Suppressor analysis of eepR mutant defects reveals coordinate regulation of secondary metabolites and serralysin biosynthesis by EepR and HexS

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Abstract
The EepR transcription factor positively regulates secondary metabolites and tissue-damaging metalloproteases. To gain insight into mechanisms by which EepR regulates pigment and co-regulated factors, genetic suppressor analysis was performed. Suppressor mutations that restored pigment to the non-pigmented ΔeepR mutant mapped to the hexS ORF. Mutation of hexS also restored haemolysis, swarming motility and protease production to the eepR mutant. HexS is a known direct and negative regulator of secondary metabolites in Serratia marcescens and is a LysR family regulator and an orthologue of LrhA. Here, we demonstrate that HexS directly controls eepR and the serralysin gene prtS. EepR was shown to directly regulate eepR expression but indirectly regulate hexS expression. Together, these data indicate that EepR and HexS oppose each other in controlling stationary phase-associated molecules and enzymes.

INTRODUCTION
In stationary phase, the Gram-negative bacterium and opportunistic pathogen Serratia marcescens synthesizes a number of secondary metabolites and secreted enzymes. Generation of these factors is highly regulated by a number of transcription factors including negative regulators CopA [1], CRP [2], HexS [3, 4], RssAB [5] and SpnR [6] and positive regulators EepR [7, 8], PigP [3] and Smal [9].

The EepR putative response regulator is a direct positive regulator of several compounds including the biologically active pigment prodigiosin, the antibiotic biosurfactant serratamolide and the cytotoxic metalloprotease serralysin (prtS) [7, 8]. The eepR gene is also important in positive regulation of chitinases and chitin binding protein Cbp21, as well as other proteins such as the SfpB protease and S-layer protein SlaA [7]. EepR-like regulators have been found in other medically relevant organisms including Burkholderia species [10]. The coordinated interplay between EepR and other transcriptional regulators that govern secondary metabolites and virulence factors has not been determined.

In this study, suppressor analysis was used to gain insight into the regulatory network of the EepR transcription factor. Transposon mutations that restored pigmentation to a ΔeepR mutant mapped to the hexS transcription factor and upstream of the eepR ORF. Subsequent analysis supports that HexS directly binds to and inhibits eepR expression and that EepR inhibits hexS expression. Together, the data presented here suggest that EepR and HexS are key regulators that oppose one another in control of secondary metabolites and the cytotoxic metalloprotease serralysin.

METHODS
Microbiological growth conditions and media
Escherichia coli and S. marcescens strains are listed in Table 1 and were grown in lysogeny broth (LB) [11, 12] at 30°C. Growth in liquid medium was performed with aeration using a tissue culture roller (TC-7). Swarming motility plates were composed of LB with 0.6 % agar, and swimming motility plates were LB with 0.3 % agar. Haemolysis detection plates consisted of tryptic soy agar with 5 % sheep erythrocytes. Antibiotics used were gentamicin at 10 µg ml⁻¹, kanamycin at 50–100 µg ml⁻¹ and tetracycline at 10 µg ml⁻¹.
**Mutagenesis and genetic manipulations**

Transposon mutagenesis was performed as previously described [13] using Himar1 delivery plasmids pBT20 [14] and pSC189 [15]. Transposons were mapped by arbitrary PCR [16] or marker rescue [15]. After eight mutations were mapped to the \( \text{hexS} \) gene, primers that amplify the \( \text{hexS} \) ORF were used to screen transposon mutants with desired phenotypes. The primer sequences were GTTATTCTTCTTCGTCCACCAGGCTGG and ATGACAACTGCAATTGCTGTCCGATACTTAATCTCG (all primer sequences are shown 5\(^\prime\) to 3\(^\prime\)).

The \( \text{hexS} \) gene was mutated by allelic replacement as previously described using plasmid pMQ296 [17]. The pMQ296 plasmid was introduced into strains CMS2089 and CMS2097 by conjugation and was resolved using pMQ240, an I-SceI delivery plasmid [18]. The \( \text{hexS} \) mutation was screened for by hyper-pigment phenotype, followed by PCR amplification and sequencing of the \( \text{hexS} \) gene to verify the \( \text{hexS}-\Delta1 \) mutation. This mutation deletes one base pair of the \( \text{hexS} \) ORF causing a frameshift mutation and a null allele [17].

The \( \text{lrhA} \) gene was amplified from \( \text{E. coli} \) strain S17-1\(^{pir} \) [19] using Phusion high-fidelity polymerase (New England Biolabs) and primers cgacggccagtgccaagcttgcatgcctgcaggtcgacT-TACTCGATATCCCTTTCAATC and gtggaattgtgagcggataacaatttcacacggaaacagATGATAAGTGCAAATCGTCC. The lower-case nucleotides target recombination and the upper-case letters direct amplification of the \( \text{lrhA} \) ORF, which was placed under control of the \( \text{E. coli} \) lac promoter on pMQ131 using yeast recombineering techniques [18, 20]. The resulting plasmid pMQ407 was introduced into \( \text{S. marcescens} \) by conjugation.

**Mass spectrometry**

Serratamolide analysis was performed as described previously [8, 21]. Bacteria were grown in LB medium for 20 h in 10\( \times \)5 ml cultures per genotype and pooled. Cultures were centrifuged for 10 min at 10 000 \( \text{g} \) and 50 ml of the supernatant was extracted three times with an equal volume of ethyl acetate. The extract was dried over sodium sulphate and evaporated in vacuo and the residue was dissolved in methanol and analysed by HPLC-MS (Shimadzu LCMS-2020) equipped with a DIONEX Acclaim 120C18 column (3 \( \mu \)m particle size, 120 \( \AA \) pore size, 2.1\( \times \)150 mm dimensions).
A previously described [8], mobile-phase gradient was used along with a column flow rate of 0.2 ml min⁻¹ at 40 °C. Serratamolide was monitored at m/z=515 with an ES-MS detector at positive mode, and purified serratamolide [21] was used as a positive control. The experiment was performed three times using independent bacterial cultures.

**Gene expression analysis and electrophoretic mobility shift assays**

β-Galactosidase assay: Bacteria with a plasmid-borne flhDC-lacZ transcriptional reporter, pMQ248, were grown in LB with kanamycin (100 µg ml⁻¹) overnight and then subcultured 1 : 100 into the same medium. After 20h, samples were taken and the OD₆₀₀ reading was determined with a spectrophotometer (Spectronic 200, Thermo Scientific). β-Galactosidase activity was determined as described by Griffith and Wolf [22].

Tdtomato assay: Bacteria with a plasmid-based eepR promoter fusion to tdTomato, pMQ412, were grown under the same experimental conditions described for the β-galactosidase assays noted above, and Tdtomato fluorescence was read as previously described [21] with a plate reader (Biotek, Synergy 2).

RNA preparation and quantitative reverse transcriptase PCR (qRT-PCR) were performed as previously described [8]. Primers for eepR (GGATTGGAAAACGTCAGCAT and CACGAAAAAGATGGCATCAC) and hexS (CGTTAAAAGCGCAGGATCTTC and AAGAACCTTTGGTGCGGTGTG) were designed to amplify DNA from the deletion alleles (all primers are listed as 5¢-3¢). Primer sequences for 16S and prtS analysis were noted in Brothers et al. [7]. Electrophoretic mobility shift assay (EMSA) reactions were performed with a commercial EMSA kit (Lightshift Chemiluminescent EMSA kit, Pierce) using previously described reagents (purified protein and promoter regions) and conditions [3, 8, 23]. The hexS promoter region was amplified using primers CCCGGTTTCTATAAGCACC and GCTCTAATCGCTGACATTGTGTG. The amplicon is 345 bp in length and includes 194 bp upstream of the hexS ORF that contains a predicted promoter determined using Softberry BPROM promoter prediction software. The eepR, flhDC and prtS promoter regions were as described previously [3, 8, 23]. Each EMSA experiment was performed three to six times with consistent results.

**Statistical analysis**

GraphPad Prism software was used for statistical analysis with significance set to P<0.05. Mann–Whitney U-tests were used for gene expression comparison and ANOVA with Tukey’s post-test was used for other experiments as noted.

**RESULTS**

**Suppressor analysis of the eepR mutant pigment defect**

To gain insight into mechanisms by which EepR regulates pigment and co-regulated factors, suppressor analysis was performed. Random transposon mutations were introduced into a previously described DeepR mutant strain [8] that is pigmentless. Eighteen pigmented colonies were isolated from among 32 independent mutagenesis pools representing approximately 50 400 mutant colonies. A sample mutant screen plate is shown in Fig. 1(a). Pigmented strains were noted as red eepR suppressors (reep). A maximum of one pigmented colony was taken from each mutagenesis pool to eliminate sibling mutant colonies.

Transposon insertion sites were mapped in the majority of the reep strains. The mutations mapped to one of two locations: in the hexS ORF and upstream of the eepR ORF. This manuscript will describe the genetic interactions and transcriptional regulation of eepR and hexS. The mutations upstream of the eepR ORF will be described in a separate study.

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**Fig. 1.** Genetic screen for eepR suppressor mutations. Transposon mutations were introduced into the CMS2097 strain (DeepR) to find pigmented suppressor mutants. (a) A portion of one plate is shown with one red suppressor (reep) mutant (black arrow) visible among the pigmentless DeepR colonies. (b) Location of DeepR suppressor mutations (vertical arrows) in the hexS gene (horizontal bar). Of the 12 insertions, 8 are shown, the other 4 are in the hexS ORF but not mapped. (c) Prodigiosin pigmentation of strains grown on LB agar for 20 h at 30 °C. WT refers to parental strain PIC3611; eepR, to CMS2097; hexS, to CMS2922; eepR hexS, to CMS2204; Nima, to parental strain CMS1787; Nima eepR, to CMS2089; and Nima eepR hexS, to CMS2320.
Eight mutants had transposon insertions in the hexS gene at base pairs 1, 45, 210, 213, 214, 214, 265 and 292. Four more had mutations in the hexS ORF whose specific insertion sites were not mapped, as noted in Methods. The specifically mapped mutations clustered near the N-terminus of the HexS protein proximal to a helix–turn–helix domain, whereas none was isolated in a predicted cd08439 (substrate-binding domain) in the C-terminus. HexS is a LysR family transcription factor that directly and negatively regulates prodigiosin and serratamolide production by S. marcescens [3, 4, 17]. HexS is closely related to the LrhA protein of E. coli [24]. LrhA homologues, found in a variety of micro-organisms including E. coli (LrhA), Erwinia species (HexA and PecT), Serratia ATCC 39006 (PigU) and Yersinia pseudotuberculosis (RovM), are involved in regulation of secreted enzymes, motility and virulence [25–29].

The robust pigment phenotypes observed in this screen suggest a regulatory relationship between EepR and HexS in the coordination of secondary metabolite biosynthesis. We investigated whether this relationship went beyond prodigiosin, as previous studies demonstrate that EepR positively and HexS negatively regulates biosynthesis of the secondary metabolite serratamolide and proteases [4, 8].

Opposing control of serratamolide and serralysin biosynthesis by EepR and HexS

An eepR hexS double mutant strain was built incorporating the previously described hexS-A1 null mutation [17] by allelic exchange into the ΔeepR strain background. The double mutant strain was used for epistasis analysis to explore the relationship between EepR and HexS in coordinated regulation of secondary metabolites and secreted enzymes.

Introduction of the hexS mutation into the ΔeepR strain suppressed the pigment-defective phenotype of the eepR mutant (Fig. 1c). There were no obvious phenotypic differences observed between the reep mutants or between the reep mutants and the directed eepR hexS double mutant (CMS2204). Serratamolide is required for haemolysis and swarming phenotypes of many strains of S. marcescens, and PIC3611 harbouring the ΔeepR mutation (strain CMS2097) is unable to accomplish either phenotype due to a severe deficiency in serratamolide biosynthesis [8]. Both swarming ability and haemolysis were restored in the eepR hexS double mutant indicating that the double mutant synthesizes serratamolide (Fig. 2a, b). Swarming zone radii measured at 24 h for strains in the PIC3611 strain background were observed to be 3.0±1.7 mm for wild-type, 19.5±2.3 mm for ΔhexS, 0±0 for ΔeepR and 4.8±1.9 mm for ΔeepR ΔhexS. Importantly, these strains grew at similar rates indicating that the difference in motility is not due to altered growth by the mutant strains (Fig. 3a).

MS analysis was used to measure serratamolide production in the wild-type (PIC3611) and derived strains. Compared to the wild-type, increased levels of serratamolide were measured in the hexS and double mutant, and reduced serratamolide in the eepR mutant (Fig. 3b). The eepR hexS
double mutant produced serratamolide similarly to the hexS mutant, both significantly more than the ΔeepR mutant (P<0.001, ANOVA with Tukey’s post-test). These data indicate epistasis of the hexS mutant phenotype over the eepR mutant phenotype for serratamolide biosynthesis.

Since EepR positively regulates secreted enzymes such as the cytotoxic metalloprotease serralysin (PrtS) [7] and HexS has been reported to regulate undetermined secreted protease(s) [4], we tested whether the overlapping regulons of HexS and EepR extend to secreted protease activity. Azo-casein was used as a quantitative substrate to detect proteases in normalized stationary-phase culture filtrates. Similar to a study by Tanikawa et al. [4] who used a different strain background, the hexS mutant version of strain PIC3611 exhibited elevated protease activity. Unlike the eepR mutant, the eepR hexS double mutant produced protease activity similar to wild-type (Fig. 4a).

To ensure that this genetic interaction was not specific to strain PIC3611, we generated an eepR hexS double mutant variant of strain Nima [30]. Nima and PIC3611 are of different biotypes [31]. Pigment, serratamolide and protease activity were restored in a manner similar to strain PIC3611 and indicate that the relationship between HexS and EepR is not strain specific (Figs 1c, 2 and 4b).

Analysis of gene regulation by EepR and HexS

The suppression of several eepR mutant phenotypes by mutation of hexS led us to test whether HexS regulates eepR gene expression. The expression of eepR was increased 2.4-fold in the ΔhexS mutant compared to the wild-type (P<0.001, Fig. 5a). This trend of increased eepR expression was reversed when hexS was added to the chromosome in cis using pMQ294 as previously described [17] (P<0.01, ANOVA with Tukey’s post-test; Fig. 5a). As a second way to measure the impact of hexS mutation on eepR expression, a P_{eepR}–tdtomato fusion was employed. Significant 4.8-fold and 3.1-fold increases in eepR expression were measured in the ΔhexS mutant relative to wild-type when measured at OD_{600} 1.2 and 3, respectively (P<0.05), data not shown. These results suggest that HexS inhibits eepR gene expression.

Experiments were carried out using qRT-PCR to test whether HexS mediates hexS expression. There was a non-significant twofold decrease in expression of the hexS gene in the hexS mutant strain compared to the wild-type strain when measured at OD_{600} 3 (0.029±0.012 for the wild-type and 0.014±0.002 for the hexS mutant, P=0.10).

A hexS mutant is known to produce more extracellular protease [4, 8], but the specific protease was not determined. EepR is known to regulate the serralysin protease, coded for by the prts gene [7]. Given the potential overlap of the EepR and HexS regulons, we tested whether mutation of hexS changes prts expression. A 37-fold increase in prts expression (P=0.009) was measured by qRT-PCR from the ΔhexS mutant compared to the wild-type at OD_{600} 3 (Fig. 5b).

The role of EepR in transcriptional regulation of hexS and eepR was also tested. The hexS gene was elevated in expression 28-fold in the eepR mutant compared to the wild-type at OD_{600} 3 (Fig. 5c, P=0.0006). The eepR gene was also elevated in the eepR mutant, but only by 3.4-fold (P=0.016, Fig. 5d).

EMSA analysis was used to examine whether HexS and EepR directly regulated the genes tested for expression above. Formerly described maltose binding protein (MBP) protein fusions to EepR and HexS were used at previously optimized concentrations in promoter binding experiments; the MBP domain was used to affinity purify the fusion proteins and complementation analysis indicated that the fusion proteins retained functionality [3, 7, 8, 17]. Whereas MBP itself did not bind to the eepR promoter, the MBP–HexS fusion caused a gel shift of the biotinylated eepR promoter DNA that could be inhibited by an excess of unlabelled eepR promoter DNA (Fig. 6a). Recombinant HexS did not bind to the hexS promoter region.

![Fig. 4](https://www.microbiologyresearch.org/)

**Fig. 4.** Genetic evidence suggests coordinated protease regulation by EepR and HexS. Protease activity in supernatants from stationary-phase bacterial cultures normalized to OD_{600} 2.0. Azocasein was used as a colorimetric protease substrate. Means and standard deviations are shown, n=3 independent experiments, each with three biological replicates. (a) Protease activity from the PIC3611 background. (b) Protease activity from the Nima strain background.
suggesting that HexS does not directly regulate expression of the hexS gene (Fig. 6a). The absence of MBP–HexS binding to the hexS promoter also serves as a control for HexS binding specificity. Since HexS regulates secreted protease activity, we tested whether HexS bound to the prts promoter, and we found evidence indicating that HexS could bind to the prts promoter in vitro (Fig. 6a). Recombinant EepR bound to the eepR promoter, but not the hexS promoter region (Fig. 6b). The binding of EepR to eepR appears to be specific as the binding could be outcompeted with unlabelled eepR promoter sequence and recombinant EepR did not bind to the hexS promoter DNA. These data suggest that HexS directly regulates eepR and prts, but not hexS expression, and that EepR directly regulates eepR expression but indirectly regulates hexS transcription.

**Multicopy expressions of hexS and lrhA inhibit flagellum and prodigiosin biosynthesis and reveal a functional conservation**

As noted above, HexS is similar to LrhA from *E. coli*. BLASTP analysis [32] indicates a 69% amino acid sequence identity between HexS and LrhA. To test whether the HexS protein and LrhA are functionally related, we cloned the *lrhA* gene under control of the *E. coli* *lac* promoter and moved it into *S. marcescens*. Wild-type *S. marcescens* bearing hexS and lrhA on a medium-copy plasmid were both able to completely inhibit prodigiosin pigment production, whereas the vector control did not (Fig. 7a). This suggested remarkably conserved function as *E. coli* does not have the prodigiosin biosynthetic operon and yet multicopy expression of *lrhA* could impair pigment production similar to multicopy expression of hexS. Thus, former studies performed with LrhA in *E. coli* may give insight into other roles of HexS in *S. marcescens*. For example, *lrhA* regulates *flhDC* expression in *E. coli* [33–35]. FlhD and FlhC are the master regulators of flagellum biosynthesis and control biosynthesis of phospholipase and other metabolites in *Serratia* species [23, 36, 37]. Therefore, we tested whether HexS also regulates *flhDC*. A plasmid-borne *flhDC* promoter–*lacZ* fusion transcriptional reporter construct was introduced into the hexS mutant and isogenic wild-type strain. β-Galactosidase activity was >10-fold higher at OD$_{600}$ 3 in the wild-type compared to the hexS mutant suggesting positive regulation of *flhDC* by HexS (Fig. 7b). EMSA analysis supports direct regulation of the *flhDC* promoter by HexS (Fig. 6a). However, this reduction in *flhDC* expression did not result in a corresponding loss in swimming motility under the tested conditions: the wild-type had a 46±7 mm swim diameter and the hexS mutant had a 44±7 mm swim zone in 24 h ($P=0.58$, Student’s $t$-test).

**DISCUSSION**

The EepR regulator of *S. marcescens* is a global positive regulator of secreted enzymes and secondary metabolites and is necessary for wild-type levels of virulence in a
rabbit keratitis model [7, 8]. The goal of this study was to use genetic suppressor analysis to find other regulatory factors that coordinate with EepR in control of the EepR transcriptional regulon. Suppressor mutations of the ΔeepR mutant pigment phenotype mapped to the hexS ORF.

Fig. 6. EepR and HexS promoter binding analyses. EMSA analysis with biotinylated promoter DNA. Labelled promoter DNA was used in each reaction at 2 ng per reaction. Specific promoters are noted in the upper left-hand corner of each panel. MBP and MBP–HexS were used at 38 µM and 28 µM, respectively. MBP–EepR was used at 17.5 µM (∗) or 35 µM (+); 500 ng of poly-dIdC was added per reaction to prevent non-specific protein–DNA interactions, and unlabelled promoters were used at 500 ng. –, indicates no addition of a particular reagent. (a) Representative EMSA using recombinant MBP or MBP–HexS. (b) Representative EMSA using recombinant MBP or MBP–EepR. Each EMSA experiment had a consistent result in at least three independent experiments.

Fig. 7. Multicopy expressions of lhrA and hexS inhibit prodigiosin biosynthesis and flhDC expression in the hexS mutant. (a) Prodigiosin pigmentation of strains grown on LB agar with kanamycin for 20 h at 30 °C. Plasmids with the hexS or lhrA genes under control of the P_{lac} promoter inhibit pigmentation in both the wild-type and the ΔhexS mutant. Experiments were performed with the PIC3611 strain background and vector indicates pMQ131. (b) β-Galactosidase activity produced by WT and ΔhexS strains bearing a plasmid-borne flhDC-lacZ transcriptional reporter after growth in LB medium to OD_{600} 3. A representative experiment with three independent biological replicates is shown. Mean and standard deviation is shown. (c) Model, described in Discussion, for coordinated regulation of secondary metabolite biosynthetic genes (pigA-N and swrW) and the prtS protease gene by EepR and HexS. The line between EepR and HexS indicates that each inhibits transcription of the other.
The results presented here indicate that the hexS mutations not only suppressed the pigment defect of the eepR mutation but also were able to reverse other eepR mutant defects including loss of protease production, serratamolide biosynthesis and associated phenotypes, haemolysis and swarming motility. Notably, this study demonstrates that the elevated protease activity due to undetermined protease(s) generated by the hexS mutants is due, at least in part, to elevated production of PrtS. Importantly, suppression of the eepR mutant defects by mutation of hexS was consistent in two different strain backgrounds suggesting that EepR and HexS have a conserved relationship in control of the tested phenotypes.

Transcriptional and EMSA analyses suggest that EepR directly and negatively regulates expression of the eepR gene but indirectly regulates hexS expression in a strong negative manner. Evidence presented here supports the model that HexS negatively regulates expression of the eepR and prtS, but HexS did not bind to the hexS promoter and mutation of hexS did not cause a significant change in hexS transcript. The observation that, in the hexS mutant, eepR expression is elevated suggests that the derepression of the eepR promoter by hexS mutation is dominant compared to the negative regulation imparted by increased EepR. Together, these data suggest a model, Fig. 7 (c), in which EepR and HexS oppose one another in transcriptional control of secondary metabolites prodigiosin and serratamolide and of the cytotoxic protease serratamolide (PrtS). In the absence of HexS, EepR is predicted to be made at higher levels, leading to increased pigmentation, serratamolide production and protease production. The opposite is also true where, in the absence of EepR, HexS is expected to be made at higher levels leading to a lack of secondary metabolite and protease production, as is seen in the eepR mutant. At this point, the signals stimulating EepR and HexS are unknown. EepR also appears to weakly inhibit the expression of the eepR gene, perhaps as a way to prevent the overproduction of energetically costly secondary metabolites, as overexpression of eepR has been shown to stimulate prodigiosin production [7, 8].

Lastly, the similarity between HexS and LrhA led to the surprising result that multicopy expression of lrhA was able to inhibit pigmentation in a similar manner to multicopy expression of hexS. This suggests that the two proteins have a highly conserved binding site and that genes controlled by LrhA in E. coli are likely to be controlled by HexS in S. marcescens. Unfortunately, the predicted LrhA binding site (AT-N9-AT) [34] is common in the S. marcescens genome. Nevertheless, as an example of how we can take advantage of this similarity, we observed that the flhDC operon is regulated by HexS in S. marcescens and a similar manner by LrhA in E. coli. However, the flhDC expression deficit in the hexS mutant did not result in a reduction in swimming motility through semisolid agar or in a reduction in swarming motility; this may be due to differences in liquid versus solid medium conditions. It is known that S. marcescens without flagella can swarm under certain conditions [38]. Importantly, these results indicate that the EepR-HexS regulon extends to FlhDC-regulated genes including flagella and phospholipase A [36], all of which may contribute to a bacterium’s success in interspecies competition and pathogenesis.

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Conflicts of interest
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References
14. Kulasekara HD, Ventre I, Kulasekara BR, Ladzunski A, Filloux A et al. A novel two-component system controls the expression of...


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