS for its cognate DNA appears reduced *in vitro*; however, reducing extracellular pH from 7.0 to 5.5 did not markedly change *pecS/pecM* expression (57).

CONCLUSION

Related bacterial species may rely on orthologous transcription factors to respond to certain cues; however, outcomes may differ, suggesting that the regulatory systems evolved to suit particular environments or lifestyles by acquiring new targets (58). Additionally, global regulators typically exhibit lower specificity for cognate DNA sites compared to local regulators, perhaps to facilitate control over a larger regulon (58). Such global regulators may then work in concert with more specific, local regulators to achieve precise control over target genes.

These considerations are relevant in the context of PecS. Although the *pecS-pecM* locus appears to have been distributed by HGT, the functional consequences of its

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integration into host transcriptional networks are variable. The most comprehensive view of PecS function comes from *Dickeya* species, where PecS has emerged as a master regulator of virulence gene expression, particularly at early stages of infection. For a horizontally acquired transcription factor to be integrated into ancestral transcriptional networks, its target genes must harbor or evolve cognate sites. It is notable that *D. dadantii* PecS appears to have the most relaxed sequence preference of characterized PecS proteins, with no clear palindrome even retained in the *pecS-pecM* intergenic DNA. We propose that PecS in *Dickeya* species may have diverged more extensively from the original donor, precisely to optimize its integration as a global regulator and regulate genes ancestral to the acquisition of *pecS-pecM*.

In other bacterial species, PecS regulatory functions remain incompletely understood. Although palindromic sequences are recognizable in most *pecS-pecM* intergenic regions, the number of sites and the PecS binding modes differ. Such differences may form the basis for the observed differential regulation of *pecS* and *pecM* in *A. fabrum* and *K. pneumoniae* (11, 49). The ability of PecS to accumulate across a larger DNA region at higher ratios of PecS to DNA, as seen for *D. dadantii, V. vulnificus*, and *S. coelicolor* PecS, may serve to maintain repression of this locus even when the PecS ligand accumulates (9, 12, 40). Assuming that such protein accretion is unique to the *pecS-pecM* region, other genes in the PecS regulon may then be selectively induced.

As argued above, the PecS regulons from different bacterial species vary. For example, *D. dadantii* PecS regulates genes that do not exist in even closely related soft-rot pathogens, and *A. fabrum* PecS is not involved in virulence. On a side note, although the substrate for PecM has only been identified for *D. dadantii* (36), the absence of this blue pigment in other species harboring the *pecS-pecM* locus would argue for the existence of distinct PecM substrates, possibly even the ligand for PecS. However, in all cases, PecS has been integrated into transcriptional networks, where it interacts with other regulatory elements to modulate gene expression. It is clear that PecS function has been optimized to match the unique regulatory needs of individual bacterial species and that its roles must be appreciated in the context of the regulatory networks into which it was recruited.

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FUNDING

Funder	Grant(s)	Author(s)
National Science Foundation (NSF)	MCB-2153410	Anne Grove

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