



Published in final edited form as:

Adv Microbiol. 2012 December 1; 2(4): . doi:10.4236/aim.2012.24065.

## The LysR Transcription Factor, HexS, Is Required for Glucose Inhibition of Prodigiosin Production by *Serratia marcescens*

Nicholas A. Stella<sup>1</sup>, James E. Fender<sup>1</sup>, Roni M. Lahr<sup>1</sup>, Eric J. Kalivoda<sup>1,2</sup>, and Robert M. Q. Shanks<sup>1,\*</sup>

Nicholas A. Stella: stellan@upmc.edu; James E. Fender: jef474@yahoo.com; Roni M. Lahr: rml40@pitt.edu; Eric J. Kalivoda: eric.kalivoda@uvm.edu

<sup>1</sup>Charles T. Campbell Laboratory of Ophthalmic Microbiology, Department of Ophthalmology, University of Pittsburgh, Pittsburgh, USA

<sup>2</sup>College of Medicine, University of Vermont, Burlington, USA

### Abstract

Generation of many useful microbe-derived secondary metabolites, including the red pigment prodigiosin of the bacterium *Serratia marcescens*, is inhibited by glucose. In a previous report, a genetic approach was used to determine that glucose dehydrogenase activity (GDH) is required for inhibiting prodigiosin production and transcription of the prodigiosin biosynthetic operon (*pigA-M*). However, the transcription factor(s) that regulate this process were not characterized. Here we tested the hypothesis that HexS, a LysR-family transcription factor similar to LrhA of *Escherichia coli*, is required for inhibition of prodigiosin by growth in glucose. We observed that mutation of the *hexS* gene in *S. marcescens* allowed the precocious production of prodigiosin in glucose-rich medium conditions that completely inhibited prodigiosin production by the wild type. Unlike previously described mutants able to generate prodigiosin in glucose-rich medium, *hexS* mutants exhibited GDH activity and medium acidification similar to the wild type. Glucose inhibition of *pigA* expression was shown to be dependent upon HexS, suggesting that HexS is a key transcription factor in secondary metabolite regulation in response to medium pH. These data give insight into the prodigiosin regulatory pathway and could be used to enhance the production of secondary metabolites.

### Keywords

Pigment; Antibiotic; Transcription Factor; Secondary Metabolite

### 1. Introduction

The production of many secondary metabolites by micro-organisms, such as antibiotics, is inhibited by growth in glucose-rich medium [1,2]. This is unfortunate as glucose is an ideal carbon source for growth of many microorganisms. The Gram-negative bacterium *Serratia marcescens* is a model to study secondary metabolites because it generates the tractable tripyrrole pigment, prodigiosin [3]. Glucose inhibits prodigiosin production by *S. marcescens* [4] due to acidification of the medium [1,2], but the mechanism of this inhibition is incompletely understood.

Fender, *et al.*, recently identified genes required for glucose dehydrogenase (GDH)-dependent inhibition of prodigiosin by medium acidification [5]. It was demonstrated that GDH activity in glucose rich medium is necessary for the rapid culture pH reduction, resulting in a complete inhibition of prodigiosin production. Expression of the prodigiosin biosynthetic operon, *pigA-N* [6,7] was inhibited by glucose-induced acidification, suggesting that prodigiosin inhibition is largely controlled at the transcriptional level. The transcription factors responsible for this regulation are unknown. However, a clue to the identification of the unknown factor(s) was revealed in a recent genetic screen [5]. Fender and colleagues observed that mutation in the gene that codes for the LysR-family transcription factor, HexS, allowed the bacterium to generate prodigiosin in glucose-rich medium in a *crp* mutant strain. However, the impact of mutation of *hexS* on glucose inhibition of secondary metabolites was not characterized and the phenotype was not verified in a strain with a functional CRP protein.

The goal of this study was to determine whether the transcription factor HexS is necessary for glucose-mediated inhibition of secondary metabolite production, using prodigiosin as a model secondary metabolite.

HexS was previously found in a genetic screen for genes that control production of the secondary metabolites, serratomolide and prodigiosin, and was also shown to directly regulate the prodigiosin biosynthetic operon [8]. The only other report featuring HexS indicates that it negatively regulates production of the antimicrobial biosurfactant serratomolide [9]. HexS is highly similar LrhA of *Escherichia coli* [10] and is well conserved among the Enterobacteriaceae. HexS is also similar to HexA proteins from *Pectobacterium* and *Photobacterium* species, and PigU from *Serratia* sp. ATCC 39006, which are regulators of secreted enzymes and metabolites [11–14]. LrhA regulates a number of processes including motility, chemotaxis, and aspects of stationary phase physiology partially through indirect control of sigma factor S [10].

This brief report provides genetic and biochemical evidence that HexS is necessary for glucose to inhibit prodigiosin production and transcription from the prodigiosin biosynthetic operon by *S. marcescens*.

## 2. Materials and Methods

### 2.1. Bacterial Strains and Culture Conditions

All microorganisms are listed in Table 1 and were grown in lysogeny broth (LB) [15,16] with or without D-glucose (110 mM), with the exception of *Saccharomyces cerevisiae* which was grown with SC-uracil or YPD broth [17]. Cultures were grown in 5 mL aliquots of medium and rotated at high speed on a TC-7 tissue culture rotor (New Brunswick Scientific). Antibiotics used were tetracycline (10 µg/mL), kanamycin (100 µg/mL) and gentamicin (10 µg/mL). Conjugations were performed using *Escherichia coli* donor strain S17-1 as previously described [5]. Time course experiments were performed as previously described [5].

### 2.2. Cloning and Genetic Manipulations

The *hexS* open reading frame (ORF) was cloned using primers gaattgtgagcggataacaattcacaggaacagctAT-GACAACGCAAATCGTCCG (uppercase describes priming DNA, and lowercase represents recombination targeting sequence) and gttttatcagaccgcttctgcttctgatttag-TTATTCTTCTTCGTCCACCAGGCTGGC using yeast *in vivo* cloning [18] into integrative plasmid pMQ236 [19] to generate pMQ294. The *hexS*-Δ1 allele was made by digesting pMQ294 with SacII, which cuts at a single site in the *hexS* ORF. The SacII digested plasmid was treated with the End-IT kit (Epicentre) to generate

blunt ends and the plasmid was recircularized using T4 DNA ligase (New England Biolabs). The resulting plasmid, pMQ296, contained a *hexS* gene with a one base pair deletion (base pair 670 out of a total of 945 for the gene) creating a frame shift mutation and truncation.

For the insertional mutagenesis of *alaT*, *hexS* and *nuoC*, internal fragments were amplified using primers tgggtaacgccagggtttccagtcacgacgttgtaCCGTTACGACA-TGCATTTGCACC and gataacaatttcacacagaaacagctat-gaccatgatGGACGATCAGCTCAGAGACGC for *alaT*, gttgggtaacgccagggtttccagtcacgacgttgtaCGCGCACGTCAATGGCCAGC and taacaatttcacacagaaacagctatgaccatgatCCTCGATCTGCTAAGAACCTTTG for *hexS*, and gacgttgtaaaacacagggatctatcatcgtgatccTGGTGATAACCGATGTCCGGCAC and cttagcgggtttccgactggaagcgggcagtgagcgcCGAACTGCGCAACCGTTTTGG for *nuoC*. The amplicons were cloned into pMQ118 or pStvZ3 [19,20] and the resulting plasmids were introduced into *S. marcescens* by conjugation and selection for kanamycin resistance. The resulting strains have the plasmid inserted before the midpoint of each gene. Mutations were verified by PCR and mutant phenotype.

For purification of recombinant HexS, a N-terminal maltose binding protein (MBP) fusion was created in which the entire *hexS* open reading frame was fused to the *malE* gene using pMal-C2 (New England Biolabs). The open reading frame was amplified with primers that incorporated EcoRI and PstI sites for subsequent digestion and T4-ligase based ligation into the pMal-C2 plasmid polylinker. The resulting construct was verified by sequencing across the *malE-hexS* junction.

Allelic replacement of *hexS* with deletion alleles was performed using pMQ296, respectively, with previously described protocols [19], and was verified by PCR, sequencing, and phenotypic analysis. Briefly, pMQ296 was introduced into the recipient strain by conjugation and selection for kanamycin resistance (100  $\mu$ g/mL) to generate a merodiploid. The I-SceI expressing plasmid pMQ240 was introduced into the merodiploid by conjugation with selection for gentamicin (10  $\mu$ g/mL). The merodiploid strain with pMQ240 was grown in LB with gentamicin and streaked to single colonies on LB agar plates. Colonies were tested individually for loss of kanamycin resistance, and kanamycin susceptible strains were assessed for deletion of *pigA* or *hexS* using PCR. The *pigA::lacZ* construct was generated using pMQ268 as previously described [20], where the pStvZ3 plasmid bearing an internal fragment of *pigA* was introduced into recipient strains by conjugation and selection for kanamycin (100  $\mu$ g/mL).

### 2.3. Enzymatic Assays and Electrophoretic Mobility Shift Assay (EMSA)

Measurement of  $\beta$ -galactosidase activity was performed as previously described using 200  $\mu$ g of cell lysate from stationary phase cultures that had grown for 20 hours at 30°C using o-nitrophenyl  $\beta$ -D-galactopyranoside (ONPG) as a chromogenic substrate [20].

D-gluconic acid concentration was measured using an enzymatic D-gluconic acid detection kit (Megazyme, product K-GATE) as described by the manufacturer, with modifications that were previously described [5].

Glucose dehydrogenase activity (GDH) was measured using a chromogenic reaction based on the method of Matsushita, *et al.* [21] using 2,6-Dichlorophenol-Indo-phenol (Sigma, D-1878) and 13 mM phenazine methosulfate (Sigma, P-9625) as substrates as previously described [5].

A maltose binding protein (MBP) fusion of HexS and the MBP alone were purified using the manufacturers specifications (pMal Protein Fusion and Purification System, New England Biolabs). EMSA reactions were performed as previously described [20] using a

comercial EMSA kit (Lightshift Chemiluminescent EMSA kit, Pierce, Rockford IL). Briefly, *pigA* promoter DNA was amplified using biotinylated (or non-biotinylated) oligonucleotide primer 5'-ggggtacccttcacctgcaaagtattcatc-3' and non-biotinylated primer cgggaattcagatcgcgagcgcattcatgcc. The resulting amplicon was gel purified and used at 2 ng per binding reaction following the manufacturers specifications. A 10  $\mu$ L aliquot of the reaction was separated in a TBE PAGE gel (Pierce), transferred to a nylon membrane, and examined through chemiluminescence according to the manufacturers specifications. MBP and MBP-HexS were used at 25 – 30  $\mu$ g per reaction, and unlabelled promoter DNA was amplified with unlabelled versions of the above listed primers and the amplicon was used at 500 ng per binding reaction where specified. The EMSA reaction was performed three times with consistent results.

### 3. Results and Discussion

#### 3.1. Mutation of *hexS* Allows Prodigiosin Production in Glucose Rich Medium

In a recently reported genetic screen, we identified mutants that produced prodigiosin on LB glucose-supplemented agar plates (LBG) unlike the parental strain that was pigmentless on LBG [5]. One mutant strain that was pigmented on LBG agar had a transposon insertion in the gene for the HexS prodigiosin-regulatory transcription factor protein. The importance of HexS in glucose inhibition of prodigiosin by glucose was not verified in that study. Furthermore the previous study was performed using a *crp* mutant rather than a wild-type strain. The goal of this brief study was to determine whether HexS is necessary to inhibit *S. marcescens* prodigiosin production in glucose rich medium.

To characterize the effect of the *hexS* mutant phenol-type in a wild-type (WT strain CMS376) background, we generated a deletion allele *hexS-Δ1*, a directed *hexS* null-mutant allele to replace the chromosomal *hexS* gene in the WT (CMS376) strain, generating strain CMS2210, referred to here as  $\Delta hexS$ . This mutant strain exhibits all of the characteristics of a *hexS* null mutant strain, such as elevated prodigiosin production and serratomolide production (data not shown). The  $\Delta hexS$  (CMS2210) mutant and WT (CMS376) grew at the same rate in LB medium (Figure 1(a)). Unlike its growth-inhibitory effect upon the WT (CMS376), glucose did not inhibit culture density of the  $\Delta hexS$  (CMS2210) mutant (Figure 1(a)). Furthermore, the  $\Delta hexS$  (CMS2210) strain produced prodigiosin when grown with glucose unlike the WT (CMS376) (Figure 1(b)).

Previously described mutants able to produce pigment in LB medium supplemented with glucose (LBG) had mutations in genes necessary for glucose dehydrogenase activity (GDH), failed to achieve WT levels of medium acidification, and medium acidification was necessary for glucose to prevent prodigiosin production [5]. To test the prediction that *hexS* mutants are also able to generate pigment in LBG because they fail to acidify the medium, we measured the impact of *hexS* mutation upon medium acidification. No difference was observed between WT (CMS376) and the  $\Delta hexS$  (CMS2210) mutant with regards to pH in LB (Figure 1(c)). In LBG, the  $\Delta hexS$  (CMS2210) mutant differed from the WT (CMS376). Up to hour 6, both the WT (CMS376) and *hexS* mutant (CMS2210) experienced a sharp drop in pH, but after 6 hours the WT (CMS376) culture continued to decrease below pH 4, whereas the  $\Delta hexS$  (CMS2210) culture reproducibly stabilized close to pH 5 and then increased to neutrality by t = 24 hours (Figure 1(c)). This result is in stark contrast to all other reported pigment positive mutants that did not support a glucose-induced pH reduction [5]. Interestingly, the HexS deficient strain produced prodigiosin in cultures with very low pH. These are important observations because it shows that prodigiosin can be produced in acidic medium, indicating that the enzymatic process of generating prodigiosin is not simply unable to occur at low pH, and implying that an active inhibition of prodigiosin production

takes place in glucose-rich medium. The mechanism by which HexS mediates culture pH in LBG will be the subject of subsequent research.

### 3.2. Quinoprotein Glucose Dehydrogenase Protein Activity is Altered in *hexS* Mutants

Previous work showed that reduced quinoprotein glucose dehydrogenase (GDH) activity, which converts glucose to gluconic acid, conferred the ability to produce prodigiosin in LBG [5]. Therefore, we tested whether the *hexS* mutant (CMS2210) would have reduced GDH activity. However, the  $\Delta hexS$  (CMS2210) mutant surprisingly produced equivalent GDH activity to the WT (CMS376) when grown in LB, and higher levels than the WT (CMS376) at later time points when cultured in LBG (Figure 1(d)) suggesting the GDH activity in LBG is regulated by HexS.

As a second assay for GDH activity, we measured gluconic acid (GA) levels. GA in LBG was maximal for the wild type (CMS376) at 6 – 9 hours [5]. Unexpectedly, gluconic acid levels in the  $\Delta hexS$  (CMS2210) mutant culture at six hours were significantly reduced compared to the WT (CMS376) (Student's T-test  $p < 0.05$ ). In the aforementioned time course (Figure 1) gluconic acid was measured at  $12.1 \pm 1.9$  mM from the  $\Delta hexS$  (CMS2210) strain, compared  $20.2 \pm 2.4$  mM for the WT (CMS376) at  $t = 6$  hours. A similar trend was observed over the time course (data not shown). These data suggest that the reduced organic acid in  $\Delta hexS$  (CMS2210) mutant cultures at late time points may account of the increased culture pH at late time points.

### 3.3. Complementation of the $\Delta hexS$ (CMS2210) Phenotype

To perform complementation analysis, we introduced a single WT copy of *hexS* into the  $\Delta hexS$  (CMS2210) chromosome with pMQ294 (diagramed in Figure 2(a)) and restored the glucose sensitivity phenotype (Figure 2(b)). We measured elevated prodigiosin levels in both the *hexS* deletion strain and a *hexS-1* (CMS1779) insertion mutant strain compared to WT (CMS376). As an additional control, *hexS* was mutated by plasmid insertion. This *hexS-1* (CMS1779) strain served as a control to be an independent mutation to confirm that mutation of *hexS* conferred the observed pigment phenotypes, and to test whether insertion of a plasmid alone into a *hexS* mutant (CMS2210) strain would restore sensitivity to glucose. In LBG, the WT (CMS376) and  $\Delta hexS$  (CMS2210) + pMQ294 results were not significantly different in prodigiosin production, whereas the *hexS* mutant's (CMS2210) pigment levels were significantly higher than either WT (CMS376) or  $\Delta hexS$  (CMS2210) + pMQ294 (ANOVA,  $p < 0.05$ , Figure 2). To further ensure that the pigment phenotype of *hexS* mutants were not due to a polar effect on adjacent genes, mutations were introduced into the operons on either side of *hexS* on the chromosome. In one direction is a divergently transcribed *alaT* homolog greater than 650 base pairs away from the *hexS* gene, and on the opposite side of *hexS*, transcribed in the same orientation, is a predicted NADH-ubiquinone oxidoreductase operon (*nuoA-N*), greater than 700 base pairs away from the *hexS* ORF. To further support that loss of the glucose inhibition of prodigiosin phenotype of *hexS* mutants was not due to a polar effect on adjacent genes, *alaT* and *nuoC* were separately mutated in the WT (CMS376) strain. The resulting mutant strains did not exhibit the pigmented phenotype of the *hexS* insertion mutant in LBG (data not shown). Together, these data support that HexS is important for glucose inhibition of pigmentation.

### 3.4. HexS Is Necessary for Glucose-Inhibition of Transcription of the Prodigiosin Biosynthetic Operon

To test the hypothesis that HexS regulates the *pigA* promoter in LBG, we used a previously characterized chromosomal *pigA-lacZ* reporter [20]. Whereas *pigA-lacZ* associated  $\beta$ -galactosidase levels were severely reduced in the WT (CMS376) in LBG compared to LB medium, the  $\Delta hexS$  (CMS2210) mutant exhibited similar expression levels regardless of

glucose (Figure 3(a)). To ensure that this was a direct transcriptional regulation, we confirmed a previous report that the HexS protein binds to *pigA* promoter DNA *in vitro* using a recombinant version of HexS in an EMSA assay [8], but using recombinant HexS protein that had been cloned from the CMS376 genome (Figure 3(b)).

The major goal of this study, to test whether HexS is required for inhibition of the secondary metabolite, prodigiosin, by glucose was achieved. Genetic analysis implicated the HexS transcription factor in the glucose inhibition of secondary metabolite phenotype. Interestingly, unlike the other glucose insensitive mutants isolated [5], *hexS* mutation neither prevented early medium acidification nor prevented glucose oxidation by glucose dehydrogenase.

Together results suggest that HexS transcriptional control of *pigA-N* is an important factor in inhibiting prodigiosin production in glucose-rich medium. Given that HexS negatively regulates multiple secondary metabolites [8], and the data from this study, we propose that HexS may be the major regulator involved of the inhibition of secondary metabolites by glucose. Future experimentation will be aimed at determining whether HexS activity responds directly to changes in pH. We propose that mutation of LrhA-like regulators may be useful for production of desired secondary metabolites.

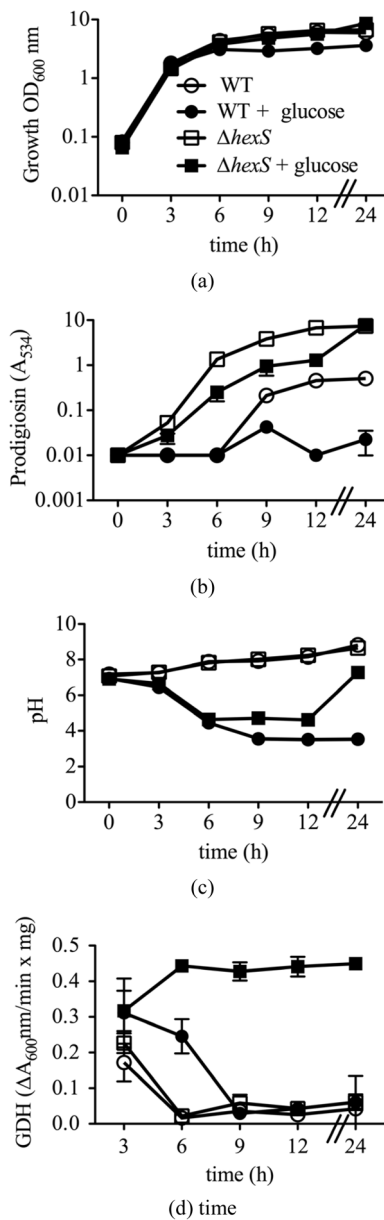
## Acknowledgments

The authors would like to thank Jean-Paul Vergnes, Kip Kinchington, Cody Bender, Aroba Sadaf, and Marissa Aston for technical help, and Pryce Haddix and Kim Brothers for critical manuscript evaluation. This study was funded by AI085570 and a Research to Prevent Blindness Career Development Award to RS. Other funding sources were NIH grant EY08098, the Eye and Ear Foundation of Pittsburgh, and unrestricted funds from Research to Prevent Blindness.

## References

1. Sole M, Francia A, Rius N, Loren JG. The Role of pH in the 'Glucose Effect' on Prodigiosin Production by Non-Proliferating Cells of *Serratia marcescens*. *Letters in Applied Microbiology*. 1997; 25:81–84.
2. Sole M, Rius N, Loren JG. Rapid Extracellular Acidification Induced by Glucose Metabolism in Non-Proliferating Cells of *Serratia marcescens*. *International Microbiology*. 2000; 3(1):39–43. [PubMed: 10963332]
3. Williamson NR, Fineran PC, Leeper FJ, Salmond GP. The Biosynthesis and Regulation of Bacterial Prodiginines. *Nature Reviews Microbiology*. 2006; 4(12):887–899.10.1038/nrmicro1531
4. Bunting MI, Robinow CF, Bunting H. Factors Affecting the Elaboration of Pigment and Polysaccharide by *Serratia marcescens*. *Journal of Bacteriology*. 1949; 58(1):114.
5. Fender JE, Bender CM, Stella NA, Lahr RM, Kalivoda EJ, Shanks RMQ. *Serratia marcescens* Quinoprotein Glucose Dehydrogenase Activity Mediates Medium Acidification and Inhibition of Prodigiosin Production by Glucose. *Applied and Environmental Microbiology*. 2012; 78(17):6225–6235.10.1128/AEM.01778-12 [PubMed: 22752173]
6. Dauenhauer SA, Hull RA, Williams RP. Cloning and Expression in *Escherichia coli* of *Serratia marcescens* Genes Encoding Prodigiosin Biosynthesis. *Journal of Bacteriology*. 1984; 158(3):1128–1132. [PubMed: 6233267]
7. Harris AK, Williamson NR, Slater H, Cox A, Abbasi S, Foulds I, Simonsen HT, Leeper FJ, Salmond GP. The *Serratia* Gene Cluster Encoding Biosynthesis of the Red Antibiotic, Prodigiosin, Shows Species- and Strain-Dependent Genome Context Variation. *Microbiology*. 2004; 150(11): 3547–3560. [PubMed: 15528645]
8. Tanikawa T, Nakagawa Y, Matsuyama T. Transcriptional Down Regulator HexS Controlling Prodigiosin and Serrawettin W1 Biosynthesis in *Serratia marcescens*. *Microbiology and Immunology*. 2006; 50(8):587–596. [PubMed: 16924143]

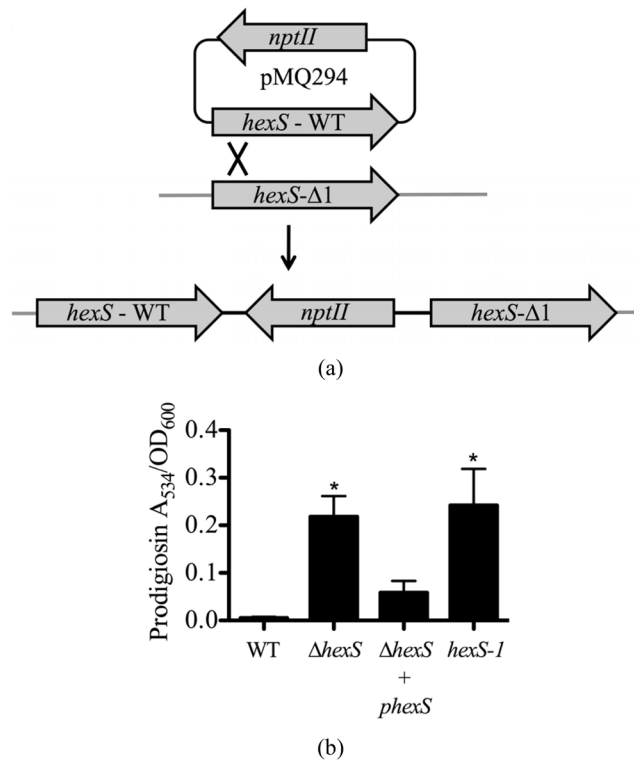
9. Shanks RM, Stella NA, Lahr RM, Wang S, Veverka TI, Kowalski RP, Liu X. Serratamolide Is a Hemolytic Factor Produced by *Serratia marcescens*. PLoS One. 2012; 7(5):Article ID: e36398.10.1371/journal.pone.0036398
10. Gibson KE, Silhavy TJ. The LysR Homolog LrhA Promotes RpoS Degradation by Modulating Activity of the Response Regulator *sprE*. Journal of Bacteriology. 1999; 181(2):563–571. [PubMed: 9882671]
11. Fineran PC, Slater H, Everson L, Hughes K, Salmond GP. Biosynthesis of Tripyrrole and Beta-Lactam Secondary Metabolites in *Serratia*: Integration of Quorum Sensing with Multiple New Regulatory Components in the Control of Prodigiosin and Carbapenem Antibiotic Production. Molecular Microbiology. 2005; 56(6):1495–1517.10.1111/j.1365-2958.2005.04660.x [PubMed: 15916601]
12. Harris SJ, Shih YL, Bentley SD, Salmond GP. The *hexA* Gene of *Erwinia carotovora* Encodes a LysR Homologue and Regulates Motility and the Expression of Multiple Virulence Determinants. Molecular Microbiology. 1998; 28(4):705–717.10.1046/j.1365-2958.1998.00825.x [PubMed: 9643539]
13. Mukherjee A, Cui Y, Ma W, Liu Y, Chatterjee AK. *hexA* of *Erwinia carotovora ssp. carotovora* Strain Ecc71 Negatively Regulates Production of RpoS and *rsmB* RNA, a Global Regulator of Extracellular Proteins, Plant Virulence and the Quorum-Sensing Signal, N-(3-oxohexanoyl)-L-homoserine Lactone. Environmental Microbiology. 2000; 2(2):203–215.10.1046/j.1462-2920.2000.00093.x [PubMed: 11220306]
14. Joyce SA, Clarke DJ. A *hexA* Homologue from *Photorhabdus* Regulates Pathogenicity, Symbiosis and Phenotypic Variation. Molecular Microbiology. 2003; 47(5):1445–1457.10.1046/j.1365-2958.2003.03389.x [PubMed: 12603747]
15. Bertani G. Studies on Lysogenesis. I. The Mode of Phage Liberation by Lysogenic *Escherichia coli*. Journal of Bacteriology. 1951; 62(3):293–300. [PubMed: 14888646]
16. Bertani G. Lysogeny at Mid-Twentieth Century: P1, P2, and Other Experimental Systems. Journal of Bacteriology. 2004; 186(3):595–600.10.1128/JB.186.3.595-600.2004 [PubMed: 14729683]
17. Burke, DD.; Dawson, DS.; Stearns, T. Methods in Yeast Genetics, a Cold Spring Harbor Laboratory Course Manual. Cold Harbor Laboratory Press; Plainview: 2000.
18. Shanks RM, Caiazza NC, Hinsa SM, Toutain CM, O'Toole GA. *Saccharomyces cerevisiae*-Based Molecular Tool Kit for Manipulation of Genes from Gram-Negative Bacteria. Applied and Environmental Microbiology. 2006; 72(7):5027–5036.10.1128/AEM.00682-06 [PubMed: 16820502]
19. Shanks RM, Kadouri DE, MacEachran DP, O'Toole AG. New Yeast Recombineering Tools for Bacteria. Plasmid. 2009; 62(2):88–97.10.1016/j.plasmid.2009.05.002 [PubMed: 19477196]
20. Kalivoda EJ, Stella NA, Aston MA, Fender JE, Thompson PP, Kowalski RP, Shanks RM. Cyclic AMP Negatively Regulates Prodigiosin Production by *Serratia marcescens*. Research in Microbiology. 2010; 161(2):158–167.10.1016/j.resmic.2009.12.004 [PubMed: 20045458]
21. Matsushita K, Shinagawa E, Adachi O, Ameyama M. Quinoprotein D-Glucose Dehydrogenase of the *Acinetobacter calcoaceticus* Respiratory Chain: Membrane-Bound and Soluble Forms Are Different Molecular Species. Biochemistry. 1989; 28(15):6276–6280.10.1021/bi00441a020 [PubMed: 2551369]
22. Miller VL, Mekalanos JJ. A Novel Suicide Vector and Its Use in Construction of Insertion Mutations: Osmoregulation of Outer Membrane Proteins and Virulence Determinants in *Vibrio Cholerae* Requires *toxR*. Journal of Bacteriology. 1988; 170:2575. [PubMed: 2836362]



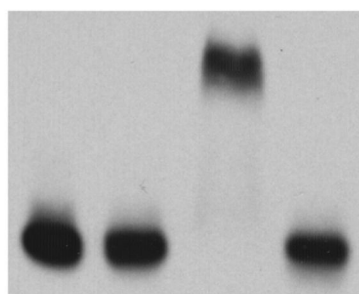
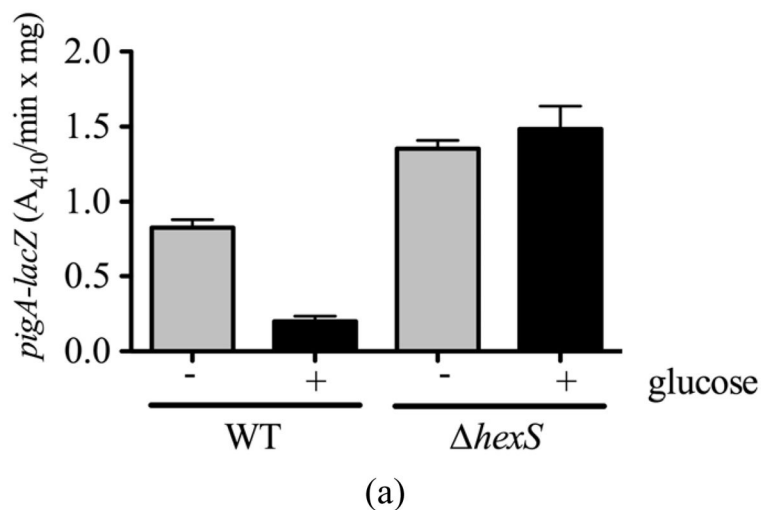
**Figure 1.**

Growth, prodigiosin production, culture pH of WT and  $\Delta hexS$  (CMS2210) mutant cells grown in LB and LBG. Genotype symbols are consistent for each panel. Cultures were grown in LB and in LBG (+glucose). The *hexS* mutant used was  $\Delta hexS$  (CMS2210). (a) Culture growth as measured by turbidity ( $OD_{600\text{ nm}}$ ). (b) Extracted prodigiosin levels were measured from 1 ml of culture ( $A_{534\text{ nm}}$ ). (c) Culture pH was determined with a pH meter. (d) Glucose dehydrogenase activity measured from crude cellular extracts. Time course experiments were performed with 4 independent biological replicates per time point per condition, and performed three times with consistent results, one representative time course is shown. Error bars indicate one standard deviation.





**Figure 2.** Complementation of the *hexS* mutant phenotype. (a) Complementation strategy through integrating a wild-type *hexS* gene on pMQ294 into a strain with a *hexS* deletion mutation (CMS3125). (b) Prodigiosin production in LBG shows that unlike the WT (CMS376), a *hexS* deletion mutant ( $\Delta hexS$ , CMS2210) and a *hexS* insertion mutant (*hexS-1*, CMS1779) were able to generate prodigiosin. A wild-type copy of *hexS* on a plasmid (pMQ294) was inserted into the chromosome of the  $\Delta hexS$  (CMS2210) strain and restored glucose sensitivity. Error bars indicate one standard deviation, and asterisks indicate significant differences from the WT (CMS376) ( $p < 0.05$ , ANOVA with Tukey's post-test).



MBP	-	+	-	-
MBP-HexS	-	-	+	+
comp.	-	-	-	+

(b)

**Figure 3.**

Glucose sensitivity of the prodigiosin biosynthetic operon is dependent upon HexS. (a) Expression from the *pigA* promoter was indirectly measured using a chromosomal *lacZ* transcriptional fusion.  $\beta$ -galactosidase activity was measured from cells grown to stationary phase (20 h) from culture grown in LB (-) or LBG (+) medium. Error bars indicate one standard deviation; (b) Representative EMSA showing MBP-HexS binding to the biotinylated *pigA* promoter. MBP = maltose binding protein, MBP-HexS = N-terminal fusion of maltose binding protein to HexS, comp. = unlabelled competitor *pigA* promoter DNA.

Table 1

Strains and plasmids used in this study.

Strain or plasmid	Description	Reference or source
<i>S. cerevisiae</i>		
InvSc1	Diploid uracil auxotroph	Invitrogen
<b>Bacterial strains</b>		
S17-1 $\lambda$ pir	<i>Escherichia coli</i> strain used for conjugation	[22]
EC100D	<i>E. coli</i> strain used for cloning, <i>pir</i> -116	Epicentre
ER2566	<i>E. coli</i> strain used for protein expression	New England Biolabs
CMS376	wild-type <i>Serratia marcescens</i> , PIC strain number 3611	Presque Isle Cultures
CMS1779	<i>hexS</i> ::pStvZ3 in WT (CMS376), <i>hexS</i> -1	This study
CMS2210	$\Delta$ <i>hexS</i> , ( <i>hexS</i> - $\Delta$ 1 allele) in CMS376	This study
CMS3125	CMS2210 + pMQ294 <i>hexS</i> complementation strain	This study
CMS3142	<i>nuoC</i> ::pStvZ3 in CMS376	This study
CMS1317	<i>alaT</i> ::pMQ118 in CMS376	This study
<b>Plasmids</b>		
pMal-C2	Maltose binding protein fusion construct	New England Biolabs
pStvZ3	<i>oriR6K lacZ nptII</i> promoter probe	[20]
pMQ118	suicide vector <i>nptII</i> , <i>rpsL</i> , <i>oriT</i> , <i>URA3</i> , <i>CEN6/ARSH4</i>	[19]
pMQ197	pMQ118 with internal fragment of <i>alaT</i>	This study
pMQ236	allelic replacement vector, pMQ118 with I-SceI site	[19]
pMQ240	I-SceI-expression plasmid, <i>aacC1</i> , <i>oripSC101</i> <sup>ts</sup>	[19]
pMQ268	pStvZ3 + <i>pigA</i> internal fragment	[20]
pMQ294	pMQ236 with <i>hexS</i> open reading frame	This study
pMQ296	pMQ294 with <i>hexS</i> - $\Delta$ 1 mutant allele	This study
pMQ342	pStvZ3 with internal fragment of <i>nuoC</i>	This study
pMQ402	pMAL-C2 + <i>hexS</i> (MBP-HexS fusion construct)	This study