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Creating and Investigating a Deletion Mutant in *Staphylococcus aureus* MarR-family Regulator MgrA

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Abstract

Staphylococcus aureus, a Gram-positive bacterium, exhibits many defense mechanisms against the host innate immune system and multiple antibiotics. *Staphylococcus aureus* is second only to *Clostridium difficile* as a cause of healthcare-associated infections in the United States; therefore, it is important to develop new therapeutics. The exposure of bacterial pathogens to nitric oxide (NO·), an immune effector, causes growth inhibition and induction of stress regulons. *Staphylococcus aureus*, however, has evolved defense mechanisms to resist NO·. The metabolic resistance of *S.aureus* to NO· is one of the adaptations that makes it unique and more pathogenic than other Staphylococcal species such as *S. epidermidis* and *S. saprophyticus*. In a previous transposon-sequencing (Tn-seq) study using a strain of a community-acquired methicillin-resistant *S.aureus* (USA300 LAC), 168 genes were identified as likely to play a role in NO· resistance. One of these genes, encoding MarR-family transcriptional regulator *mgrA*, was investigated in this research with the goal of better understanding the control of gene expression during nitrosative stress. A deletion mutant of the gene, *ΔmgrA,* was constructed and investigated to elucidate its phenotype in the presence of NO·. The mutant was also investigated during peroxide (oxidative) stress, because potentiation of oxidative stress is one of the major ways NO· impacts bacteria. Although the previous Tn-seq data suggested that MgrA is required for NO· resistance, our data revealed that the *ΔmgrA* mutant exhibited enhanced NO· resistance relative to wildtype, but not peroxide resistance. This suggests that MgrA regulates genes associated with specific aspects of NO· stress that are separate from oxidative stress. Further studies will investigate what genes *MgrA* regulates during NO· stress and whether these could represent potential antibiotic targets.

1. Introduction

The spread of antibiotic resistance is well demonstrated in *S.aureus*. In addition to asymptomatically colonizing one-third of the human population, *S.aureus* is increasingly becoming multi-drug resistant [3,19]. The hypervirulence of methicillin-resistant *S.aureus* (MRSA) and community-acquired MRSA (CA-MRSA) has been shown to cause skin and soft tissue infections, which can become more invasive when not treated[12]. One leading cause of *S.aureus* persistence in mammalian hosts is due to its resistance to NO·.

NO· is a membrane-permeable, antimicrobial gas that is produced by the host's activated phagocytes during a bacterial infection. The released gas can disrupt the proliferation of bacterial infection by destroying bacterial DNA, proteins, and lipids[8]. NO·, among many other reactive nitrogen intermediates, is derived from arginine and can be produced by the inducible form of NO· synthase in immune cells. Upon release in response to a bacterial infection, NO· easily diffuses through the bacterial cell membrane and targets the metal or redox centers of bacterial enzymes such as heme iron, iron-sulfur clusters, and other transition metal cofactors[8,18]. The release and binding of NO· to these highly reactive redox centers can then arrest respiratory pathways, replication, and consequently cause the inhibition of bacterial proliferation and growth during infection[18,20].

Staphylococcus aureus overcomes the toxic effects of NO· by activating a wide variety of different resistance mechanisms that are not ubiquitous in other bacteria species. For example, *S.aureus* activates the staphylococcal respiratory response AB (SrrAB) two-component system which is essential for survival under hypoxic conditions and during oxidative and nitrosative stress. It also regulates the expression of many of the NO·-induced metabolic genes. Compared to other staphylococcal species, another unique mode of metabolic resistance to nitrosative stress in *S.aureus* is the induction of L-lactate dehydrogenase (ldh1) which allows *S.aureus* to maintain its redox homeostasis. Ldh1 shares a regulation region (regulated by SrrAB) with the NO.-detoxifying flavohemoglobin (Hmp), whose role as a virulence determinant depends on the host's ability to produce NO·.The efficiency of Hmp has been demonstrated by its ability to consume and detoxify the majority of NO· in *S.aureus*, in order to attenuate the nitrosative stress induced by phagocytic cells of the host[16,17].

Other transcriptional regulatory genes involved in NO· resistance that have previously been identified and characterized, using targeted mutagenesis and transcriptomics, include *sarA, codY, fur* and *srrAB*[6]. The deletion mutants of these regulator proteins were found to show metabolic disruption and transcriptional dysregulation upon exposure to nitrosative stress. The *S.aureus* Δ*sarA* mutant, for example, was considered NO· sensitive due to its significantly extended lag time, reduced ATP levels, and the loss of L-lactate reassimilation which is important for growth during NO· stress[5,6]. While these characterized regulons showed involvement in *S.aureus'* response to NO· stress, their response also suggests that there are other unidentified regulons.

A previous Tn-Seq study in the strain USA300 LAC, a CA-MRSA, identified 168 genes that were identified as likely to play a role in NO· resistance in *S.aureus*[8]. Dr. Grosser's lab is interested in exploring the genes that contribute to *S.aureus* NO· resistance and determining their specific functions through individual deletion. One of the genes identified in this Tn-seq as potentially playing a role in NO· resistance is *mgrA*, which is part of the multiple antibiotic resistant regulator family (MarR)[8]. The marR family transcriptional genes encode DNA-binding proteins that exist as dimers and bind palindromic sequences and a variety of ligands. MarR family proteins have winged helix motifs associated with DNA binding and are widespread in prokaryotes with large genome size including both bacteria and archaea. MarR regulators are diverse and regulate a wide variety of genes, including other genes involved in responding to antibacterial stress. The cellular processes that other MarR family regulators control includes resistance to antibiotics, organic solvents, household disinfectants, and oxidative-stress agents[9,10]. They also regulate the synthesis of virulence factors in microbes that infect humans and plants. MarR family regulators are generally involved in responses to environmental conditions, and this makes them remarkably capable of regulating virulence genes in pathogenic bacteria such as *S.aureus*. MarR, for example, is characterized in *E.coli* as a group of transcriptional regulators that are involved in regulating an operon that encodes a drug efflux pump. This is also true in *S.aureus* with *mgrA'*s ability to regulate efflux pumps. A previous study showed that MgrA, a 147-amino acid protein regulates NorA, NorB, and tet38, which are all efflux pump-encoding genes known to cause an increase in resistance against several antibiotic drugs [2,21].The efflux pump plays a vital role in both gram positive and gram negative bacteria species such as pumping out solutes from the cell, pumping out metabolites and antimicrobial agents, as well as regulating quorum sensing signals[15,20]. Another mode of regulation reported is the co-regulation of nitric oxide reductase (Nor) by *srrAB* and *mgrA*. Upon induction by NO· stress and anaerobic growth, *nor* is expressed and upregulated under anaerobic conditions, and links metabolism, oxygen sensing, NO· sensitivity, and virulence in the strain of *S.aureus* studied[4].

Many articles have been published on transcriptional regulators and how they impact both Gram positive and Gram negative bacteria species, but none have investigated the role of *mgrA* under nitrosative stress in *S.aureus*. This research will focus on creating a chromosomal deletion of *mgrA* in the CA-MRSA strain USA300 LAC.

The versatile human pathogen *Staphylococcus aureus* is known to colonize and spread at an alarming rate, therefore, exploring ways to counter its prominence could result in developing effective novel antibiotics. Here, we focus on the effect of a marR-family transcription regulator, MgrA, that plays a role in NO· resistance in *S.aureus*. Additionally, the elucidation of NO· resistance mechanisms and the understanding of the importance of transcriptional regulatory proteins such as MgrA can reveal novel aspects of the unique ways *S.aureus* coordinates its response to innate immune system antimicrobials such as NO· and hydrogen peroxide.

2. Materials and Methods

2.1. Mutagenesis Strategy

To create a chromosomal deletion in *mgrA*, allelic exchange was used to replace the chromosomal copy of this gene with *ermB*, conferring an erythromycin resistance marker[5]. Briefly, this was done by amplifying 1000 base pair flanking regions of *mgrA*, cloning these into a plasmid on either side of the antibiotic resistance gene, transforming the knockout plasmid into *S. aureus*, and selecting for mutants in which the antibiotic resistance gene (*ermB*) has replaced *mgrA* in the chromosome through homologous recombination[5]. These steps (Figure 2) are described below in detail.

2.1.1. construction of knockout plasmid for mutagenesis

S. aureus LAC gDNA was purified using Epicentre MasterPure Gram Positive DNA Purification Kit. To create regions of homology between the knockout plasmid (KO) and chromosome, about 1000 base pair flanking regions upstream and downstream of *mgrA* were PCR amplified from the LAC genomic DNA template using the primers

mgrA 5' FWD (5' CACTAGGATCCGGTTGATCTCTAAATGACAC 3'), 5' REV (5' CACTAGGATCCGGTTGATCTCTAAATGACAC 3'), 3' REV (5' CACTAGAATTCATGTCGCGTATTATAAAGCG 3'), and 3' FWD (5' CACTAGAATTCGCATTTGATGAAACAAAGGA 3').

The PCR was performed in a T100 thermal cycler using NEB Q5 High-Fidelity DNA Polymerase and a touchdown protocol (initial denaturation at 98ºC for 30 seconds, 25-35 cycles at 98ºC for 5-10 seconds, 50-72ºC for 20-30 seconds, 72ºC for 20-30 per kilobases, final extension at 72ºC for 2 minutes, and hold at 4-10ºC). A gel electrophoresis was performed to confirm if the PCR worked, and the products were purified with IBI Gel/PCR DNA Fragments Extraction Kit.

The 3' flanking PCR fragment was digested with EcoRI and cloned into the EcoRI site of pBTE**.** Digested pBTE was treated with Antarctic phosphatase (NEB) to keep the plasmid restriction sites from self-ligating. Ligation was done using the NEB standard T4 ligase protocol**.** The ligation product was transformed into NEB DH 5-alpha High-Efficiency Chemically Competent *E.coli* cells using the standard NEB heat shock transformation protocol, and plated on LB agar + 100µg/ml ampicillin . Because a single digest was used to clone in the 3' flanking region, colony PCR was used to confirm both that the insert was present and in correct orientation, yielding plasmid pBTE_*mgrA*-5'. A plasmid-specific forward primer (BT2.2a) was purchased from Eurofins Genomics to confirm directionality of the insert. Successful colonies (indicated by 1kb PCR product) containing pBTE_*mgrA*-5' were grown in Lysogeny broth (LB) liquid culture and 100µg/ml ampicillin. Freezer stocks were made from overnight liquid cultures adjusted to a final concentration of 25% glycerol and then stored in -80ºC freezer. Plasmid was extracted using the IBI High-Speed Plasmid Mini Kit.

The 5' flanking region of *mgrA* was next cloned into the BamH1 site of pBTE_*mgrA*-5' using the same procedures as described for cloning in the 3' fragment. Colony PCR was used to confirm the insert was present and in correct orientation in plasmid. A plasmid-specific reverse primer (BT2.2b) from Eurofins Genomics was used to ensure the directionality of the insert. Successful colonies were grown in Lysogeny broth (LB) liquid culture + Amp 100. Freezer stock was made using 50% glycerol and 500µl O/N liquid culture and then stored in -80ºC freezer. The complete KO plasmid pBTE_*mgrA* (Figure 1) was extracted using the IBI High-Speed Plasmid Mini Kit.

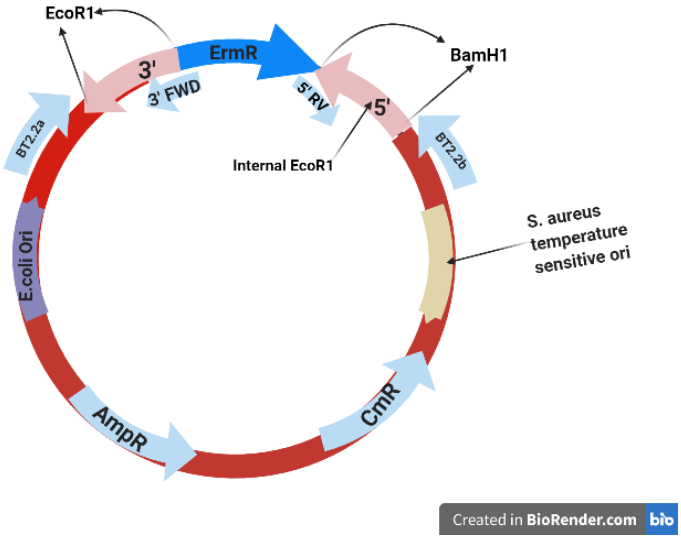

Figure 1. Complete KO plasmid

2.1.2. use of knockout plasmid to delete mgra from the s. aureus *lac chromosome*

The complete KO plasmid, pBTE_*mgrA* was first transformed into *S. aureus* RN4220. Due to a mutation in the *sau1 hsdR* gene, RN4220 is restriction-deficient[13]; this allows it to take up *E. coli* DNA and methylate it appropriately for uptake by other *S. aureus* strains. Using an established electroporation procedure, RN4220 electrocompetent cells were prepared and electroporated according to Grosser et al., 2016. pBTE has a temperature sensitive *S. aureus* origin of replication, so the product was then plated and grown in a 30ºC incubator, at the permissive temperature for plasmid replication, for 48hours. Individual colonies grown in O/N cultures were grown in TSB and 10µg/ml chloramphenicol (Cm 10) at 30°C and miniprepped using standard IBI miniprep protocol (modification of adding 2500 µg/ml lysostaphin to lyse the *S. aureus* cell wall after PD1 resuspension).

The miniprep product (complete KO plasmid, now methylated appropriately for uptake by *S. aureus*) was next transformed into *S. aureus* LAC electrocompetent cells (prepared as described above) and plated on TSA + Cm 10 at a permissive temperature of 30ºC. Isolated colonies were then grown at a non-permissive temperature of 43ºC on TSA + Cm10 to force recombination of the plasmid into the chromosomes at either the *mgrA* 5' or 3' regions of homology. Colonies were picked and grown with 5µg/ml erythromycin (Erm5) but without Cm10 at 30ºC to allow for a second recombination event that would remove the plasmid from the chromosome, with a 50% chance of generating a chromosomal deletion of *mgrA* and a 50% chance or regenerating wild-type (Figure 3). The presence of erm5 ensured that replicating cells were either mutants, or wild-type that had not yet lost the plasmid. A three-day passage at 30ºC without cm10 was performed to cure the plasmid.

Finally, an enrichment was performed to preferentially isolate mutant clones that had successfully lost the plasmid (and thus become Cm-sensitive). The O/N culture was diluted 1:1000 in fresh TSB lacking antibiotics and incubated at 30°C. The new culture was further diluted 1:100 into fresh TSB and incubated at 37° C to OD_{660} ~1.0 (~1 X 10^8 cfu/ml) before adding Cm10. Chloramphenicol is a bacteriostatic antibiotic, so it stops growth of (but does not kill) any cells which have lost the plasmid containing Cm-resistance, while cells that still have the plasmid continue growing. 30-minutes later, a 1:100 (100 µg/ml) cycloserine was added; this is a bactericidal antibiotic that kills only growing cells, resulting in preferential killing of cells that still have the Cm-resistant plasmid, and thus enriching for the cells that have lost it. Serial dilutions were plated on TSB + Erm5 at 37° C O/N. Colonies were simultaneously patched onto TSA + Cm10 plates and TSA + Erm5 plates to find Cm-sensitive recombinants, which should be mutants (Figure 4). A confirmation PCR was performed to check the replacement of the chromosomal copy of *mgrA* with *ermB* (Figure 5). This was done by using a primer internal to the *ermB* cassette (erm.1a) and a primer flanking the 5' chromosomal region of *mgrA* to check for the presence of the *ermB* cassette in the chromosome as well as its correct replacement of the *mgrA* gene.

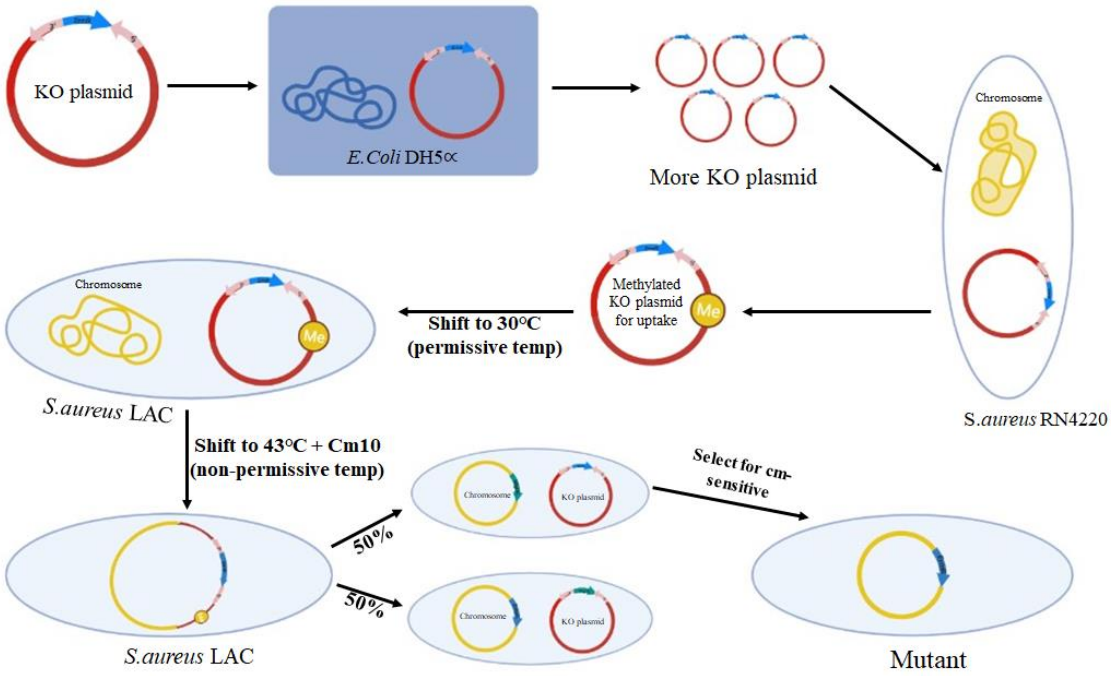

Figure 2**:** Layout for making *⊿mgrA* mutant.1000 base pair flanking regions of *mgrA* were amplified and cloned into either side of an ermB (erythromycin resistance gene) in the plasmid pBTE. Construction and subcloning of pBTE-*mgrA* was performed in *E. coli*. pBTE-*mgrA was* transformed into *S. aureus* RN4220, a restriction-deficient strain, to appropriately methylate the DNA for uptake by other *S. aureus* strains. pBTE-*mgrA* was transformed into *S. aureus* LAC and cultured at a permissive temperature of 30°C. Transformants were forced to recombine pBTE-*mgrA* into the chromosome in order to maintain resistance to Cm10 at a temperature of 43°C, which is non-permissive for pBTE plasmid replication. Recombinants were serially passaged at 30°C without Cm 10 to allow for a second recombination event and loss of plasmid.

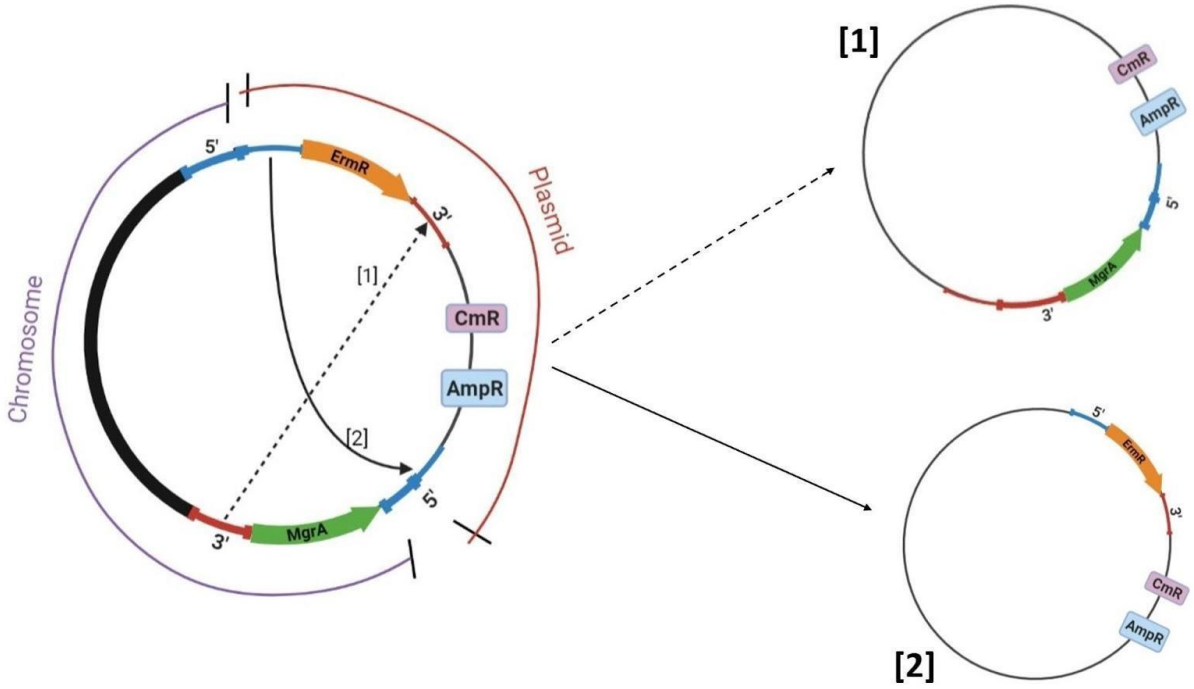

Figure 3. Schematic of possible recombination events in *S. aureus* during generation of the *mgrA* mutant. Homologous recombination can occur in bacteria between any two areas of DNA sequence homology. Here, the plasmid was incorporated into the chromosome via a recombination event between the blue 5' *mgrA* flanking region in the chromosome (thick line) and the blue 5' *mgrA* flanking region in the plasmid (thin line). Arrows indicate two possible recombination events that could subsequently occur during passage at a temperature permissive for plasmid replication, to once again remove the plasmid from the chromosome. (1) shows a possible recombination event between the two *mgrA* 3' regions of sequence homology (red), which would result in removal of the *mgrA* gene from the chromosome, replacing it with *ermB*, and generating a chromosomal deletion of *mgrA* once the plasmid is cured. (2) shows a possible recombination event between the two *mgrA* 5' regions of sequence homology (blue) which would result in removal of the original plasmid and regeneration of WT. These two possibilities each have equal probability of occurring.

2.1.3. no· growth curves

To compare growth phenotypes of the Δ*mgrA* mutant relative to wild-type, 24-hour growth curves were generated under a variety of conditions using 96-well plates, where each well serves as an independent culture. To prepare bacteria for growth curves, three independently isolated *mgrA* mutants and wild-type (WT) were grown in 5ml TSB O/N and incubated at 37°C and 250rpm of shaking for 24hrs. The culture OD was measured at 650nm and was diluted to a final OD650 of 0.01 for each strain in fresh TSB. We transferred 200ul of each culture into wells of a 96-well plate. Select wells were inoculated with 5mM or 10mM DETA/NO (a slow-release NO· donor), and growth curves were generated using the BioTek biolog plate reader to track absorbance (a proxy for growth) at 15-minute intervals (Growth OD_{650} : 37°C, 97 kinetic cycles; First shaking: 830 seconds shaking, orbital mode, 1mm amplitude; 2nd shaking: 30 sec duration, linear mode, 1mm amplitude, 10 sec settle time, 5 flashes).

3. Results

A previous Tn-seq in the CA-MRSA strain USA300 LAC identified *mgrA* as likely to contribute to NO· resistance in *S.aureus*[8]. The Tn-Seq was performed with a pool of ~100,000 mutants and was therefore also a competition assay, so the specific function of MgrA during NO· stress next needed to be investigated by comparing a pure culture of an Δ*mgrA* mutant to wild-type. Although isolated transposon mutants in many *S.aureus* genes are available for purchase from the University of Nebraska Medical Center, an *mgrA* mutant is not among them. Additionally, true deletion

mutants represent a more reliable way to study gene function than transposon-insertion mutants. We therefore created a chromosomal deletion mutant of *ΔmgrA* in USA300 LAC.

3.1. Generation of chromosomal deletion mutant of *ΔmgrA*

A chromosomal deletion mutant, *ΔmgrA* was created to characterize the phenotype of *mgrA* in the presence of NO·. A knockout plasmid was first constructed in *E. coli* that included an erythromycin resistance gene (*ermB)* flanked by DNA matching the chromosomal flanking sequences of *S. aureus mgrA*. From *E. coli*, the resulting KO plasmid (Figure 1) was purified and electroporated into *S.aureus* RN4220, a restriction deficient strain and a suitable cloning host for LAC, and subsequently transformed into *S.aureus* LAC electrocompetent cells. In LAC, sequence homology between the *mgrA* flanking regions of the plasmid and chromosome allowed recombination to occur, first integrating the entire plasmid into the chromosome via a single recombination event, then recombining a second time to remove the plasmid from the chromosome. The net effect was replacement of the chromosomal *mgrA* gene with the *ermB* gene from the plasmid. Curing of the chloramphenicol-resistant plasmid was ensured by serial passage and screening of resulting erythromycin-resistant colonies for sensitivity to Cm10. Cm10-sensitive, but Erm5-resistant colonies were presumed to be mutants (Figure 2, Figure 4), but were further screened by PCR for confirmation. The replacement of *mgrA* with an *ermB* cassette was confirmed (Figure 5), and loss of the knockout plasmid was also confirmed by PCR.

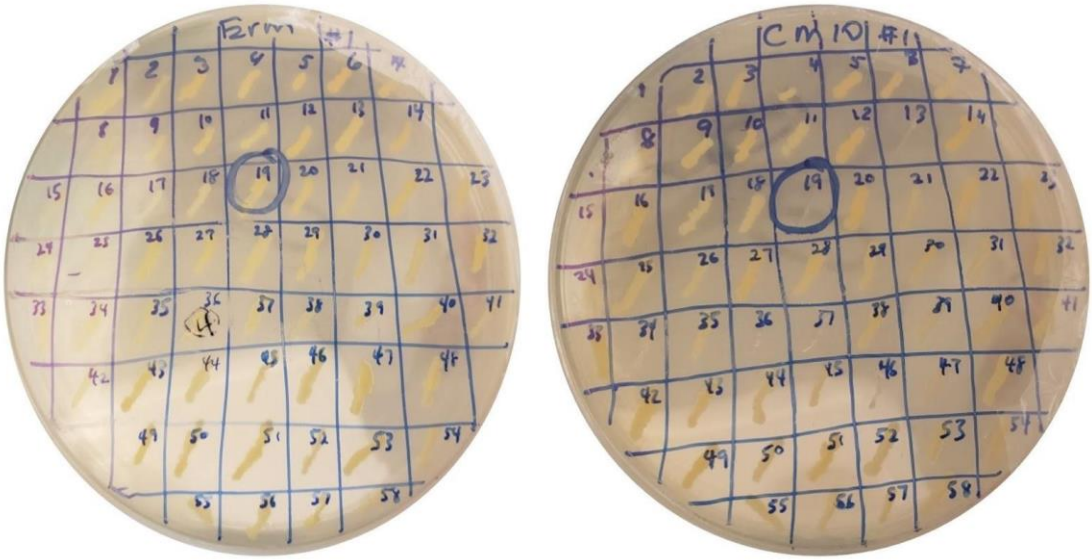

Figure 4**. S**imultaneously patched colonies (1 to 58) on TSA + Cm10 plate and TSA + Erm5 plate to find Cm-sensitive but Erm5-resistant recombinants (circled) which indicates an *mgrA* mutant that has been cured of the knockout plasmid.

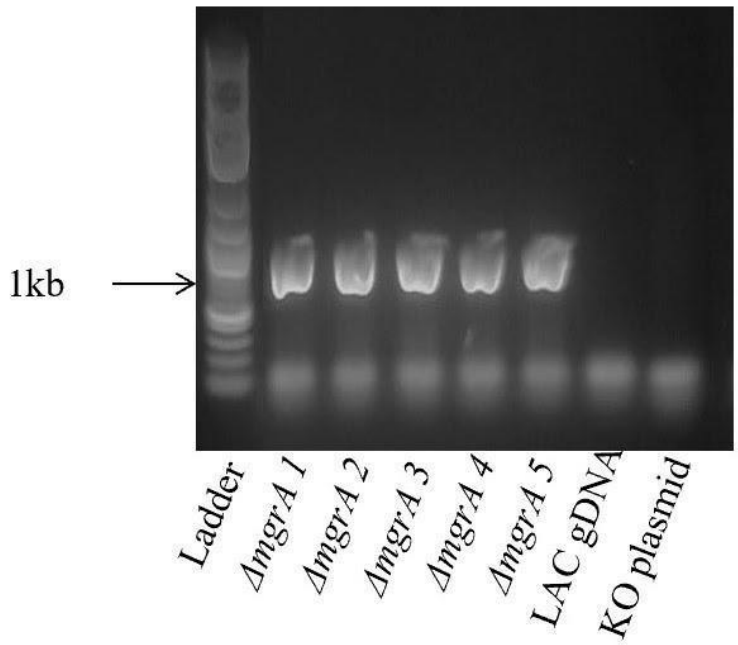

Figure 5. Mutant confirmation PCR. We used primer erm.1a (internal to the erm cassette), and a primer specific to the 5' flanking chromosomal DNA of *mgrA*. A successful product indicates that the *ermB* cassette is present and in the correct location in the chromosome. We only see products for confirmed mutants because wild-type LAC gDNA does not have the *ermB* cassette, and the control, which is the knockout plasmid, lacks the full flanking portion. Any apparent differences in size are due to the amount of DNA loaded because of variability of amplification.

3.2. The *ΔmgrA* mutant exhibited faster recovery from the effects of NO· during lag phase relative to wildtype.

The growth of the LAC *ΔmgrA* mutants was next compared to wild-type LAC in the presence of different NO· concentrations. The NO· donor DETA/NO extends lag time when it is added at the beginning of a growth curve, so we compared the recovery time (time to reach mid-exponential growth, or an OD650 of 0.5) between WT and *ΔmgrA* after DETANO exposure. We found that *ΔmgrA* mutants begin exponential growth significantly faster than WT in the presence of NO· (Time to OD650 of 0.5 was 10.06 +/- 3.57 for *ΔmgrA* mutants and 16.12 +/- 1.60 for WT in 10mM DETANO, $p<0.0001$ two-way ANOVA; Figure 6, Figure 7). This suggests that the *ΔmgrA* mutant exhibits enhanced NO· resistance relative to WT, at least under the specific conditions of this assay.

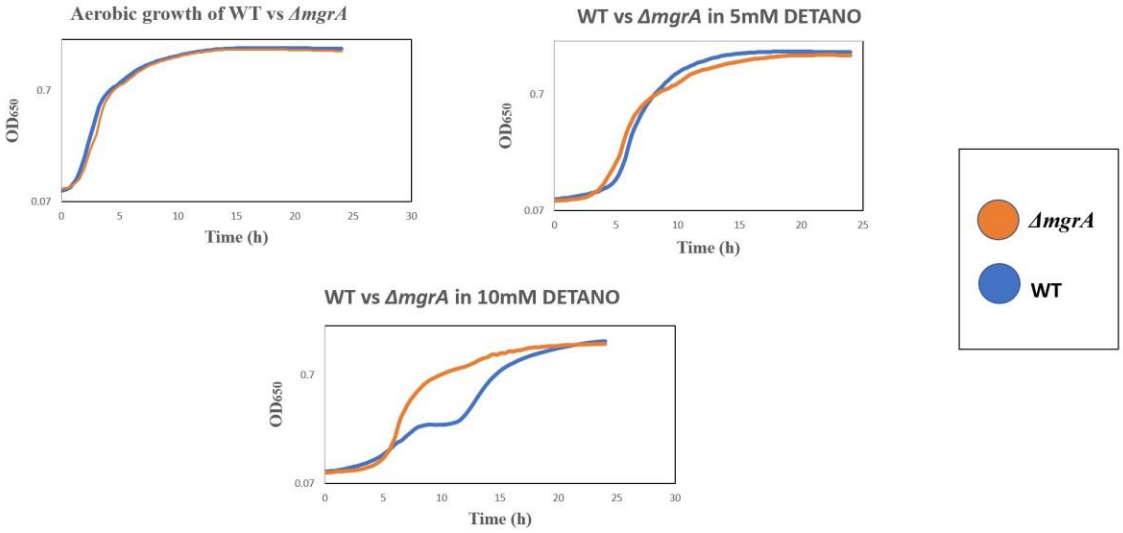

Figure 6. *ΔmgrA* had a faster recovery time compared to WT in the presence of NO·. Representative growth curves are shown for each condition (n=3 biological replicates, performed independently on different days, with 3 technical replicates of each treatment per day). (Note: we chose to show representative curves rather than average curves because day-to-day variation in lag time was significant. The same trend was observed in each biological replicate and is quantified in Figure 7).

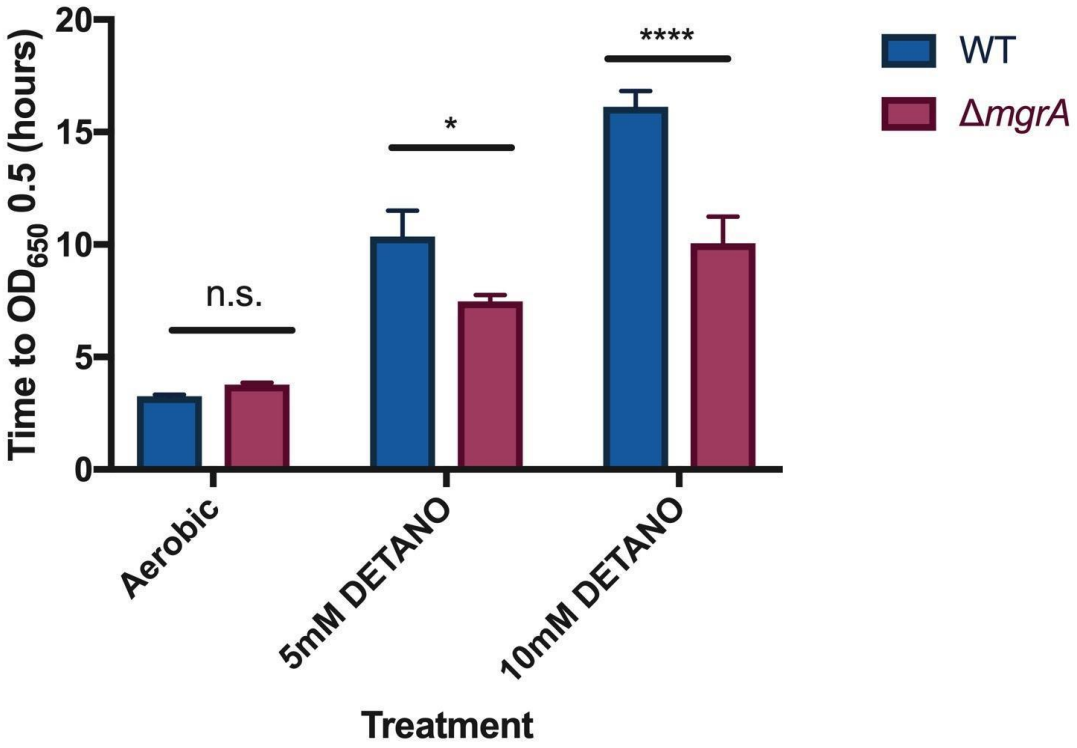

Figure 7. The average time to reach exponential growth (OD650 0.5) of WT LAC and *ΔmgrA* strains after aerobic growth in TSB or exposure to 5mM or 10mM DETANO. (n=3 biological replicates, performed independently on different days, with 3 technical replicates of each treatment per day; bars represent mean +/- SEM). Significance for the experiment was determined by a two-way analysis of variance test (ANOVA) with a *post hoc* Bonferroni multiple-comparison test (*, $p \leq 0.05$; ****, $p \leq 0.0001$); n.s., not significant)

3.3. The *ΔmgrA* mutant phenotype in the presence of hydrogen peroxide relative to WT.

We further tested and compared the phenotypes of WT and the *ΔmgrA* mutant upon the addition of hydrogen peroxide. Hydrogen peroxide causes oxidative stress in bacteria. While many of the effects of NO· stress (nitrosative stress) is distinct from oxidative stress, one component of nitrosative stress is that it induces and potentiates simultaneous oxidative stress within bacteria[14]. Therefore, testing *ΔmgrA* with hydrogen peroxide will help deduce if the induction of oxidative stress by NO· is the sole reason *ΔmgrA* has a faster recovery time than WT, or if other effects of NO· are responsible for the phenotype. Our approach was to determine the minimum inhibition concentration (MIC) of peroxide that Δ*mgrA* versus WT can grow in, starting with 1% peroxide. Preliminary data (n=2) did not show a difference in peroxide MIC between WT and Δ*mgrA* (Figure 2). This suggests that the faster recovery time in the presence of NO· for Δ*mgrA* compared to WT is likely due to *mgrA* regulation of genes that impact other aspects of NO· resistance unrelated to oxidative stress.

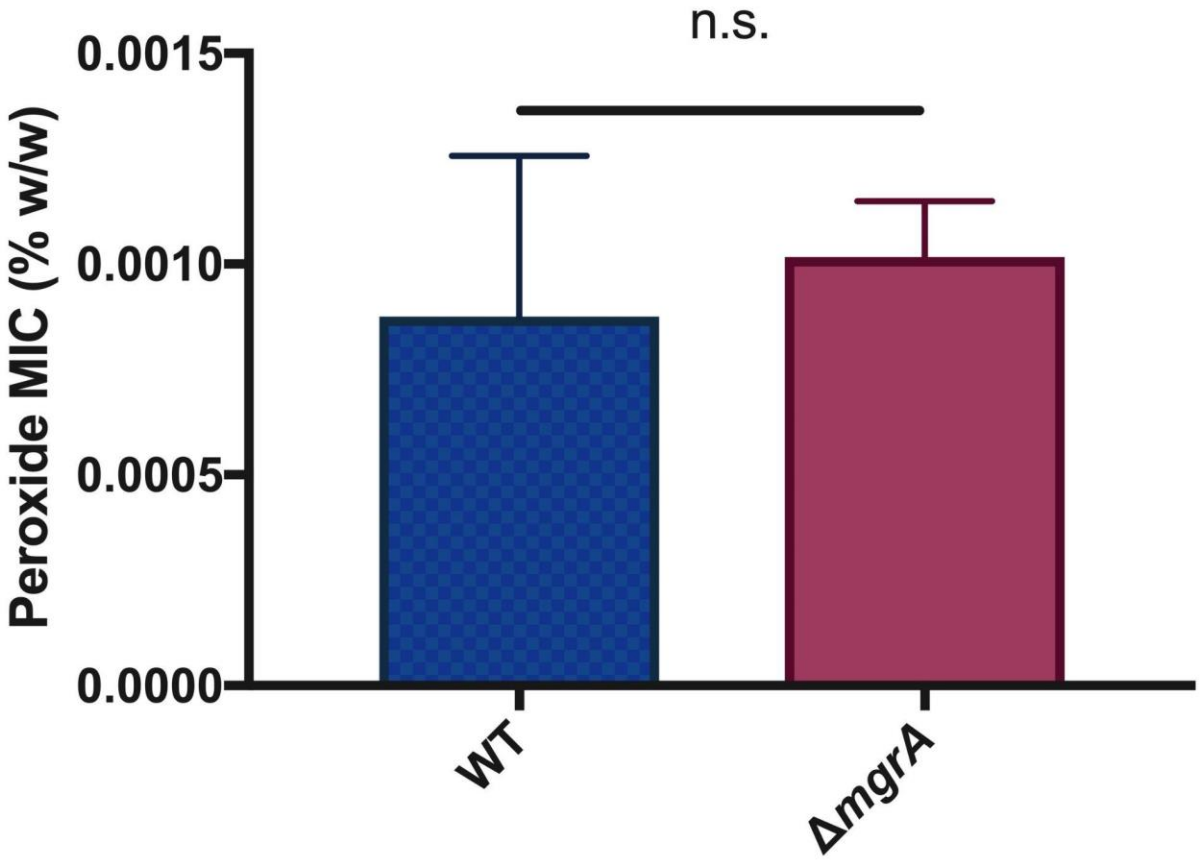

Figure 8. There is no significant difference in the minimum inhibition concentration (MIC) of peroxide that Δ*mgrA* versus WT can grow in. Significance for the experiment was determined by a two-sided t test (n.s., not significant).

4. Discussion

Staphylococcus aureus overcomes specific components of the innate immune system response, such as the toxic effects of NO·, by activating a wide variety of different resistance mechanisms that are not ubiquitous in other bacteria species. Here, we studied how *mgrA* is involved in the response to NO·. The results from this study provide information on this transcriptional regulon that has not yet been investigated in the context of NO· stress response. Previous studies [2,11] have reported *mgrA* to broadly contribute to multiple aspects of *S.aureus* physiology, and our data extend that contribution to include the *S. aureus* NO· response.

Interestingly, our data seemingly contradict the finding in Grosser et al. 2018 that *mgrA* transposon mutants have a fitness defect during NO· stress. However, this discrepancy may be explained by the fact that Grosser et al., 2018 examined fitness in a pool of transposon mutants rather than deletion mutants. Transposon mutagenesis involves the insertion of a transposon into the bacterial chromosome, which presumably disrupts gene function if inserted into a gene. The gene itself is not deleted, and depending on where the insertion occurs, can retain partial function. Additionally, an *mgrA* transposon mutant was not previously studied on its own for its NO· phenotype. Its fitness was assessed within and relative to a pool of ~100,000 other mutants. Mutant fitness can vary when studied in pure culture versus in a competition assay with wild-type and other mutants. We are confident in the reported *mgrA* phenotype in our new finding since we created a novel deletion mutant of *mgrA* and ensured the complete lack of the gene in our knockout mutants (Figure 2).

Because MgrA is a repressor of many genes, it is likely that overexpression of specific genes in the Δ*mgrA* mutant may explain its faster recovery time in the presence of DETANO. Previous studies have performed RNA-Seq or qRT-PCR to identify genes regulated by MgrA (though not during NO· stress specifically). Some of the genes found to be repressed by *mgrA* are efflux pump transporters such as *norA* [1,2]. Another gene is *scdA*, which is involved in repairing iron-sulfur clusters and has been shown to play a role in resistance to oxidative stress, so it is interesting that Δ*mgrA* does not seem to differ from wild-type during oxidative stress. The gene *nor*, encoding a nitric oxide reductase, has also been found to be partially regulated by MgrA. Nor contributes to detoxifying NO·, so the possible overexpression of Nor in the Δ*mgrA* mutant is worth further investigation[4]. However, this study found that *nor* is only regulated by MgrA under anaerobic conditions. The true reason for the NO· phenotype observed in Δ*mgrA* cannot yet be determined since any of the genes or combination of the genes it regulates could potentially be contributing to this phenotype. Further studies will attempt to investigate mutants in these genes with *mgrA* individually as well as together to elucidate if and how those genes contribute to resistance to NO·. It would also be of great interest to use RNA-Seq and qRT-PCR to investigate the set of genes regulated by *mgrA* in the USA300 LAC strain specifically when NO· is present.

In the early stages of this study, attempts were made to characterize the phenotype of SAUSA300_1583, another gene encoding a transcriptional regulator that was identified to play a role in resistance to NO· in *S.aureus*[8]. Elucidating the phenotype of this gene might provide further insight into other aspects of the *S. aureus* transcriptional response under nitrosative stress.

5. Acknowledgement

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6. References

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