Spring 2015

Regulation of the Surfactant Serrawettin W2 in Serratia Sp. Strain SCBI

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Regulation of the Surfactant Serrawettin W2 in *Serratia* Sp. Strain SCBI

Honors Senior Thesis

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May 2015

Abstract:

A novel *Serratia* sp (termed SCBI) forms an association with nematodes (South African *Caenorhabditis briggsae*) that were isolated by bait trapping in the greater wax moth, *Galleria mellonella*. This association represents a new microbe-nematode relationship and potentially a nascent entomopathogenic lifestyle. Inactivation of the hemolysin in *Serratia* sp. SCBI resulted in loss of hemolysis, but did not attenuate insecticidal activity. Unexpectedly, inactivation of the hemolysin gene in *Serratia* sp. SCBI resulted in significantly increased motility and a dramatic increase in mRNA levels of a nonribosomal peptide synthetase gene, *swrA*, which produces the surfactant serrawettin W2. A series of mutations in the 17 Kb *swrA* gene gave variable results in antibiotic activity, motility, virulence and hemolysin activities. The locations of three of the four mutations were confirmed by PCR analysis. The regulation of the expression of the *swrA* and hemolysin genes in *swrA* and hemolysin mutants was investigated by RT-qPCR using primer sets for different regions of these two genes. Furthermore, the cDNA of the 17 Kb transcript of *swrA* was generated by reverse transcription and was analyzed by PCR with the primers used to confirm the *swrA* mutants. Results indicate that the *swrA* transcript is expressed differently at various points in its sequence. Mutations appear to affect expression differently depending on the location, suggesting complex regulatory mechanisms and the presence of multiple promoters within the transcript.

Introduction:

The Genus *Serratia*

The genus *Serratia* is ubiquitous in nature, and survives in a wide range of habitats including water, soil, humans, insects, and plants (3). *Serratia* spp. are opportunistically pathogenic to humans; the most well know human pathogen being *S. marcescens,* which causes infection in immunocompromised patients. Outside of human hosts, *Serratia spp.* are found to be pathogenic towards plants, insects, and cold-blooded vertebrates. The ubiquity of *Serratia* spp. is achieved through the secretion of a wide range of extracellular enzymes; characteristic enzymes produced by *Serratia* spp. include gelatinase, lecithinase, and DNase (3,7). Many *Serratia* spp. secrete biomolecules with anti-bacterial properties that are crucial for success. *Serratia* spp. are also known to secrete a range of pigments, biosurfactants, and fatty acids. Insect pathogenic *Serratia* spp. possess high toxicity towards hemocytes and strong protease activity that contributes to this toxicity and immunosuppression (5).

A novel *Serratia* species

Some *Serratia* spp. are known to form entomopathogenic relationships with nematodes similar to the *Photorhabdus-Heterorhabditis* relationship (5, 12). One such relationship can be found in the novel *Serratia* sp. (termed SCBI), which has been found to form a symbiotic relationship with a new strain of South African *Caenorhabditis briggsae* nematodes (KT0001). This symbiotic nematode-bacteria pair was isolated from the greater wax moth, *Galleria mellonella,* isolated from soil samples through bait traps in three South African provinces (1). The association represents a new microbe-nematode relationship and potentially a nascent entomopathogenic lifestyle. *Serratia* sp. strain SCBI shares a 99% 16S rRNA sequence similarity with *S. marcescens* Db11, a known nematode pathogen (1,5). Many of the virulence factors of *Serratia* sp. strain SCBI are similar to those found in *S. marcescens* Db11, including hemolysin, lipase, and chitinase activities. Although both strains possess these factors they are regulated differently, possibly contributing to the evolution from a nematode pathogen to symbiont (5,6).

The association between *Serratia* sp. strain SCBI and *C. briggsae* represents a nascent entomopathogenic association similar to well known nematode-bacteria associations. Figure 1 depicts the proposed life cycle of this association.

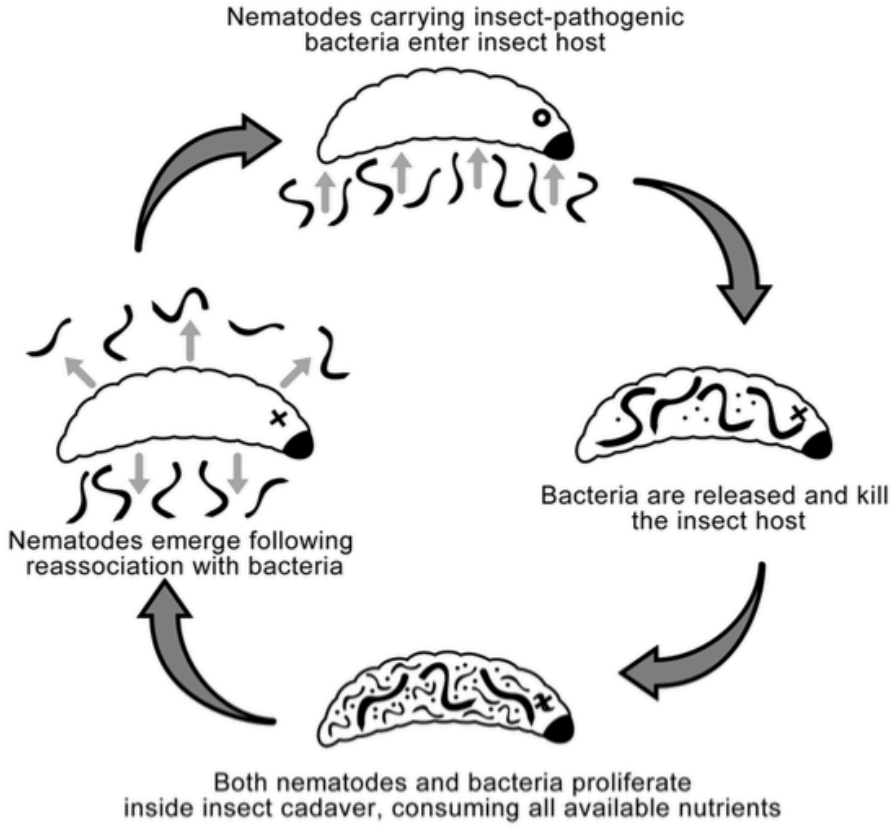

Fig. 1: Proposed life cycle of the *Serratia* sp. strain SCBI and *C. briggsae* association. Bacteria colonize the intestine or surface of the nematode, and the nematode enters the insect larva. The nematode penetrates the insect and the bacteria are released and kill the insect host. Once the insect is killed nematode reproduces while the bacteria consume the nutrients of the insect, before the two reassociate and search for new insect prey (5).

During this infection cycle, *Serratia* sp. SCBI associates internally with the *C. briggsae* nematode, and the pair infect the insect host (2,5). Although it is proposed that *Serratia* sp. strain SCBI associates with *C. briggsae* and the pair subsequently infect an insect larva, it is still not known how *C. briggsae* gains entry into the insect host (1). Once inside the host, *Serratia* sp. SCBI is able to kill the insect. Through direct injection of *Serratia* sp. strain SCBI

into the hemocoel of *Galleria mellonella* it was found that the bacterium is lethal in numbers of less than 1,000 CFU (1). *C. briggsae* KT001 was unable to kill then insect when unassociated with *Serratia* sp. SCBI, indicating that *Serratia* sp. SCBI is critical for death of the insect host (2).. *Serratia* sp. SCBI is a potent insect pathogen that does not affect the longevity and reproduction of *C. briggsae* KT0001 supporting the proposed symbiotic model (4).

Serratia sp. strain SCBI

Serratia sp. SCBI shares a high 16s rRNA and genome similarity to *S. marcescens* Db11 (5,7). This similarity extends to virulence factors, and studies on virulence factors found that temperature also plays a role in regulation of virulence factors in *Serratia* sp. SCBI. Although *Serratia* sp. SCBI and *S. marcescens* Db11 share many virulence factors, there are differences in hemolytic activity and motility, with *Serratia* sp. SCBI displaying greater swarming and hemolytic activities than *S. marcescens* Db11 (5,6). *Serratia* sp. SCBI contains a two-gene hemolysin operon that shares a 95% identity to the hemolysin secretion system found in *S. marcescens* Db11. Although they share a high sequence similarity, the regulation of hemolytic activity is different between the two species in order to account for the different associations with nematode hosts (6).

Along with the hemolysin gene, it was found that *Serratia* sp. SCBI produces a surfactant protein, termed serrawettin W2, similar to the serrawettin surfactants produced by *S. marcescens* Db11. (6). Serrawettin W2 is encoded by a 17,775 bp nonribosomal-peptide synthetase gene, *swrA,* and the structure of this surfactant was determined by MALDI-Orbtrap-MS, as shown in figure 2 (Peterson et al., unpublished). Although *Serratia* sp. SCBI shares high hemolysin and serrawettin W2 sequence similarities to *S. marcescens* Db11, the regulation of these is drastically different. Inactivation of the hemolysin did not attenuate virulence of *Serratia* sp. SCBI, indicating that hemolysis is not the driving factor in *Serratia* sp. SCBI pathogenesis. Unexpectedly, inactivation of the hemolysin gene it lead to drastically increased swimming and swarming motility, and the overproduction of serrawettin W2 indicating that hemolysin is regulated differently in *Serratia* sp. SCBI compared to *S. marcescens* Db11. Mutations within the *swrA* gene had variable affects in

motility, hemolysin activity, antibiotic activity and virulence. The observed phenomena indicate that the hemolysin gene and *swrA* influence the expression of each other, but how this influence is achieved has yet to be discovered (6).

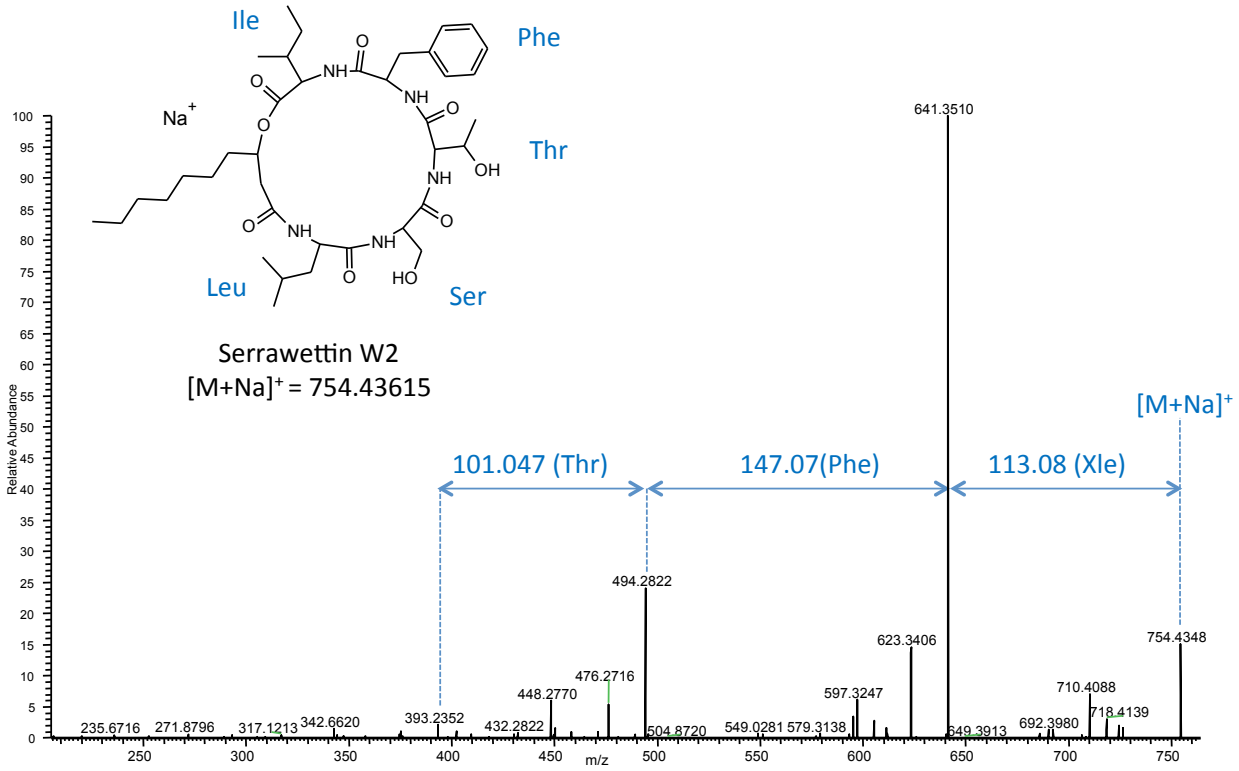

Fig. 2: MALDI-Orbitrap-MS^2 analysis of *m/z* 754.43 [M+Na]$^+$. Structure of Serrawettin W2. (Peterson et al., unpublished)

Previous studies proposed that the beginning of the *swrA* gene is responsible for producing serrawettin, while the remaining majority of the gene may responsible for regulation or modification (6). This project seeks to explore the regulation of the *swrA* gene. Using previously generated transposon mutants, containing mutations in the *swrA* gene, expression will be analyzed through PCR. Understanding the expression and regulation of the *swrA* gene could have important implications on better understanding *Serratia* sp. SCBI symbiosis and pathogenesis.

Materials and Methods:

Bacterial Strains and Growth Conditions. Table 1 lists all of the bacterial strains used in this study. Transposon mutants containing mutations in the *swrA* or hemolysin genes were used. The strains were grown on LB agar (1% tryptone, 0.5% yeast extract, 1% NaCl, 1.5% agar) at 37°C for 24 hours (6) and stored at 4°C. Liquid cultures were grown in LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl) at 37°C for 24 hours as needed (6).

Table 1: Bacterial strains used in this study

Strain/Mutant	Description	Source
WT *Serratia* sp. SCBI	Wild-Type *Serratia* sp. SCBI	Abebe et al., 2010
1-A4	Transposon mutant, disrupted *swrA*	Peterson et al., 2014
1-C4	Transposon mutant, disrupted hemolysin gene	Peterson et al., 2014
11-B8	Transposon mutant, disrupted *swrA*	Peterson et al., 2014
11-C1	Transposon mutant, disrupted *swrA*	Peterson et al., 2014
13-G2	Transposon mutant, disrupted *swrA*	Peterson et al., 2014

Genomic DNA Extraction: Cultures were grown in LB medium at 37°C for 18 hours. Genomic DNA was extracted using the phenol-chloroform extraction technique (6), see appendix 1 for detailed protocol. The cells were pelleted and the supernatant decanted, followed by resuspension in 1X TE buffer (565.8 µL). Once resuspended, lysozyme (1.2 µL), proteinase K (3 µL), 5M NaCl (100 µL), CTAB/NaCl solution (80 µL), and chloroform:isoamyl:alcohol (800 µL) were added and incubated when necessary. The mixture was centrifuged and the aqueous phase was isolated, followed by the addition of phenol:chloroform:isoamyl alcohol (500 µL). Once again the mixture was centrifuged and the aqueous phase was isolated, treated with isopropanol (0.6 volume), and frozen at -20°C overnight. The samples were centrifuged and the supernatant was discarded, isolating the pellet. The pellet was washed with ice cold 70% ethanol (600 µL), followed by centrifugation and isolation of the pellet. The pellet was resuspended in 1X TE buffer (99

µL) and treated with RNase (1 µL). The DNA was quantified using a NanoDrop spectrophotometer, diluted to a concentration of 250 ng/µL, and stored at -20ºC until use.

Confirmation of *swrA* and hemolysin mutants: Confirmation of *swrA* mutants was performed using PCR. Four primers at the site of the transposon insertions were used, see table 2 for a complete list of primers. Wild type genomic DNA was used as a positive control in these experiments. The PCR reactions were done using 500 ng of genomic DNA, primers, 2X Reaction Mix, and OneTaq Hot Start 2X Master Mix with the Standard Buffer (New England Biolabs) in a 50 µL reaction mix. The thermocycler settings were as follows: 30 seconds at 94°C for an initial denaturation, 30 cycles of 30 seconds at 94°C, 30 seconds at 50°C, and 1 minute at 68°C, followed by a final extension for 10 minutes at 68°C. The PCR products were viewed through gel electrophoresis using a 1% agarose gel run at 120V, followed by staining with ethidium bromide.

Table 2: Primers used for the PCR confirmation of the *swrA* and hemolysin mutants

Primer Name	**Location**	**Sequence (5'➔ 3')**
F-SCBI_swrA 11-C1	*swrA* 5561	TGGAACACACGCCAGAAA
R-SCBI_swrA 11-C1	*swrA* 6428	CGCCTCCATACAGCAGAAA
F-swrA_8700	*swrA* 8595	ATGACATGACGCTGGATCTG
R-swrA_8700	*swrA* 8922	GATAGCTGAGGCGTTCTTCTC
F-swrA 13-G2	*swrA* 9654	CCGTGGTATGCCCGTAATC
R-swrA 13-G2	*swrA* 10489	CCAACTGTACCGCCAACA
F-SCBI_swrA 11-B8	*swrA* 15894	CCGCGTTGTTCAATCAGTATG
R-SCBI_swrA 11-B8	*swrA* 16872	GATCAAAGATCGCCTGTACCT
F-1-C4 mutant	hemolysin 923	GCGACAACTACCAGAACTACC
R-1-C4 mutant	hemolysin 1367	GTTCGCTGGACTTATCCTTCTC

RNA Extraction: Cultures were grown in LB medium at 37°C for 18 hours and treated with RNA protect (11), following manufacture's instructions. The RNA extraction was performed using the RNeasy Mini Kit (10), following manufacturer's instructions. After extraction, the RNA was treated with DNase to remove any contaminating DNA. The RNA was quantified

using a Qubit 2.0 Fluorometer (Invitrogen), following manufacturer's instructions, diluted to a concentration of 250 ng/µL and stored at -80ºC until use.

cDNA Synthesis using random primers: cDNA of total RNA was synthesized to be used in qRT-PCR experiments. cDNA was synthesized from total RNA using the GoScript Reverse Transcriptase and random primers (9), following to manufacturer's instructions. The cDNA was quantified using a NanoDrop spectrophotometer, diluted to a concentration of 10 ng/µL and stored at -20ºC until use.

cDNA synthesis of the *swrA* transcript: cDNA of the *swrA* gene was synthesized in order to evaluate expression at different points within the transcript. cDNA was synthesized using the GoScript Reverse Transcriptase (9), following manufacturer's instructions. For synthesis of the *swrA* transcript the swrA_17037 reverse primer was used (see table 3). The cDNA was quantified using a NanoDrop spectrophotometer, diluted to a concentration of 250 ng/µL and stored at -20ºC until use.

Analysis of *swrA* expression using RT-PCR with total RNA: Analysis of *swrA* expression was performed using RT-PCR. 6 primers at different sites in the *swrA* gene were used (see table 3) for a complete list of primers. Wild type genomic DNA was used as a positive control in these experiments. The RT-PCR reactions were done using 500 ng of total RNA, primers, 2X Reaction Mix, and SuperScript III RT/Platinum Taq Mix (Invitrogen) in a 50 µL reaction mix. The thermocycler settings were as follows: 30 seconds at 94°C for an initial denaturation, 30 cycles of 30 seconds at 94°C, 30 seconds at 50°C, and 1 minute at 68°C, followed by a final extension for 10 minutes at 68°C. The PCR products were viewed through gel electrophoresis using a 1% agarose gel run at 120V, followed by staining with ethidium bromide.

Analysis of *swrA* expression using RT-PCR with *swrA* cDNA: Analysis of *swrA* expression was performed using RT-PCR. Six primers at different sites in the *swrA* gene were used (see table 3 for a complete list of primers). Wild type genomic DNA was used as a positive control in these experiments. The RT-PCR reactions were done using 1000 ng of

swrA cDNA (generated using the swrA_17037 reverse primer), primers, and OneTaq Hot Start 2X Master Mix With Standard Buffer (New England Biolabs) in a 25 µL reaction mix. The thermocycler settings were as follows: 30 seconds at 94°C for an initial denaturation, 30 cycles of 30 seconds at 94°C, 30 seconds at 50°C, and 1 minute at 68°C, followed by a final extension for 10 minutes at 68°C. The PCR products were viewed through gel electrophoresis using a 1% agarose gel run at 120V, followed by staining with ethidium bromide.

Table 3: Primers used for the RT-PCR analysis of the *swrA* gene

Primer Name	**Location**	**Sequence (5'➔3')**
F-swrA-qRT	*swrA* 616	CCCGCTTCACTACCGATAAA
R-swrA-qRT	*swrA 723*	GCTGATTTGCATGGACGAATAA
F-swrA_2907	*swrA* 2907	CCTGGCGTATGTGGTTTACA
R-swrA_2907	*swrA* 3338	CCGTCGGCCCATAGTTATTC
F-SCBI_swrA 11-C1	*swrA* 5561	TGGAACACACGCCAGAAA
R-SCBI_swrA 11-C1	*swrA* 6428	CGCCTCCATACAGCAGAAA
F-swrA_8700	*swrA* 8595	ATGACATGACGCTGGATCTG
R-swrA_8700	*swrA* 8922	GATAGCTGAGGCGTTCTTCTC
F-swrA 13-G2	*swrA* 9654	CCGTGGTATGCCCGTAATC
R-swrA 13-G2	*swrA* 10489	CCAACTGTACCGCCAACA
F-SCBI_swrA 11-B8	*swrA* 15894	CCGCGTTGTTCAATCAGTATG
R-SCBI_swrA 11-B8	*swrA* 16872	GATCAAAGATCGCCTGTACCT

Quantification of *swrA* expression using qRT-PCR: Amplification and quantification of *swrA* expression was performed using the Strategene Mx3000 qPCR system (Agilent Technologies). Three primers at different sites in the *swrA* gene were used (see table 4 for a list of primers). The *rplU* ribosomal gene was used as a normalizer for all qRT-PCR experiments. To determine primer efficiency, standard curves were generated using *Serratia* sp. Strain SCBI gDNA with each primer set. The comparative threshold-cycle method was used to quantify gene expression, and reactions were performed in triplicate. The qRT-PCR reactions were performed using 50 ng of template cDNA, SYBR Green PCR

master mix (Applied Biosystems), and primer mix (0.3 µM) in a 25 µL reaction mixture. Parameters for the Agilent MP3000 were as follows: 95ºC for 15 min; 40 cycles of 95ºC for 15 s and 60ºC for 30 s; and a thermal disassociation cycle of 95ºC for 60 s, 55ºC for 30 s , and incremental increases of temperature to 95ºC for 30s.

Table 4: Primers used for the qRT-PCR quantification of *swrA* gene expression

Primer	Location	Sequence (5'➔3')
F-swrA-qRT	*swrA* 616	CCCGCTTCACTACCGATAAA
R-swrA-qRT	*swrA* 723	GCTGATTTGCATGGACGAATAA
F-swrA_13810	*swrA* 13808	GTTGAGATCGACAGCATCGT
R-swrA_13810	*swrA* 13924	GATTCTGTTCCACCACCTGT
F-swrA_17037	*swrA* 17031	TGCGGAGTTCCCGATTTATG
R-swrA_17037	*swrA* 17176	GAGTAACCGAACAGGTGGTAAG

Results:

Confirmation of *swrA* mutations in previously generated transposon mutants.

Previous studies indicated the presence of four mutants in the miniHimar RBI library containing inserts within the swrA gene. PCR was conducted with *Serratia* sp. SCBI wild type and mutant gDNA, and primers for the proposed insertion sites. The presence of increased bands on the electrophoresis gel of the PCR products indicated a positive confirmation for the insert site (see appendix 2 for PCR results). Of the four mutants, three insertion sites were confirmed. These inserts were present in the 11-C1, 1-A4, and 13-G2 mutants, and the insertion sites can be seen in figure 3. The insert in the 1-C4 hemolysin mutant was confirmed to be located at the 1,086 bp site of the 4,827 bp major hemolysin gene.

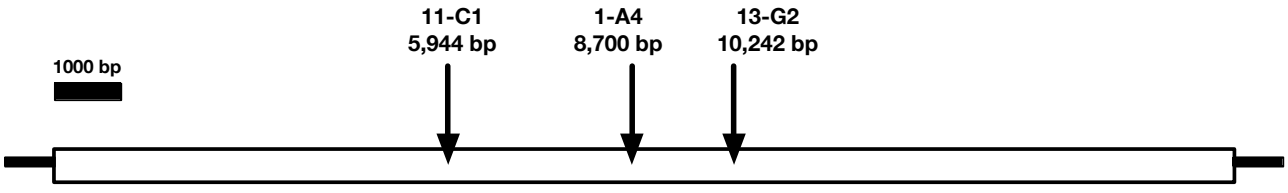

Fig. 3: PCR with genomic DNA confirmed the transposon insertion sites in the *swrA* gene. Transposon insertion sites are indicated on the gene map of the 17,775 bp *swrA* gene.

Identification of *swrA* expression patters using RT-PCR with total RNA. RNA was extracted from the confirmed mutants and RT-PCR was conducted using primers at different sites in the *swrA* gene. The presence of a band on the electrophoresis gel of the PCR products indicated a positive result (see appendix 3 for representative PCR data). The expression levels at each point in the *swrA* gene for the different mutants can be seen in figure 4.

The RT-PCR reactions with total RNA produced variable results in expression patterns. Wild type SCBI showed expression at each point in the transcript, indicating that the complete transcript was being produced. Unexpectedly, the 11-C1 mutant, which contained an insert at the 5,944 bp site, also showed expression of the complete transcript. The 1-A4 mutant showed expression at sites beyond the transposon insert, while the 13-G2 mutant only showed expression of the beginning of the *swrA* transcript.

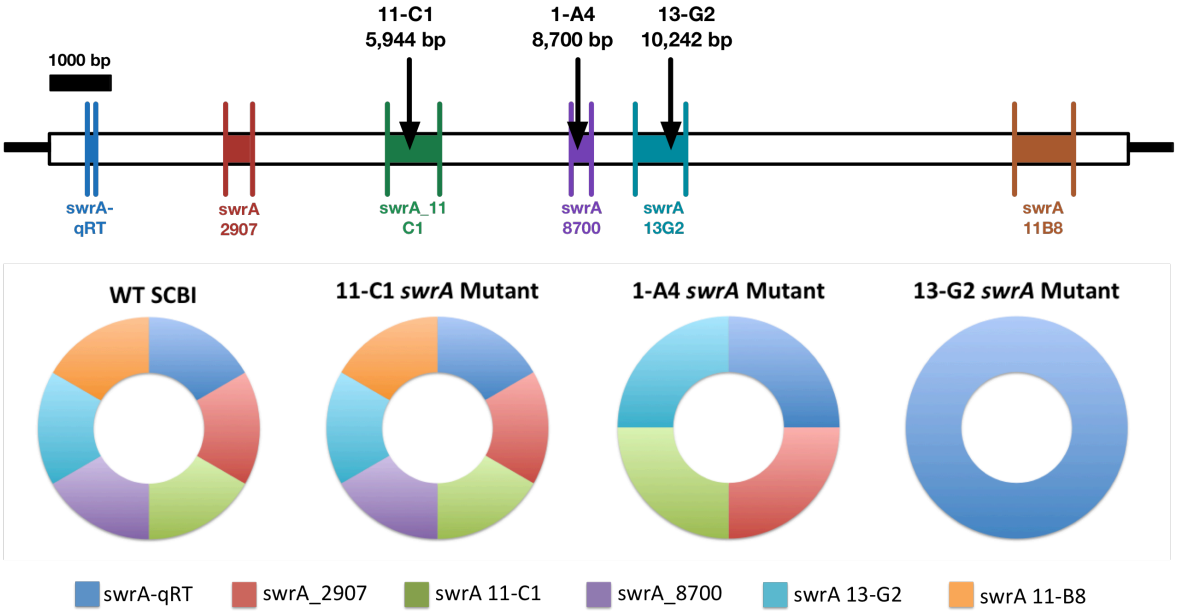

Fig. 4: RT-PCR with total RNA determined the expression of the *swrA* gene at different points in each of the transposon mutants. Primer sites are indicated in the gene map, and expression is depicted in the circle graphs. Each graph represents one *swrA* mutant. A region that is expressed is included in the graph for the mutant, see appendix 3 for representative PCR data.

Identification of *swrA* expression patterns using RT-PCR with the cDNA of the 17 Kbp *swrA* transcript. cDNA of the complete 17 Kbp *swrA* transcript was synthesized for the

confirmed mutants using the swrA_17037 reverse primer (see table 3). RT-PCR was conducted on the cDNA to amplify six different regions within the *swrA* gene. The presence of a band on the electrophoresis gel of the PCR products indicated a positive result (see appendix 4 for representative PCR data). The expression levels at each point in the cDNA of the *swrA* transcript for the different mutants can be seen in figure 5.

The RT-PCR reactions with cDNA of the *swrA* transcript also produced variable results in expression patterns. Wild type SCBI showed expression at each point in the transcript, indicating that the complete transcript was being produced. Unexpectedly, the 11-C1 mutant again showed expression of the complete transcript, with the exception of the transposon insertion site. Again, the 1-A4 mutant unexpectedly showed expression at sites beyond the transposon insert, while the 13-G2 mutant only showed expression of the beginning of the *swrA* transcript.

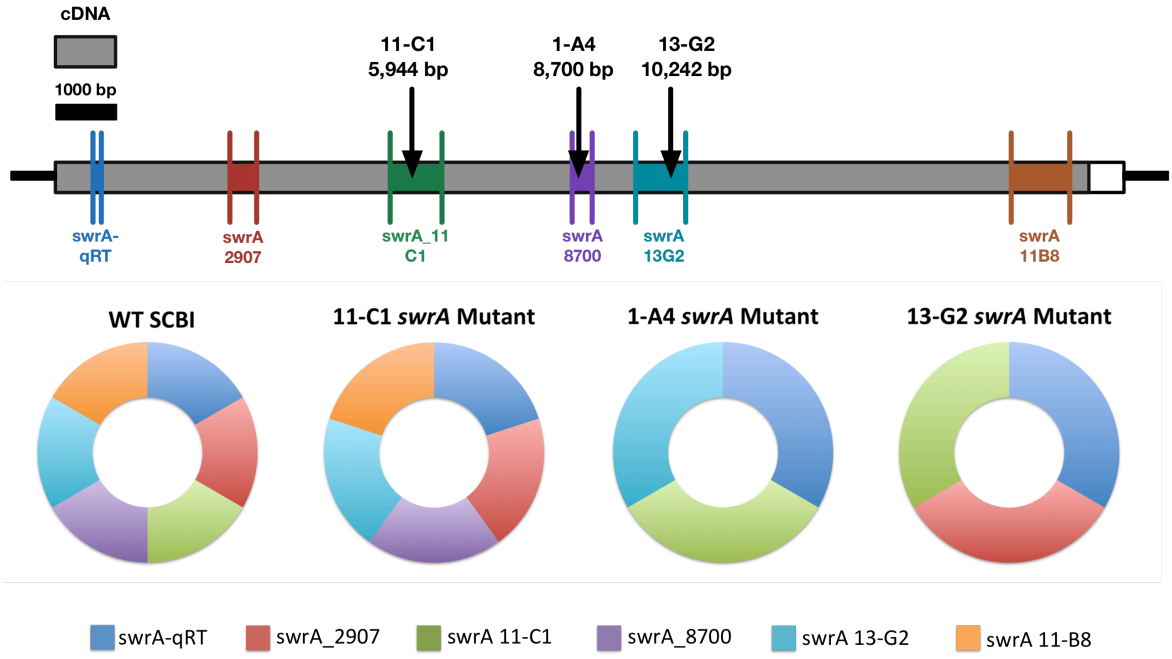

Fig. 5: RT-PCR with the *swrA* cDNA transcript determined the expression of the *swrA* gene at different points in each of the transposon mutants. The region of the *swrA* gene that was transcribed to cDNA using the swrA_17037 reverse primer is indicated in grey. Primer sites are indicated in the gene map, and expression is depicted in the circle graphs. Each graph represents one *swrA* mutant. A region that is expressed is included in the graph for the mutant, see appendix 4 for representative PCR data.

Quantifying expression of the *swrA* transcript through qRT-PCR. Total RNA was converted to cDNA for each of the mutants and qPCR was conducted. The relative expression at different locations in the *swrA* transcript was quantified using the comparative threshold-cycle method. The expression levels from this study can be seen in figure 6.

The quantification of *swrA* expression levels produced variable results. Consistent with previous studies, it was found that the hemolysin mutant leads to a dramatic increase in the production of *swrA* transcript (6). Unexpectedly, expression was remarkably increased for the end of the transcript. Also consistent with previous studies it was found that the 11-C1 mutant produces increased levels of the *swrA* transcript (6). The 1-A4 mutant did not show any expression of the *swrA* transcript, and the 13-G2 mutant showed consistent expression levels throughout the entire transcript.

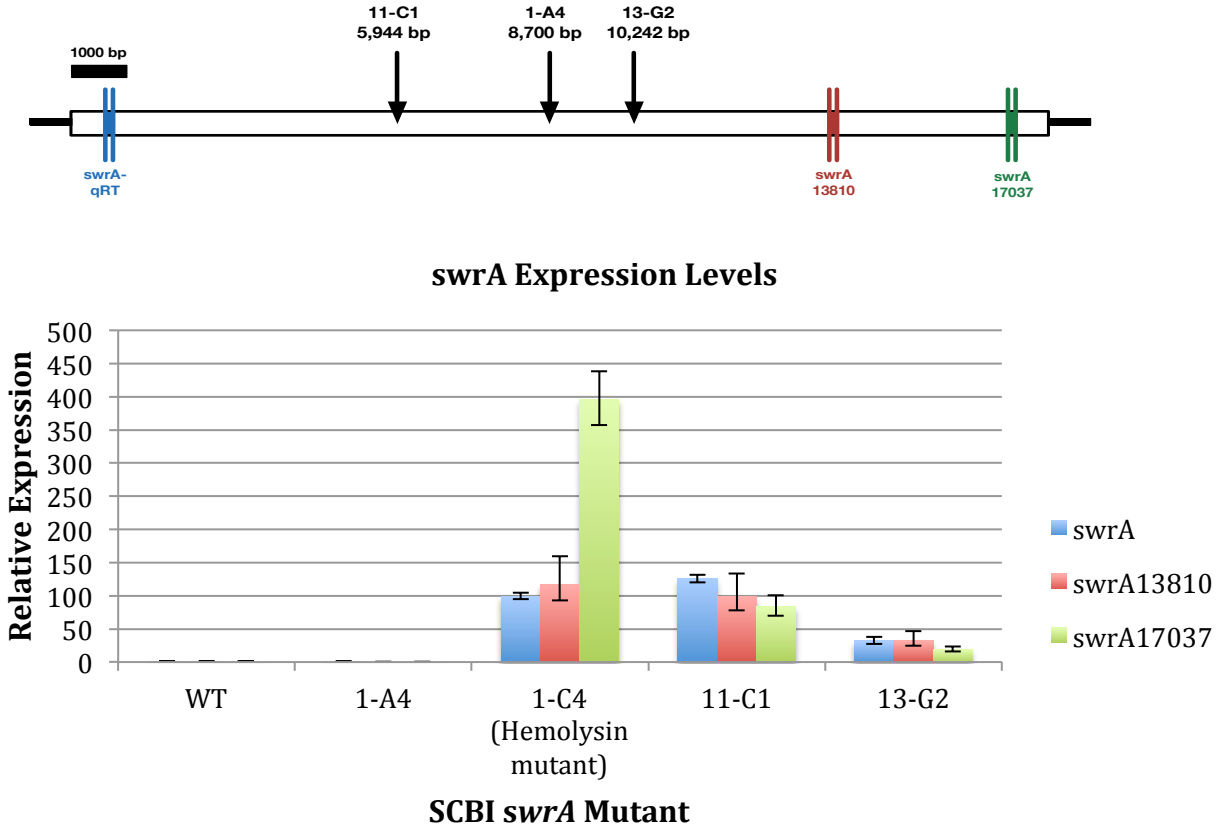

Fig. 6: qPCR was used to determine the the expression levels of the *swrA* gene at different points in each of the *swrA* mutants, as well as one hemolysin mutant. Primer sites are indicated on the gene map, and relative expression levels at each site are shown in the graph.

Discussion:

Serrawettin W2 is a surfactant of *Serratia* sp. SCBI and it is known to have effects on motility, hemolysis, antibiotic activity, and virulence, all of which play an important role in the symbiotic relationship with *C. briggsae.* The identification of three *Serratia* sp. SCBI miniHimar RBI mutants with altered *swrA* transcripts provided insight into how this gene is regulated. Through PCR it was found that many complex regulatory mechanisms are involved in the regulation of *swrA* expression. The RT-PCR results indicate that each mutation affects the transcript differently, with some mutants expressing the transcript far past the transposon insertion site. These results with both the RNA and cDNA of the *swrA* transcript (synthesized with the swrA_17037 reverse primer) indicate the possibility of undiscovered promoter regions within the transcript. Since the expression is not consistently disrupted by the transposon insertions, promoter regions may be located downstream of these inserts which allow the entire transcript to be produced even with the disruption.

The qPCR experiments also indicate that complex regulatory mechanisms may be involved in controlling the expression of *swrA.* As shown in previous studies, the inactivation of the hemolysin gene leads to overproduction of the *swrA* transcript, but the mechanism behind this is unknown. The expression patterns of the other mutants are also consistent with the multiple promoter hypothesis, as these also show expression beyond transposon insertion sites. Overall, this study uncovered complex regulatory mechanisms involved in the regulation of the *swrA* transcript. It can be shown that the *swrA* transcript is expressed differently at various points in its sequence, and mutations appear to affect the expression differently. Much remains to be learned about the complex regulation of the *swrA* gene and in order to better understand the regulation further experiments must be conducted to continue looking for a second promoter.

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Appendix 1: Genomic DNA Extraction Protocol

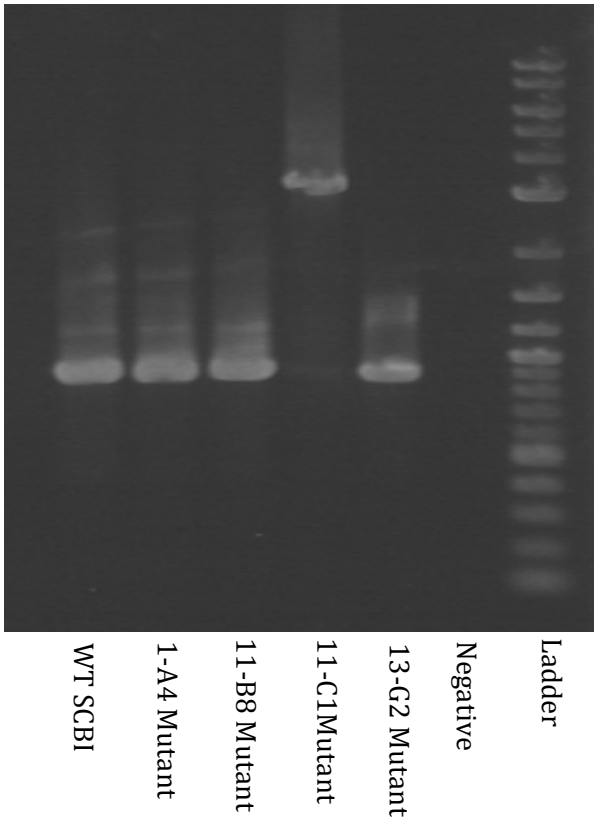
Materials:

- 1X TE buffer (10 mM tris HCl; 1 mM EDTA, ph 8.0)
- Lysozyme (50 mg/mL)
- Proteinase K (20 mg/mL)
- 10% SDS
- 5M NaCl
- CTAB/NaCl Solution (10% CTAB/0.7M NaCl)
- Chloroform: isoamyl alcohol (24.1)
- Phenol: chloroform: isoamyl alcohol (25:24:1)
- Isopropanol
- 70% ethanol
- RNase (10mg/mL)

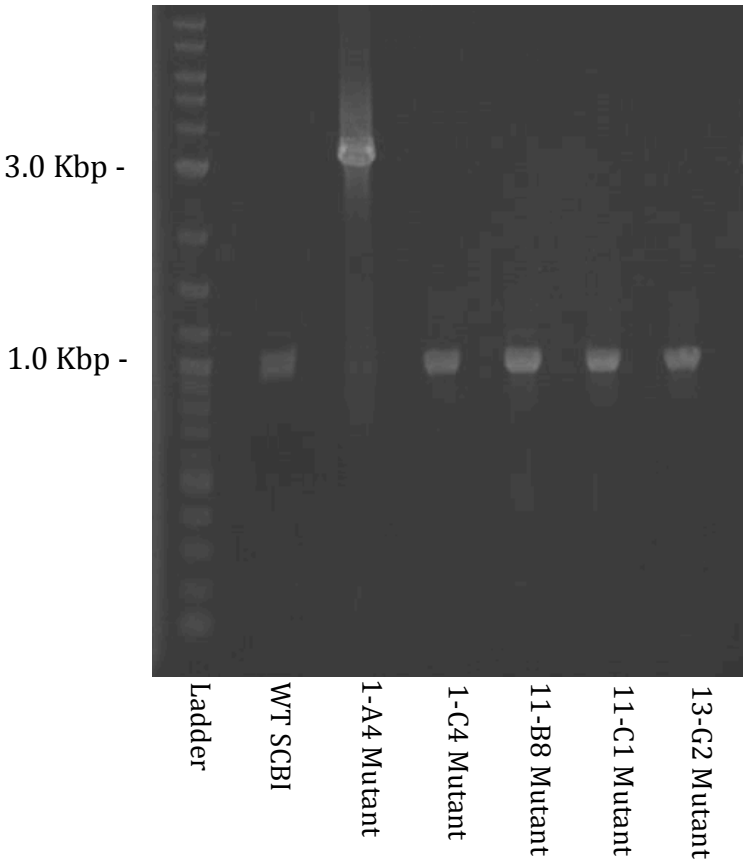
Procedure:

1. Add 1.5 mL overnight culture to microfuge tubes
2. Pellet cells by centrifuging in a bench top centrifuge at 13,000 rpm for 2 minutes
3. Discard supernatant
4. Resuspend cells by pipetting in 565.8 µL of 1X TE buffer
5. Add 1.2 µL of lysozyme (50 mg/mL). Mix by vortexing briefly
6. Incubate for 5 minutes at room temperature
7. Add 3 µL of Proteinase K (20 mg/mL). Mix by vortexing briefly
8. Incubate at 37°C for 2 hours
9. Add 100 µL of 5M NaCl. Mix by inverting several times.
10. Heat CTAB/NaCl solution to 65°C and add 80 µL. Mix by inverting several times
11. Incubate at 65°C for 10 min.
12. Add 800 µL of chloroform:isoamyl alcohol (24:1). Mix by inverting several times
13. Centrifuge in a bench top centrifuge at 13,000 rpm for 20 min.
14. Transfer aqueous phase to a sterile microfuge tube (white CTAB/protein and CTAB/polysaccharide complexes should be avoided)
15. Add 600 µL chloroform: isoamyl alcohol (24:1). Mix by inverting several times
16. Centrifuge in a bench top centrifuge at 13,000 rpm for 5 minutes
17. Transfer aqueous supernatant to a sterile microfuge tube (white CTAB/protein and CTAB/polysaccharide complexes should be avoided)
18. Add 500 µL of phenol: chloroform: isoamyl alcohol (25:24:1). Mix by inverting several times
19. Centrifuge at 13,000 rpm for 15 minutes.
20. Transfer aqueous phase to a sterile microfuge tube
21. Add 0.6 volume isopropanol
22. Place samples in a -20° freezer for 24 hours
23. Incubate samples at room temperature for 30 minutes
24. Centrifuge in a bench top centrifuge at 13,000 rpm for 15 minutes
25. Remove and discard supernatant by pipetting
26. Wash pellet with 600 µL of ice cold 70% ethanol. Mix by vortexing briefly.
27. Centrifuge at 13,000 rpm for 15 minutes
28. Remove and discard supernatant. Air dry pellet for 7 minutes at room temperature
29. Resuspend pellet in 99 µL of 1X TE buffer
30. Add 1 µL of RNase. Incubate at 37°C for 20 minutes

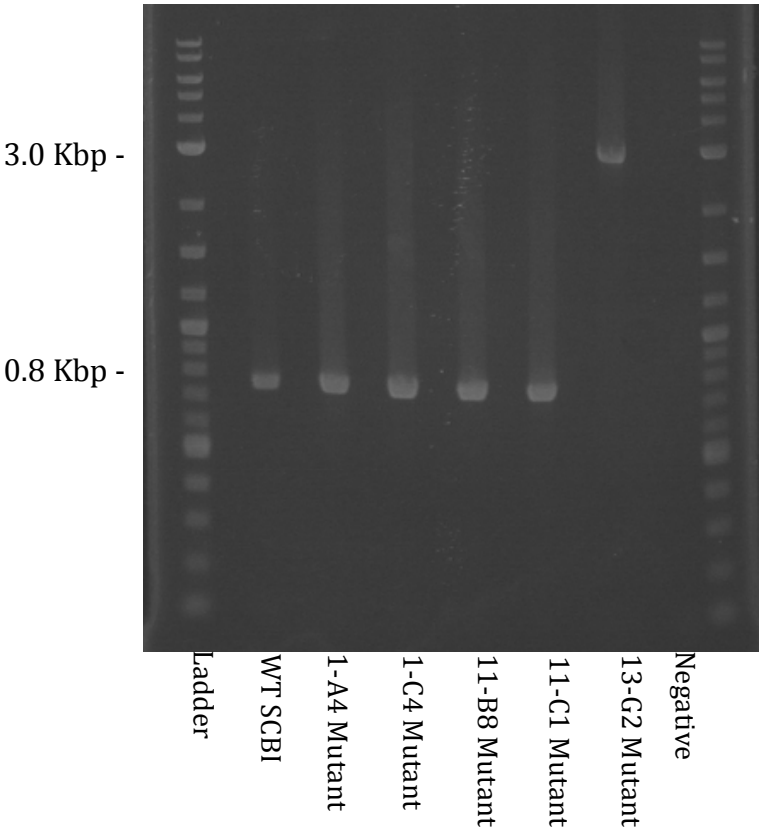
Appendix 2: Confirmation PCR Results



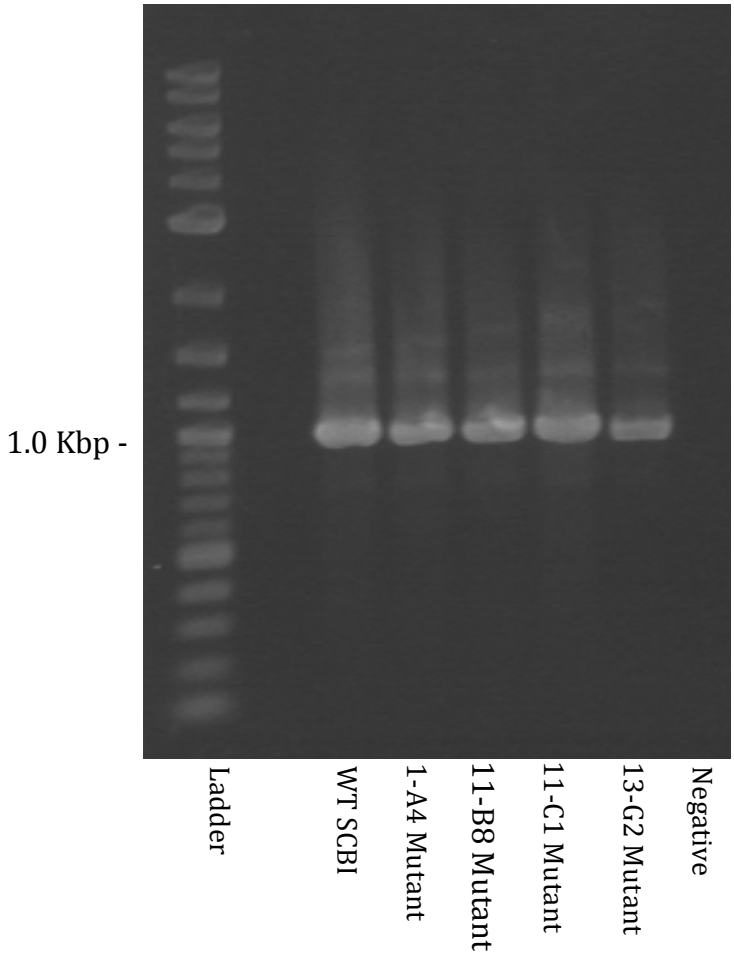
Confirmation of the 11-C1 mutant. An ethidium stained agarose gel showing DNA fragments produced by the PCR amplification of the 11-C1 transposon insert site from *Serratia* sp. SCBI mutant genomic DNA. Lane 7 contains a 2-log DNA ladder (New England Biolabs). 900 bp bands can be seen for all mutants, with the exception of 11-C1 mutant, which displays a 3000 bp product, indicating the presence of a transposon insert. For the positive control WT *Serratia* sp. SCBI was used, and for a negative control sterile distilled water was used in place of template DNA.



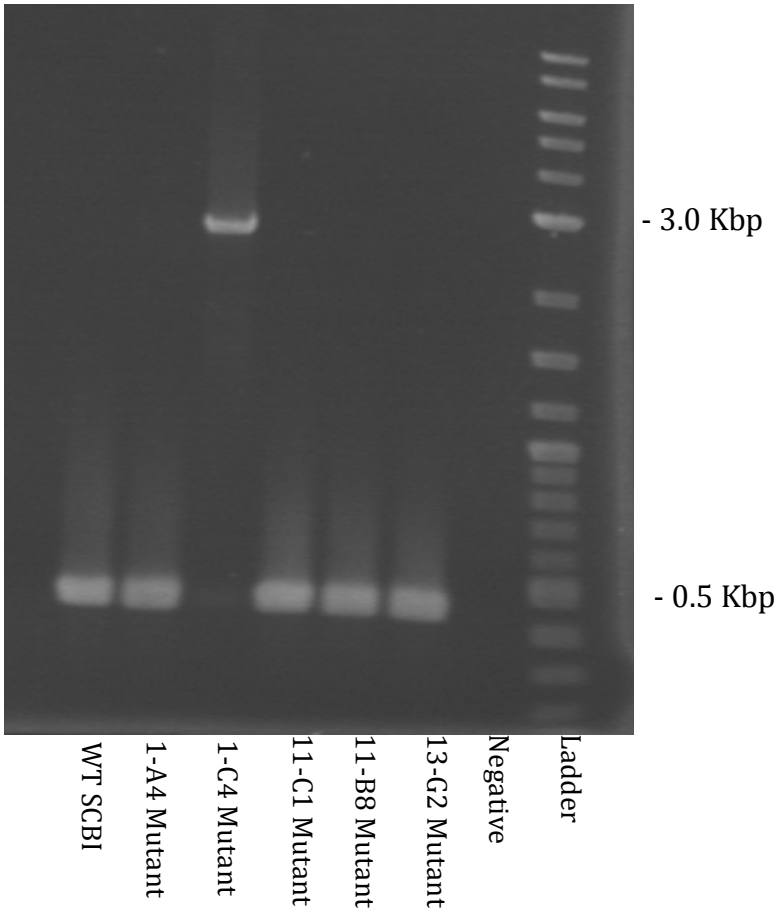
Confirmation of the 1-A4 mutant. An ethidium stained agarose gel showing DNA fragments produced by the PCR amplification of the 1-A4 transposon insert site from *Serratia* sp. SCBI mutant genomic DNA. Lane 1 contains a 2-log DNA ladder (New England Biolabs). 1000 bp bands can be seen for all mutants, with the exception of 1-A4 mutant, which displays a 3000 bp product, indicating the presence of a transposon insert. For the positive control WT *Serratia* sp. SCBI was used, and for a negative control sterile distilled water was used in place of template DNA. .



Confirmation of the 13-G2 mutant. An ethidium stained agarose gel showing DNA fragments produced by the PCR amplification of the 13-G2 transposon insert site from *Serratia* sp. SCBI mutant genomic DNA. Lane 1 contains a 2-log DNA ladder (New England Biolabs). 800 bp bands can be seen for all mutants, with the exception of 13-G2 mutant, which displays a 2000 bp product, indicating the presence of a transposon insert. For the positive control WT *Serratia* sp. SCBI was used, and for a negative control sterile distilled water was used in place of template DNA.

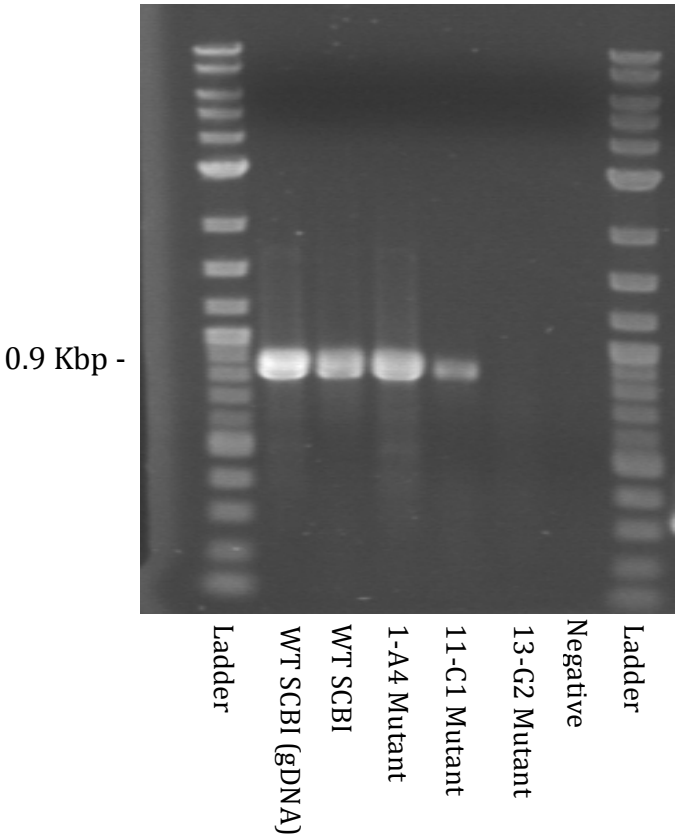


Confirmation of the 11-B8 Mutant. An ethidium stained agarose gel showing DNA fragments produced by the PCR amplification of the 11-B8 transposon insert site from *Serratia* sp. SCBI mutant genomic DNA. Lane 1 contains a 2-log DNA ladder (New England Biolabs). 1000 bp bands can be seen for all mutants, including 11-B8 indicating that the transposon insert cannot be confirmed. For the positive control WT *Serratia* sp. SCBI was used, and for a negative control sterile distilled water was used in place of template DNA.



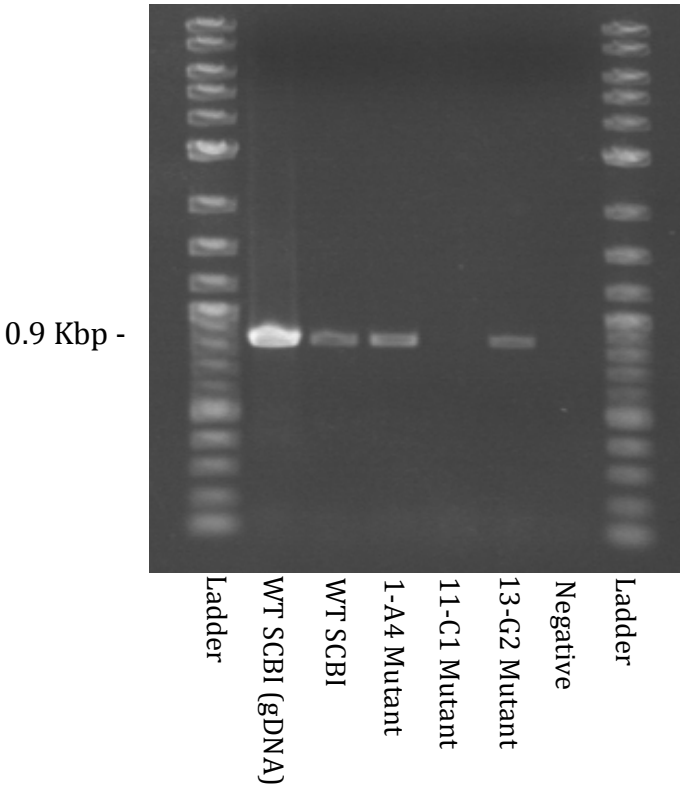
Confirmation of the 1-C4 Mutant. An ethidium stained agarose gel showing DNA fragments produced by the PCR amplification of the 1-C4 transposon insert site from *Serratia* sp. SCBI mutant genomic DNA. Lane 1 contains a 2-log DNA ladder (New England Biolabs). 500 bp bands can be seen for all mutants, with the exception of 1-C4 mutant, which displays a 3000 bp product, indicating the presence of a transposon insert. For the positive control WT *Serratia* sp. SCBI was used, and for a negative control sterile distilled water was used in place of template DNA.

Appendix 3: Representative gel of the *swrA* expression studies using RT-PCR with total RNA



11-C1 primer on RT-PCR An ethidium stained agarose gel showing DNA fragments produced by the PCR amplification of the 11-C1 transposon insert site from *Serratia* sp. SCBI mutant total RNA. Lanes 1 and 8 contain a 2-log DNA ladder (New England Biolabs). The presence of a band indicates positive expression at this location within the transcript, and is included in figure 4. For the positive control WT *Serratia* sp. SCBI was used, and for a negative control sterile distilled water was used in place of template DNA.

Appendix 4: Representative gel of the *swrA* expression studies using RT-PCR with cDNA of the *swrA* transcript synthesized with the swrA_17037 reverse primer



11-C1 primer on *swrA* transcript cDNA. An ethidium stained agarose gel showing DNA fragments produced by the PCR amplification of the 11-C1 transposon insert site from *Serratia* sp. SCBI mutant cDNA of the *swrA* transcript (synthesized using the swrA_17037 reverse primer). Lanes 1 and 8 contain a 2-log DNA ladder (New England Biolabs). The presence of a band indicates positive expression at this location within the transcript, and is included in figure 5. For the positive control WT *Serratia* sp. SCBI was used, and for a negative control sterile distilled water was used in place of template DNA.