Research Article

Regulation of Expression of Oxacillin-Inducible Methionine Sulfoxide Reductases in Staphylococcus aureus

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Cell wall-active antibiotics cause induction of a locus that leads to elevated synthesis of two methionine sulfoxide reductases (MsrA1 and MsrB) in Staphylococcus aureus. To understand the regulation of this locus, reporter strains were constructed by integrating a DNA fragment consisting of the msrA1/msrB promoter in front of a promoterless lacZ gene in the chromosome of wild-type and MsrA1-, MsrB-, MsrA1/MsrB-, and SigB-deficient methicillin-sensitive S. aureus strain SH1000 and methicillin-resistant S. aureus strain COL. These reporter strains were cultured in TSB and the cellular levels of β-galactosidase activity in these cultures were assayed during different growth phases. β-galactosidase activity assays demonstrated that the lack of MsrA1, MsrB, and SigB upregulated the msrA1/msrB promoter in S. aureus strain SH1000. In S. aureus strain COL, the highest level of β-galactosidase activity was observed under the conditions when both MsrA1 and MsrB proteins were absent. The data suggest that the msrA1/msrB locus, in part, is negatively regulated by MsrA1, MsrB, and SigB in S. aureus.

1. Introduction

Staphylococcus aureus is part of the microbiome of roughly 30% of people who show no clinical symptoms [1]. It is an opportunistic human pathogen that can cause a wide variety of diseases and can involve any organ system in the human body. Diseases caused by S. aureus may include mild skin infections such as folliculitis and impetigo to fatal conditions such as pneumonia, osteomyelitis, and endocarditis [2]. Treatment of S. aureus infections has become problematic as it has developed numerous mechanisms to become resistant to almost all known antibiotics [3, 4].

It was previously reported that exposure of S. aureus to oxacillin and other cell wall-active antibiotics increases the expression of msrA1 and msrB both at the transcriptional and at the protein level [5, 6]. Pathogenic bacterial species are exposed to a variety of extremely potent reactive oxygen species (ROS) by the host phagocytic cells during the course of phagocytosis that are damaging to all cellular macromolecules. ROS can cause damage to proteins by the oxidation of sulfhydryl groups, reduction of disulfides, oxidative adduction of amino acid residues close to metal-binding sites, and peptide fragmentation [7]. In particular, ROS oxidize the sulfur atom of protein-bound methionine residues resulting in methionine sulfoxide (MetO) and loss of protein function. However, almost all biological species possess the ability to reduce oxidized methionines [8]. MsrA and MsrB proteins reduce S- and R-epimers of methionine sulfoxides (MetO), respectively [8].

In S. aureus, genes encoding MsrA1 and MsrB are the first and second genes of a four-gene cluster that are cotranscribed [6]. A mutation in the msrA1 gene increased the susceptibility of S. aureus to oxidative stress [6, 9]. More recently, it was shown that the MsrA1 protein was critical for S. aureus in establishing an infection in mice [10]. Interestingly, the MsrA1-deficient S. aureus was shown to possess an elevated level of MsrB [9] giving rise to the speculation of autoregulation of the msrA1/msrB locus. Additionally, sigma factor B (SigB) is an alternative sigma factor that is involved in regulating the expression of stress response genes in S. aureus [11].
2. Materials and Methods

Thus, it seems plausible that SigB may have a role in the regulation of the msrA1/msrB locus. Findings of this study provide evidence that the msrA1/msrB locus is negatively regulated by the products of this locus and SigB.

### 2.1. Bacterial Strains, Antibiotics, and Growth Conditions

The bacterial strains used in this study are shown in Table 1. S. aureus cultures were grown aerobically at 37°C in tryptic soy broth (TSB) in a shaking incubator (220 rpm) or on tryptic soy agar (TSA) by incubation for 24–48 h. Overnight cultures of S. aureus reporter strains were prepared in the presence of erythromycin at 10 µg mL⁻¹. Oligonucleotide primers used in this study were obtained from Eurofins and are shown in Table 2.

### 2.2. Transduction of msrA1/msrB Promoter-lacZ into S. aureus Strains

Construction of msrA1/msrB promoter-lacZ reporter strain has been previously described [6]. In this construct, a 1.3 kb DNA fragment starting 44 nucleotides downstream of the msrA1 gene cloned in front of a lacZ gene in the vector pAZ106 [12] was integrated in the chromosome of S. aureus strain RN450 [5, 6]. The msrA1/msrB promoter-lacZ reporter was transduced into various strains of S. aureus using a phage 80a transduction procedure. Strains used in this study were verified by PCR.

| Strains | Characteristics | Reference |
|---------|----------------|-----------|
| SH1000  | S. aureus strain 8325-4 with functional RsbU | [11] |
| COL     | Homogeneous in methicillin-resistance expression | [37] |
| SH1000ΔmsrA1 | msrA1 mutant of SH1000 | [10] |
| SH1000ΔmsrB | msrB mutant of SH1000 | [10] |
| SH1000ΔmsrA1-msrB | msrA1-msrB double mutant of SH1000 | [10] |
| SH1000ΔsigB | sigB mutant of SH1000 | [14] |
| COLΔmsrA1 | msrA1 mutant of SH1000 | [6] |
| COLΔmsrB | msrB mutant of SH1000 | This study |
| COLΔmsrA1-msrB | msrA1-msrB double mutant of SH1000 | This study |
| COLΔsigB | sigB mutant of SH1000 | [38] |
| SH1000-(A1/B)P-lacZ | msrA1/msrB promoter-lacZ fusion in SH1000 (Erm<sup>R</sup>) | [13] |
| SH1000ΔmsrA1-(A1/B)P-lacZ | msrA1/msrB promoter-lacZ fusion in msrA1 mutant of SH1000 (Kan<sup>R</sup>, Erm<sup>B</sup>) | This study |
| SH1000ΔmsrB-(A1/B)P-lacZ | msrA1/msrB promoter-lacZ fusion in msrB mutant of SH1000 (Kan<sup>R</sup>, Erm<sup>B</sup>) | This study |
| SH1000ΔmsrA1-msrB-(A1/B)P-lacZ | msrA1/msrB promoter-lacZ fusion in msrA1-msrB mutant of SH1000 (Kan<sup>R</sup>, Erm<sup>B</sup>) | This study |
| SH1000ΔsigB-(A1/B)P-lacZ | msrA1/msrB promoter-lacZ fusion in sigB mutant of SH1000 (Kan<sup>R</sup>, Erm<sup>B</sup>) | This study |
| COL-(A1/B)P-lacZ | msrA1/msrB promoter-lacZ fusion in COL (Erm<sup>B</sup>) | This study |
| COLΔmsrA1-(A1/B)P-lacZ | msrA1/msrB promoter-lacZ fusion in msrA1 mutant of COL (Kan<sup>R</sup>, Erm<sup>B</sup>) | This study |
| COLΔmsrB-(A1/B)P-lacZ | msrA1/msrB promoter-lacZ fusion in msrB mutant of COL (Kan<sup>R</sup>, Erm<sup>B</sup>) | This study |
| COLΔmsrA1-msrB-(A1/B)P-lacZ | msrA1/msrB promoter-lacZ fusion in msrA1-msrB mutant of COL (Kan<sup>R</sup>, Erm<sup>B</sup>) | This study |
| COLΔsigB-(A1/B)P-lacZ | msrA1/msrB promoter-lacZ fusion in sigB mutant of COL (Kan<sup>R</sup>, Erm<sup>B</sup>) | This study |

Kan<sup>R</sup>: kanamycin resistant; Erm<sup>B</sup>: erythromycin resistant.

| Oligo | Sequence (5' → 3') |
|-------|-------------------|
| P1    | GCTAAGCTCATATGAATATG |
| P2    | GGAAGTAAACCTGATCAG |
| P3    | GATCACAGAAAGACGGCA |
| P4    | TACATCGTGGTTTTTG |
| P5    | AGGATGTGTTCTGATG |
| P6    | GACCAACTCTCCCTCAG |
| P7    | CCTTAGAAGCAGTGG |
| P8    | TCAATAGAACCACCTC |
| P9    | GCTAAGCTCATATGAATATG |
| P10   | GATGAGCTTGCAATG |
| P11   | ATCGACAGGTATG |
| P12   | CTTCAGATTAAGGACCA |
| P13   | GAGCAATCGACGTGATC |
| P14   | GAAATTACCTCTGATCAG |
| P15   | GGTATGGTAAGAACTGAAGT |
| P16   | ATTCAGCAGGAATTGACAG |
| P17   | TCTCCAAATTCGACAG |
| P18   | ACACATTCAAATTCACCGT |
| P19   | TCCAAATTCGAGCAGT |
| P20   | GGAAGGCTTGCATACAT |

Table 2: Oligonucleotide primers used in this study.

2.3. Determination of the msrA1/msrB Promoter Strength in S. aureus.

To determine if the msrA1/msrB locus is autoregulated, the expression of lacZ from the msrA1/msrB promoter-lacZ fusion was investigated in MsrA1-, MsrB-, and MsrA/MsrB-deficient strains of S. aureus strains SH1000 and COL.
In addition, SigB is a major regulator of stress response in S. aureus. Therefore, the strength of the msrA1/msrB promoter was also assessed in a sigB mutant. Overnight cultures of these strains were diluted (1:100) and grown at 37°C with shaking. These cultures were grown to OD_{600} = 0.5 that was considered time 0 and the levels of \( \beta \)-galactosidase activity in these cultures were measured at different time points (0, 90, 180, 270, and 360 min) as an indicator of the strength of the msrA1/msrB promoter.

2.4. Expression of msrA1/msrB Promoter in the Presence of a Cell Wall-Active Antibiotic, Oxacillin. Previous studies [5, 6, 9, 10, 13] have shown that, in the presence of oxacillin, there is an increased production of MsrA1 and MsrB in S. aureus. To further investigate the regulation of the msrA1/msrB locus and to see if it can be magnified in the presence of oxacillin, overnight cultures of wild-type and the derivative msrA1/msrB mutant of S. aureus strain COL were diluted (1:100) in fresh TSB and grown to OD_{600} of 0.5. 10.0 mL of the culture was split into two 15 mL tubes. To one of the cultures, oxacillin was added to the final concentration of 1.0 mg mL^{-1}. Both cultures with and without oxacillin were allowed to grow for an additional 2 h at 37°C with shaking. Bacterial cells were harvested by centrifugation and \( \beta \)-galactosidase activities in these cells were measured.

2.5. Measurement of \( \beta \)-Galactosidase Activity. The OD_{600} of the culture was determined as a measure of cell density and cells were subsequently collected by centrifugation. For precise optical density readings, cultures were diluted appropriately to bring density into measurable range. The cell pellet was used to measure \( \beta \)-galactosidase activity as described previously using O-nitrophenyl-\( \beta \)-D-galactopyranoside (ONPG) as the substrate [5, 6, 13].

2.6. Quantitative Real-Time PCR (qRT-PCR) Assays. qRT-PCR assays were used to verify induced expression of the genes of the msrA1/msrB locus under oxacillin stress and to validate the lacZ reporter expression data in sigB mutants. Cultures of S. aureus strain COL were grown to OD_{600} = 0.3 and divided into two tubes. One tube was stressed with oxacillin at a concentration of 1.0 mg mL^{-1} for 2 h. Total RNA was extracted from these oxacillin stressed and control cultures as described previously [14]. For the validation of lacZ data, the wild-type and sigB mutant strains of S. aureus were allowed to grow for 90 min and 6 h after reaching the OD_{600} = 0.5 and total RNA from these cultures were extracted. cDNA from DNase treated 0.5 \( \mu \)g of total RNA was synthesized in a 20 \( \mu \)L reverse transcription reaction containing random hexamers and SuperScript III reverse transcriptase (Invitrogen). All real-time PCR reactions were carried out with Bio-Rad iCycler (iQ5 system). The transcript level of msrA1 was quantified using primers P13 and P14, that of msrB was quantified using P15 and P16, and that of the gene encoding the IIa(PTS) was quantified using primers P17 and P18. Transcript levels of genes were normalized to DNA gyrase mRNA using primers P19 and P20 based on a previous report [15, 16]. Changes in gene expression were calculated using the formula \( 2^{-\Delta\Delta Cq} \) as described [17].

2.7. Statistical Analysis. All results are reported as the mean ± SE of at least three independent experiments. Data were analyzed with Student’s t-test using R Studio for Windows (version 0.98.1103, 3.1.3). Statistical significance was set at \( p \leq 0.05 \).

3. Results

3.1. Construction of msrA1/msrB Promoter-lacZ Reporter in Wild-Type and msrA1, msrB, msrA1-msrB, and sigB Mutants of S. aureus. Previously created msrA1, msrB, msrA1-msrB, and sigB knockout mutants of S. aureus strain SH1000 [6, 9, 10] were transduced in the methicillin-resistant S. aureus strain COL. These mutants and the presence of mecA gene in these strains were verified by PCR (see Supplementary Figures S1-S2 in Supplementary Material available online at http://dx.doi.org/10.1155/2015/617925). The msrA1/msrB promoter-lacZ fusion was subsequently integrated into the chromosome of these mutant strains using a bacteriophage transduction procedure. Overall, five msrA1/msrB promoter-lacZ reporter strains were created in methicillin-resistant as well as methicillin-sensitive S. aureus backgrounds. Proper integration of the msrA1/msrB promoter-lacZ fusion was also confirmed by PCR (Supplemental Figure S3).

3.2. Regulation of msrA1/msrB Locus in S. aureus. Previously, we reported higher MsrB levels in MsrA1-deficient S. aureus cells [9, 10]. This led to the speculation that the msrA1/msrB locus may in part be regulated by the products of this locus. To investigate this possibility, the level of \( \beta \)-galactosidase was measured in MsrA1-, MsrB-, and MsrA1-MsrB-deficient strains of S. aureus. \( \beta \)-galactosidase activity levels were higher in these strains compared to the activity level in the wild-type S. aureus strain SH1000 (Figure 1). The msrA1/msrB promoter-lacZ reporter was also studied in the methicillin-resistant strain COL. Overall, the expression of lacZ was lower in methicillin-resistant S. aureus compared to the methicillin-sensitive S. aureus (Figures 1(b) and 2(b)). In addition, \( \beta \)-galactosidase activity comparison revealed that only the msrA1/msrB double mutant strains had higher activity levels compared to the wild-type COL at the various time points (Figure 2(b)). In the individual msrA1 or msrB mutant strains, a significant increase in \( \beta \)-galactosidase activity was not observed compared to wild-type S. aureus COL (Figure 2(b)).

3.3. Role of SigB in the Regulation of msrA1/msrB Locus in S. aureus. Measurement of \( \beta \)-galactosidase activity demonstrated that there was increased expression of lacZ from the msrA1/msrB promoter when S. aureus was deficient of SigB in strain SH1000 (Figure 3(b)). However, in S. aureus COL, no such increase in the expression of lacZ was observed from the msrA1/msrB promoter under SigB-deficient conditions compared to the wild-type strain (Figure 4(b)). In qRT-PCR assays, a relatively higher level of msrA1 transcripts was observed in sigB mutant of S. aureus strain SH1000 compared
Figure 1: Regulation of msrA1/msrB locus in a methicillin-sensitive S. aureus strain SH1000. The msrA1/msrB promoter-lacZ reporter strains were cultured in TSB and growth was measured as OD$_{600}$ (a). β-galactosidase activity levels were measured in wild-type S. aureus strain SH1000 (open triangles) and its derivatives msrA1 (open circles), msrB (closed triangles), and msrA1-msrB (open square) mutants during different stages of growth (b). Values indicate averages of data from at least three independent experiments ± standard error (SE) (∗ significant at $p \leq 0.05$).

Figure 2: Regulation of msrA1/msrB locus in a methicillin-resistant S. aureus strain COL. The msrA1/msrB promoter-lacZ reporter strains were cultured in TSB and growth was measured as OD$_{600}$ (a). β-galactosidase activity levels were measured in wild-type S. aureus strain COL (open triangles) and its derivatives msrA1 (open circles), msrB (closed triangles), and msrA1-msrB (open square) mutants during different stages of growth (b). Values indicate averages of data from at least three independent experiments ± standard error (∗ significant at $p \leq 0.05$).
Figure 3: Regulation of msrA1/msrB locus in a methicillin-sensitive S. aureus strain SH1000 by SigB. The msrA1/msrB promoter-lacZ reporter strains were cultured in TSB and growth was measured as OD_{600} (a). β-galactosidase activity levels were measured in wild-type S. aureus strain SH1000 (open triangles) and its derivative sigB mutant (closed squares) during different stages of growth (b). Values indicate averages of data from at least three independent experiments ± standard error (* significant at p ≤ 0.05).

Figure 4: Regulation of msrA1/msrB locus in a methicillin-resistant S. aureus strain COL by SigB. The msrA1/msrB promoter-lacZ reporter strains were cultured in TSB and growth was measured as OD_{600} (a). β-galactosidase activity levels were measured in S. aureus strain COL (open triangles) and its derivative sigB mutant (closed squares) during different stages of growth (b). Values indicate averages of data from at least three independent experiments ± standard error (* significant at p ≤ 0.05).
Table 3: Expression levels of msrA1 in sigB mutants relative to wild-type S. aureus strains SH1000 and COL.

| Strain          | Fold increase in expression |
|-----------------|-----------------------------|
| SH1000ΔsigB1.30 | 3.16                        |
| COLΔsigB        | 0.98                        |

Values indicate averages of three independent experiments.

Table 4: Induced expression of msrA1/msrB locus genes in S. aureus strain COL under oxacillin stress.

| Gene         | Fold increase in expression under oxacillin stress |
|--------------|----------------------------------------------------|
| msrA1        | 22.9                                               |
| msrB         | 18.97                                              |
| IIa(PTS)     | 13.45                                              |

Values indicate averages of three independent experiments.

3.4. Induction of msrA1/msrB Locus in the Presence of Cell Wall-Active Antibiotic, Oxacillin. Previous studies have shown that the msrA1/msrB locus is induced by the cell wall-active antibiotics, oxacillin, vancomycin, and D-cycloserine, in a methicillin-sensitive S. aureus strain [5]. In a later study, while the msrA1/msrB locus remained inducible in the presence of D-cycloserine and vancomycin, no induction of this locus was noted in the presence of oxacillin, when similar experiments were carried out in a methicillin-resistant S. aureus strain COL [18]. However, in our experiments, a significantly increased β-galactosidase activity clearly indicates a significant induction of msrA1/msrB locus in the presence of oxacillin, even in a methicillin-resistant S. aureus (Figure 5). We also investigated the expression of the downstream genes of msrA1 locus in qRT-PCR assays. We determined that the oxacillin stress dramatically induced the expression of msrA1, msrB, and the gene encoding IIa(PTS) (Table 4). The expression level of the fourth gene of this locus was not investigated due to its very small size. This finding further supports our previous observation of cotranscription of the four genes of the msrA1/msrB locus [5, 6].

3.5. Expression of msrA1/msrB Locus in MsrA1-MsrB-Deficient S. aureus Strain COL in the Presence of Oxacillin. While studying the regulation of the msrA1/msrB locus in a methicillin-resistant S. aureus strain COL, oxacillin was added during the growth of the msrA1/msrB promoter-lacZ reporter to investigate any magnification of the regulation. In these studies, while an increased lacZ expression was observed in wild-type S. aureus strain COL after oxacillin treatment, a more dramatic increase in the lacZ expression in response to oxacillin was seen in MsrA1-MsrB-deficient COL (Figure 6).
4. Discussion

Cell wall-active antibiotics have been used extensively for the treatment of infections caused by bacterial pathogens. *S. aureus* is a major human pathogen and is resistant to most commonly available antibiotics. Interestingly, cell wall–active antibiotics cause induction of a locus in *S. aureus* that leads to elevated synthesis of two methionine sulfoxide reductases (MsrA1 and MsrB) [5, 6]. These enzymes reduce methionine sulfoxide and play important roles in maintaining protein integrity and function particularly under oxidative stress. These two proteins have also been shown to have roles in the virulence of bacterial pathogens [19–23]. Msr-deficient bacterial mutants show a reduction in the ability to adhere to eukaryotic cells and are thus less likely to establish an infection [21, 22, 24, 25]. It is speculated that the lack of the Msr enzymes compromises the integrity of the bacterial surface proteins responsible for adherence to eukaryotic cells. Reduced Msr activity decreases bacterial survival inside the phagocytic cells [20]. In addition to increased levels of MsrA1 and MsrB specifically in response to cell wall-active antibiotics, these proteins in *S. aureus* have been shown to play roles in the survival of bacterial cells under oxidative stress as well as in mice [6, 10].

We previously demonstrated that when the *msrA1* gene is deleted in *S. aureus*, there is an increase in MsrB synthesis suggesting a possible role in the regulation of this locus [9]. Findings of this study suggest that, in a methicillin-sensitive *S. aureus* strain SH1000, MsrA1 and MsrB individually can downregulate the *msrA1/msrB* locus. However, in methicillin-resistant *S. aureus* strain COL, MsrA1 and MsrB both are needed to downregulate the expression of the *msrA1/msrB* locus. It is speculated that the *msrA1/msrB* locus, to some extent, is differentially regulated between methicillin-resistant and methicillin-sensitive *S. aureus* strains. It is not uncommon to observe a differential gene expression pattern between different *S. aureus* strains. It has been demonstrated that the growth of methicillin-resistant *S. aureus* is slower than that of methicillin-sensitive *S. aureus* in the lag phase but not during the exponential phase and that the alterations in virulence between these two strains may at least partially be due to the growth rate differences [26]. Deletion of a gene encoding nitric oxide synthase (NOS) in a methicillin-resistant *S. aureus* reduced virulence as seen by decreased bacterial survival and smaller abscess formation [27]. However, NOS was shown to have a limited role in a methicillin-sensitive *S. aureus* [28]. Expression of genes encoding staphylococcal superantigen-like (SSL) proteins also varies between *S. aureus* strains [29, 30]. Significant differences were also noted between the protein profiles of the methicillin-resistant and methicillin-sensitive *S. aureus* strains exposed to Triton X-100 [31].

It is well established that the *msrA1/msrB* locus is selectively induced in the presence of cell wall-active antibiotics [5]. These antibiotics interfere with the bacterial cell wall synthesis and, as a result, the cells become fragile and susceptible to lysis. Expression of *msrA1/msrB* locus is not induced by antibiotics that target other bacterial metabolic pathways [5]. In a previous report, it was shown that the *msrA1/msrB* locus was not induced by the presence of oxacillin but was induced by the presence of D-cycloserine and vancomycin in a methicillin-resistant *S. aureus* [18]. However, data from our study provide clear evidence that oxacillin does in fact induce the *msrA1/msrB* locus in a methicillin-resistant background of *S. aureus*. The previous report [18] did not observe any induction because the bacterial cells were not exposed to a high enough concentration to impose antibiotic stress in a methicillin-resistant *S. aureus* strain. Furthermore, we explored the induction of *msrA1/msrB* genes in *msrA1/msrB* double mutant in methicillin-resistant strain COL. An increase in induction of the *msrA1/msrB* locus was further magnified in *msrA1/msrB* double mutant exposed to oxacillin compared to the wild-type *S. aureus* COL in response to oxacillin. This further confirms the notion of downregulation of the *msrA1/msrB* locus by MsrA1 and MsrB and this is more likely an indirect effect. This speculation of an indirect regulation is based on the fact that, after conducting a protein domain search (http://prosite.expasy.org/), no specific DNA-binding domain was observed in MsrA1 and MsrB proteins. It is possible that the MsrA1 and MsrB enzymes are critical in maintaining the integrity of a cytoplasmic transcriptional regulator that is involved in the regulation of expression of this locus.

In recent years, regulation of *msrA* and *msrB* has been studied extensively across multiple species; however, none have shown that MsrA or MsrB directly or indirectly regulates its own expression. It has been demonstrated that RynB regulates the synthesis of *Escherichia coli* MsrB but not MsrA by binding to the 5′ untranslated region of *msrB* mRNA and interfering with its binding to the ribosome [32]. Nitric oxide, which is induced in *Ulva fasciata* upon exposure to light, upregulates the expression of *msr* genes in the intertidal macroalga [33]. In *Saccharomyces cerevisiae*, calcium phospholipid binding protein (CPBP) interacts with the *msrA* promoter and enhances its expression [34]. In *Bacillus subtilis*, a transcriptional regulator, Spx, is shown to significantly upregulate the expression of *msrA* and *msrB* [35]. Spx also upregulates *msrA* expression in *S. aureus*. Teicoplanin induces *msrA1/msrB* expression in *S. aureus*. However, in *S. aureus* spx mutant, teicoplanin exposure resulted in no significant induction of this locus, whereas, in the spx mutant strain complemented with the wild-type *spx* gene, *msrA1/msrB* induction in response to teicoplanin exposure was restored [36]. Additionally, in the spx mutant, basal *msrA1* mRNA was significantly lower than *spx* complemented strain [36].

SigB is the alternative sigma factor in *S. aureus* that plays a role in the regulation of expression of stress responsive genes in *S. aureus* [11]. In addition, SigB is also associated with the regulation of expression of the virulence genes in *S. aureus* [11]. In a previous report, the level of expression of *msrA1/msrB* locus was investigated between RN450 (SigB−) and SH1000 (SigB+) [18]. It was shown that, in *S. aureus* strain SH1000, *msrA1/msrB* expression was 30% more induced than in *S. aureus* strain RN450 in the presence of oxacillin [18]. In contrast, our study shows that SigB in fact downregulates the expression of *msrA1/msrB* locus in *S. aureus* in the methicillin-sensitive *S. aureus* strain SH1000 and plays no
role in the regulation of this locus in methicillin-resistant strain COL.

In summary, this study provides evidence that the expression of the msrA1/msrB locus is enhanced when *S. aureus* is deficient in MrxA1, MrxB, or both in a methicillin-sensitive *S. aureus*. However, in methicillin-resistant *S. aureus*, increased expression of the msrA1/msrB locus was apparent only when the bacterial cells were deficient in both MsrA1 and MrxB. In addition, SigB also in part downregulates the expression of this locus in methicillin-sensitive *S. aureus* but not in methicillin-resistant *S. aureus*.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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