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Sydney Wolin April 9, 2019

Characterization of the role of Hfq in *ABUW_1645* expression and phenotypic switching in *Acinetobacter baumannii*

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

Characterization of the role of Hfq in *ABUW_1645* expression and phenotypic switching in *Acinetobacter baumannii*

By Sydney Wolin

Acinetobacter baumannii is a Gram-negative nosocomial pathogen that predominately affects immunocompromised individuals. Recently, *A. baumannii* strains have exhibited a stark increase in antibiotic resistance. Therefore, new treatment methods are crucial for overcoming infection. Prior research has shown that *A. baumannii* interconverts between two colony variants, opaque (O) and translucent (T). It has been demonstrated that the O variant is more virulent and antibiotic resistant than the T variant. Therapeutics that force *A. baumannii* into the T state could be an effective means of treating infection. The purpose of this study was to better comprehend the regulation of phase variation in *A. baumannii*. Several regulatory genes have been identified that regulate the O to T switch. The TetR transcriptional regulator *ABUW_1645* exhibits a 60-fold increase in expression in the T variant, and overexpression of *ABUW_1645* drives the O to T switch. Hfq is an RNA chaperone protein that simultaneously binds sRNA and mRNA acting as a post-transcriptional regulator. Hfq has also been previously identified to positively regulate O to T switching. In this study, we found that overexpression of Hfq increases *ABUW_1645* expression, and confirmed that Hfq positively regulates O to T switching. Site-directed mutagenesis of both the distal and proximal faces of Hfq reversed its effects on *ABUW_1645* expression and phenotypic switching. These results demonstrate the importance of Hfq in regulating these processes, and show that both mRNA and sRNA binding are essential for Hfq to have an effect on *ABUW_1645* expression and phenotypic switching. This work establishes Hfq as a potential target to combat *A. baumannii* infection. This research suggests that interventions manipulating Hfq may effectively reduce *A. baumannii* virulence as well as antibiotic resistance by forcing cells into the T state.

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Introduction

Acinetobacter baumannii is a Gram-negative nosocomial pathogen.[22] This bacterium is prevalent in hospital settings, and predominantly affects immunocompromised or severely injured individuals.[2,4,11,14,21,23,27-28] *A. baumannii* infections also persist in the wounds of soldiers who serve in dry climates overseas.[5] Due to resistance to multiple types of antibiotics, it has become increasingly difficult to treat these infections.[9,12,20,24-26] Multiple lines of antibiotics, such as colistin and the carbapenems, are losing their effectiveness.[8,16,18] Therefore, alternative methods to combat *A. baumannii* infections are essential.

A novel phase-variation mechanism has recently been discovered in *A. baumannii* strain AB5075 that leads to an interconversion between translucent (T) and opaque (O) colony variants.[29] These variants exhibit differences in virulence, biofilm formation, cell shape, motility and antibiotic resistance.[7,29] The O variant demonstrates greater virulence than the T variant. The O variant is also more resistant to specific types of antibiotics, such as gentamicin and tobramycin, than the T variant.[29] The multiple phenotypes affected by the switch between O and T variants convey that the mechanism controlling the switch is complex and highly regulated.[29] The TetR-type transcriptional regulator, *ABUW_1645*, and the RNA chaperone protein, Hfq, have both been shown to regulate O to T switching.

The TetR-type transcriptional regulator *ABUW_1645* in *A. baumannii* strain AB5075 exhibits a 60-fold increase in expression in avirulent T colonies.[7] When colonies convert from T to O, the expression of *ABUW_1645* is significantly reduced.[7] Overexpression of *ABUW_1645* in virulent

O colonies increases O to T switching and decreases virulence.[7] From these findings, it is evident that *ABUW_1645* is a key regulator in the switch between virulent O and avirulent T colonies.

Hfq is an RNA chaperone protein first discovered in *Escherichia coli* and found in 50% of all bacterial species.[15,30] sRNAs are small RNAs that can target specific mRNAs and regulate their expression.[31] Hfq facilitates sRNA-mRNA interactions, thereby acting as a post-transcriptional regulator.[30] The structure of Hfq consists of a proximal face and a distal face to which sRNA and mRNA bind, respectively.[30] The Hfq-sRNA-mRNA complex can either negatively or positively regulate translation through various mechanisms. These include blocking the ribosome binding site (RBS) or exposing the RBS by removing an inhibitory secondary structure.[15] Deletion of *hfq* in *A. baumannii* ATCC 17978 affects multiple virulence factors such as biofilm formation, stress resistance, cell adhesion, and invasion.[17] Our laboratory has recently shown that an *hfq::Tc* mutant exhibits decreased O to T switching, demonstrating that Hfq positively regulates O to T switching (unpublished results).

The aim of this study was to determine whether Hfq regulates *ABUW_1645* expression, and to clarify the role of Hfq in mediating O to T switching. To examine the effects of deleting *hfq* on *ABUW_1645* expression, we transformed the genomic DNA of an AB5075 *hfq::Tc* mutant into the ATCC 17978 *1645-lacZ* reporter strain, generating the ATCC 17978 *1645-lacZ, hfq::Tc* mutant. β-galactosidase assays revealed no difference in expression between the 17978 *1645-lacZ, hfq::Tc* mutant and wild type reporter strain. On the other hand, overexpression of *hfq* in the 17978 *1645-lacZ* reporter strain led to increased *ABUW_1645* expression visualized on agar

plates containing X-gal. X-gal turns blue when cleaved by β-galactosidase. Blue colonies were apparent in transformants containing the *hfq* overexpression vector; however, β-galactosidase assays displayed no difference in expression between the overexpression vector and empty vector transformants. *Hfq* was also overexpressed on a plasmid in LSO 5075, a wild-type O variant of AB5075 that exhibits extremely low rates of O to T switching. Overexpression of *hfq* in LSO 5075 increased O to T switching. In addition, we characterized the importance of the different Hfq binding domains in *ABUW_1645* expression and O to T switching. Mutations were introduced in either the distal or proximal face of Hfq, and overexpressed in 17978 *1645-lacZ* and LSO 5075. Both mutations reversed the effects of Hfq on *ABUW_1645* expression and phenotypic switching, demonstrating the importance of both mRNA and sRNA binding in these processes. The effects of Hfq on *ABUW_1645* expression and O to T switching presents Hfq as a possible target for 'locking' *A. baumannii* in the avirulent T state in order to minimize infection.

Materials and Methods

Bacterial Strains, Plasmids, Primers and Growth Conditions. The bacterial strains and plasmids used in this study are summarized in Table 1. The primers used in this study are listed in Table 2. *A. baumannii* was cultured in 2 ml sterile LB broth and supplemented with 5 μg/ml tetracycline (Tc) when necessary. 0.5 x LB plates supplemented with 0.8% agar were used for visualizing switching. LB plates supplemented with 1.5% agar were used for all other experiments.

Table 1 | Bacterial strains and plasmids used in this study

Strain or Plasmid	Genotype/Characteristics	Source/Reference
***A. baumannii* strains**		
AB5075	Wild-type	10
AB5075 *hfq::Tc*	Δ*hfq* strain	Unpublished Work
ATCC 17978 *1645-lacZ*	Wild-type *ABUW_1645* reporter strain	Unpublished Work
ATCC 17978 *1645-lacZ hfq::Tc*	*ABUW_1645* Δ*hfq* reporter strain	This study
LSO 5075	Low-switching opaque variant	Unpublished work
***E. coli* strain**		
EC100D	Used for cloning purposes	Lucigen (Middleton, WI)
Plasmids		
pWH1266	Amp^r Tc^r; expression vector	13
pWH1266_*hfq*-2	pWH1266 with *hfq* gene insertion in +/+ orientation[a]	This study
pWH1266_*hfq*-9	pWH1266 with *hfq* gene insertion in +/- orientation[b]	This study
pWH1266 *hfq*-2 F25A	pWH1266 with *hfq* harboring distal face mutation	This study
pWH1266_*hfq*-2 Q8A	pWH1266 with *hfq* harboring proximal face mutation	This study

[a]*hfq* insertion oriented in the same direction as bla_{TEM-1}(ampicillin resistance gene). [b]*hfq* insertion oriented in the opposite direction as bla_{TEM-1}.

Table 2 | Primers used in this study

Primer	Genotype	Source/Reference
Hfq_for	5'-aagcaaaatttgcacactgtcttttt-3'	This study
Hfq_rev	5'-attttaagaaacagagatggaccgc-3'	This study
oSA143	5'-taatttaataccgttaacaagggcaatagaaactgggatgcgttctttacg-3'	This study
oSA144	5'-cgtaaagaacgcatcccagtttctattgcccttgttaacggtattaaatta-3'	This study
oSA155	5'-gagaatttaagaacggatctgctaaagtttgacctttagacatttttaactccaaaaa-3'	This study
oSA156	5'-tttttggagttaaaaatgtctaaaggtcaaactttagcagatccgttcttaaattctc-3'	This study

To generate strain ATCC 17978 *1645-lacZ hfq::Tc*, cultures of ATCC 17978 *1645-lacZ* were grown overnight by shaking at 37°C. The cultures were spun down and prepared for transformation by washing multiple times in 10% glycerol solution. AB5075 *hfq::Tc* genomic DNA was isolated, as described below, and electroporated into ATCC 17978 *1645-lacZ* to generate the ATCC 17978 *1645-lacZ hfq::Tc* mutant. The transformation was plated on LB media plates containing tetracycline (5 μg/ml).

To generate *hfq* overexpression vectors, AB5075 genomic DNA was isolated and *hfq* was PCR amplified using the primers hfq_for and hfq_rev. PCR product was run out on 0.7% agarose gel, and gel purified using a gel extraction kit (Monarch DNA Gel Extraction Kit, New England Biolabs). The *hfq* PCR was ligated into the ScaI site of pWH1266, which disrupted the ampicillin resistance gene carried on this plasmid. The recombinant plasmid was transformed into EC100D, plated on LB media plates containing tetracycline (5 μg/ml), and incubated overnight at 37°C. EC100D transformants were patched on two separate LB media plates, one supplemented with ampicillin (Amp, 300 μg/ml) and the other supplemented with tetracycline (5 μg/ml). Colonies that did not grow on ampicillin, but did grow on tetracycline were cultured and prepared for QIAprep miniprep. Plasmids were isolated via QIAprep miniprep and digested with PvuII and BamHI. Digests were run out on 0.7% agarose gel to check for an insertion. Plasmids containing insertions were sent for sequencing to confirm the insertion was *hfq*, and to determine the orientation of the insert.

Plasmids overexpressing mutated alleles of *hfq* were generated with the Agilent Technologies QuickChange II XL Site-Directed Mutagenesis Kit. For Hfq distal face mutagenesis, 50 ng of pWH1266 *hfq*-2 was PCR amplified using the primers oSA143 and oSA144. Mutagenesis generated an F25A amino acid change located on the distal face of Hfq. For Hfq proximal face mutagenesis, 50 ng of pWH1266 *hfq*-2 was PCR amplified using the primers oSA155 and oSA156. Mutagenesis generated a Q8A amino acid change located on the proximal face of Hfq. Potential plasmids carrying the desired form of mutated Hfq were transformed into EC100D, plated on LB media plates containing tetracycline (5 μg/ml), and incubated overnight at 37°C. Plasmids from transformants were isolated via QIAprep miniprep and sent for sequencing.

To introduce plasmids into *A. baumannii* strains, cultures were grown overnight by shaking at 37°C. Cultures were spun down and prepared for transformation by washing multiple times in 10% glycerol solution. Electroporations into LSO 5075 were plated on 0.5 x LB media plates containing tetracycline (5 μg/ml). Electroporations into ATCC 17978 *1645-lacZ* were plated on LB media plates containing tetracycline (5 μg/ml) and X-gal (200 μg/ml).

Genomic DNA Isolation. Cells were pelleted from cultures by centrifugation and resuspended in 560 μl of 1x TE buffer. 3 μl of proteinase K and 15 μl of 20% SDS were added to the sample. The sample was incubated at 37°C for 1 hour. 100 μl of 5 M NaCl was added to the sample after incubation. 600 μl of phenol-chloroform isoamyl alcohol (PCI) was added and shaken rapidly until the solution was milky. The solution was centrifuged at 15,000 rpm for 10 minutes. The top clear layer of the sample was removed into a clean Eppendorf tube. This process was repeated

with 600 μl of PCI. The tube was filled with 750 μl of 95% ethanol (EtOH), inverted until DNA was visible, and centrifuged for 5 minutes. The EtOH was decanted off and 1 ml of 75% EtOH was added to the sample. The sample was inverted multiple times and EtOH was decanted off. This step was repeated, and any remaining EtOH was removed. The tube was left open on the bench allowing the pellet to air dry. When the pellet was clear, 50 μl of diH_2O was added to the sample and stored overnight at 4°C to resuspend the pellet.

β-galactosidase assays. For the data presented in Figure 1, colonies were grown on plates containing tetracycline for 24 hours and resuspended in 2 ml of LB. The absorbance of each suspension was immediately measured at OD_{600} and divided into two 900 μl aliquots. For the data presented in Figure 2 and Figure 4, cultures were grown overnight by shaking at 37°C. The absorbance of each culture was measured at OD_{600} and cultures were divided into two 900 μl aliquots. For all sets of data, the suspensions were pelleted by centrifugation at 15,000 rpm for 1 minute. The supernatant was removed and the cell pellets were frozen at -20°C for 1 hour. Pellets were resuspended in 0.9 ml of Z buffer containing β-mercaptoethanol (BME, 27 μl of BME per 10 ml of Z buffer). 10 μl of 0.1% SDS and 20 μl of chloroform were added to each suspension for lysis and permeabilization of cell membranes. The suspensions were inverted several times and immediately vortexed for 30 seconds. After starting the timer, 200 μl of ONPG was added to each suspension and vortexed in 30 second intervals. When the tubes appeared yellow, the timer was stopped, and 500 μl of 1 M sodium carbonate was added to each tube in Figure 2. For the data in Figure 1 and Figure 4, the timer was stopped and no sodium carbonate was added. After stopping the timer, the suspensions were centrifuged for 8-12 minutes at 15,000 rpm. The

supernatants of each suspension were carefully decanted into clear plastic cuvettes. The absorbance of each sample was read at 420 nm.[19]

Visualization of switching in LSO 5075. Switching phenotypes were analyzed using a dissecting microscope with oblique lighting, as previously described.[1]

Results

***hfq* deletion does not affect *ABUW_1645* expression.** Genomic DNA from the AB5075 *hfq::Tc* deletion mutant was transformed into the 17978 *1645-lacZ* reporter strain. This strain contains a transcriptional *ABUW_1645-lacZ* fusion in which β-galactosidase activity is a reflection of *ABUW_1645* expression. β-galactosidase assays were performed to assess the level of *ABUW_1645* expression in the 17978 *1645-lacZ hfq::Tc* mutant compared to the wild-type reporter strain by measuring β-galactosidase activity. Figures 1 and 2 show that for cells assayed from both agar plates and broth culture, deleting Hfq does not significantly affect the level β-galactosidase activity.

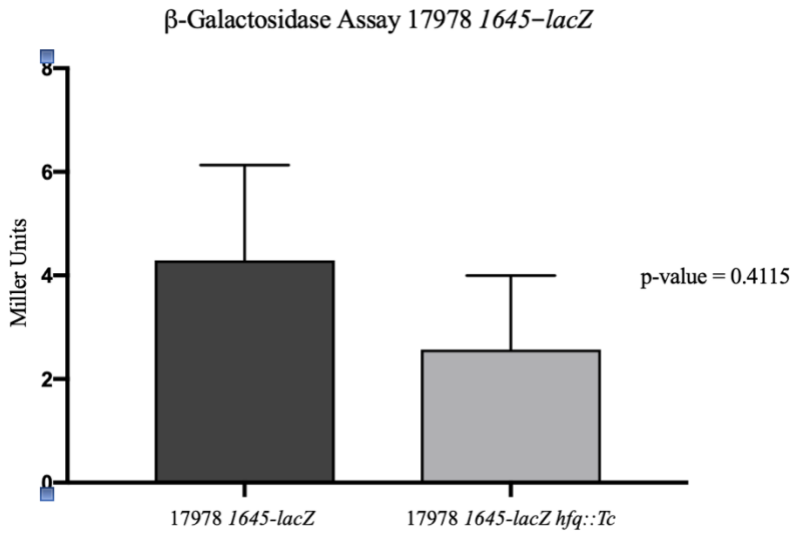

Figure 1 | Effect of an *hfq* deletion mutant on *ABUW_1645* expression on agar. The averages and standard deviations from two independent experiments are shown. Using Welch's t-test, it was shown that there is no significant difference in β-galactosidase activity between the wild-type strain, 17978 *1645-lacZ,* and the Hfq deletion mutant when assayed from 24-hour colonies.

Figure 2 | Effect of an *hfq* deletion mutant on *ABUW_1645* expression in broth culture. The averages and standard deviations from two independent experiments are shown. Using Welch's t-test, it was shown that there is no significant difference in β-galactosidase activity between the wild-type strain and the Hfq deletion mutant when assayed from broth cultures.

Overexpression of *hfq* causes increased *ABUW_1645* expression on agar plates. *hfq* was cloned into the beta-lactamase gene of pWH1266 generating two distinct overexpression vectors, pWH1266 *hfq*-2 and pWH1266 *hfq*-9. The promoter of *hfq* in pWH1266 *hfq*-2 runs in the same direction of the beta-lactamase gene, while the promoter of pWH1266 *hfq*-9 runs in the opposite direction. Therefore, *hfq* expression in pWH1266 *hfq*-2 should be enhanced by the beta-lactamase promoter, making this the stronger overexpression vector. pWH1266, pWH1266 *hfq*-2, and pWH1266 *hfq*-9 were transformed into the 17978 *1645-lacZ* reporter strain and plated on X-gal. Figure 3 shows that transformants containing pWH1266 *hfq*-2 appeared dramatically bluer when plated on X-gal compared to transformants containing pWH1266. Transformants containing pWH1266 *hfq*-9 looked the same as empty vector control (data not shown). This demonstrates that on plates, overexpression of *hfq*-2 increases *ABUW_1645* expression. β-galactosidase assays from liquid culture were also performed to assess the level of *ABUW_1645* expression via measurement of β-galactosidase activity. Figure 4 shows that overexpression of

hfq-2 does not affect the level β-galactosidase activity. The contrasting results obtained from cells grown on plates and in broth imply that the effects of *hfq* on *ABUW_1645* expression depend on the cellular environment.

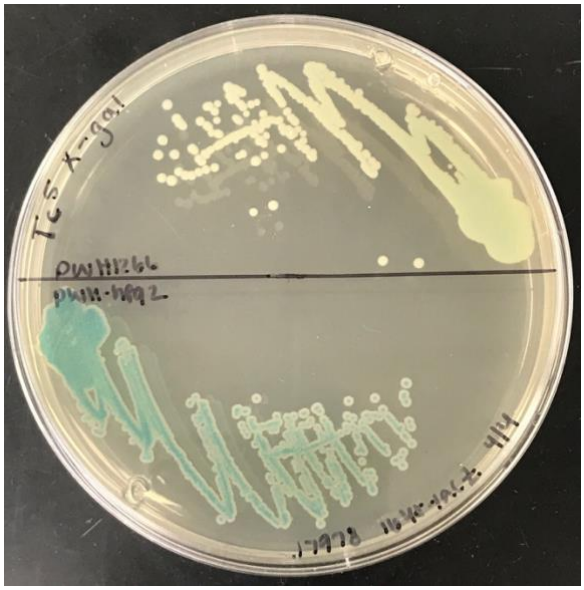

Figure 3 | Visualization of *ABUW_*1645 expression with empty vector control and *hfq*-2 overexpression vector. ATCC 17978 *1645-lacZ* transformants containing pWH1266 exhibited baseline *ABUW_1645* expression (top half of plate) while ATTC 17978 *1645-lacZ* transformants containing pWH1266 *hfq*-2 exhibited increased *ABUW_1645* expression (bottom half of plate).

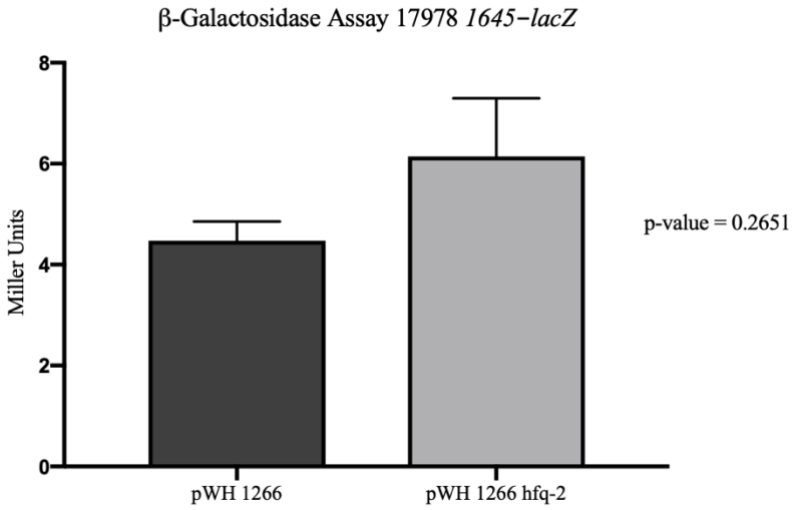

Figure 4 | *hfq* overexpression effects on *ABUW_*1645 expression. The averages from two independent experiments are shown. Using Welch's t-test, it was shown that there is no significant difference in β-galactosidase activity between the wild-type strain and the Hfq overexpression mutant when assayed from broth cultures.

Overexpression of *hfq* increases phenotypic switching. pWH1266, pWH1266 *hfq*-2, and pWH1266 *hfq*-9 were transformed into the low-switching opaque variant of AB5075, LSO 5075. Colonies transformed with either pWH1266 or pWH1266 *hfq*-9 remained opaque; however, colonies transformed with pWH1266 *hfq*-2 switched from opaque to translucent. Figures 5a and 5b show the different phenotypes of LSO 5075 transformed with the empty vector compared to the *hfq*-2 overexpression vector. These data indicate that *hfq* positively regulates phenotypic switching.

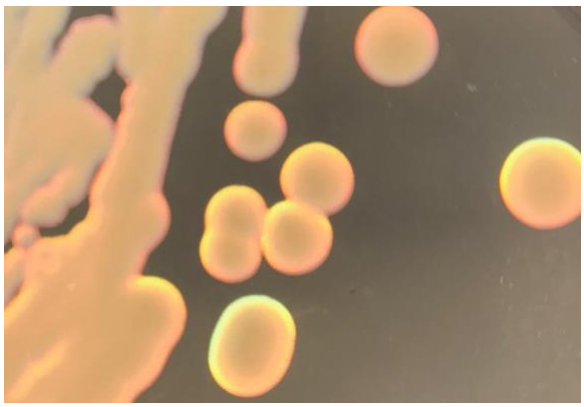

Figure 5a | Visualization of phenotypic switching with empty vector control. LSO 5075 transformed with empty vector control did not exhibit O to T switching.

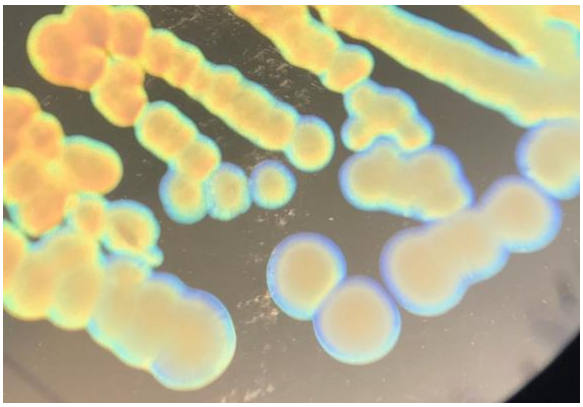

Figure 5b | Visualization of phenotypic switching with *hfq*-2 overexpression vector. LSO 5075 transformed with pWH1266 *hfq*-2 exhibited O to T switching.

Mutation of both Hfq distal face and Hfq proximal face reversed *ABUW_1645* expression and phenotypic switching. The distal and proximal faces of Hfq facilitate the binding of Hfq to mRNA and sRNA, respectively. Overexpression of a mutated allele on each domain of Hfq was performed to assess the importance of both mRNA and sRNA binding in Hfq-mediated *ABUW_1645* expression and phenotypic switching. Site-directed mutagenesis was used to create an F25A amino acid change on the distal face of Hfq. An equivalent mutation was previously shown to be important for mRNA binding in *E. coli*.[6] Site-directed mutagenesis was also used to

create a Q8A amino acid change on the proximal face of Hfq. This mutation was previously shown to be important for sRNA binding in *E. coli*.[6] pWH1266 *hfq*-2 F25A and pWH1266 *hfq*-2 Q8A were transformed into the 17978 *1645-lacZ* reporter strain and plated on X-gal. Figure 6 shows that transformants containing either the *hfq* distal face mutation or the *hfq* proximal face mutation displayed the same white phenotype as empty vector transformants on X-gal. This result implies that both mRNA and sRNA binding is required for *hfq* to regulate *ABUW_1645* expression. pWH1266 *hfq*-2 F25A and pWH1266 *hfq*-2 Q8A were also transformed into LSO 5075. Figures 7a, 7b, 7c, and 7d demonstrate that colonies harboring the empty vector, the *hfq* distal face mutation, or the *hfq* proximal face mutation remained opaque, while colonies containing the *hfq-2* overexpression vector switched from opaque to translucent. This suggests that both mRNA and sRNA binding are also important for Hfq in mediating phenotypic switching.

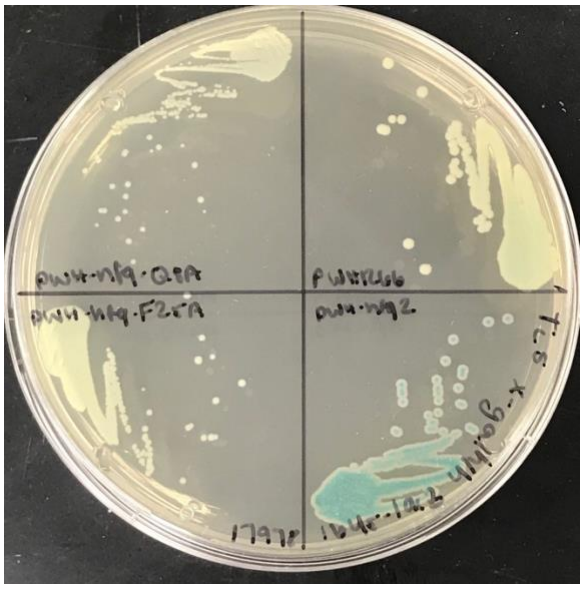

Figure 6 | Visualization of *ABUW*_1645 expression with *hfq*-2 F25A and *hfq*-2 Q8A overexpression vectors. ATCC 17978 *1645-lacZ* transformants containing pWH1266 exhibited baseline *ABUW_1645* expression (top right of plate) while ATTC 17978 *1645-lacZ* transformants containing pWH1266 *hfq*-2 exhibited increased *ABUW_1645* expression (bottom right of plate). ATCC 17978 *1645-lacZ* transformants containing both pWH1266 *hfq*-2 F25A (bottom left of plate) and pWH1266 *hfq*-2 Q8A (top left of plate) exhibited baseline *ABUW_1645* expression.

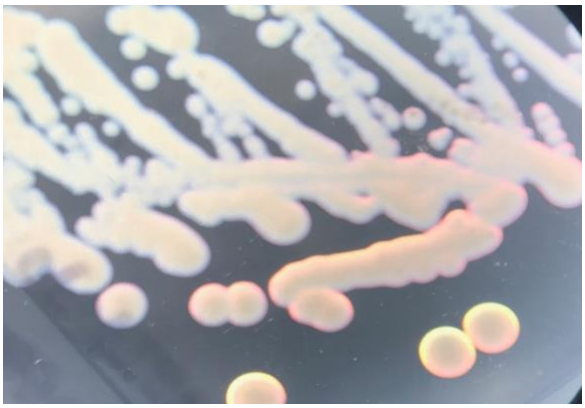

Figure 7a | Visualization of phenotypic switching with empty vector control. LSO 5075 transformed with pWH1266 did not exhibit O to T switching.

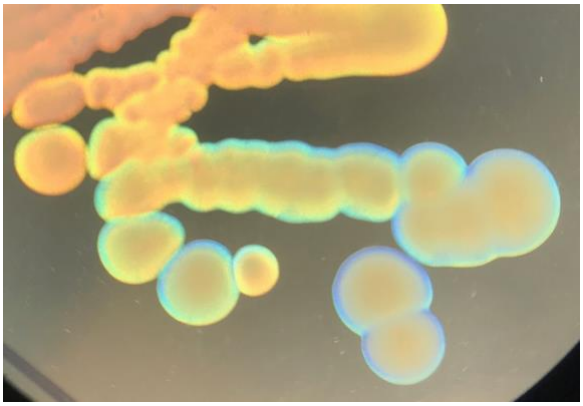

Figure 7b | Visualization of phenotypic switching with *hfq*-2 overexpression vector. LSO 5075 transformed with pWH1266 hfq-2 exhibited O to T switching.

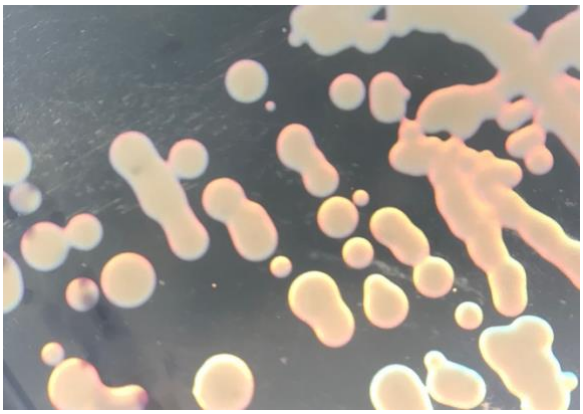

Figure 7c | Visualization of phenotypic switching with *hfq*-2 F25A overexpression vector. LSO 5075 transformed with pWH1266 *hfq*-2 F25A did not exhibit O to T switching.

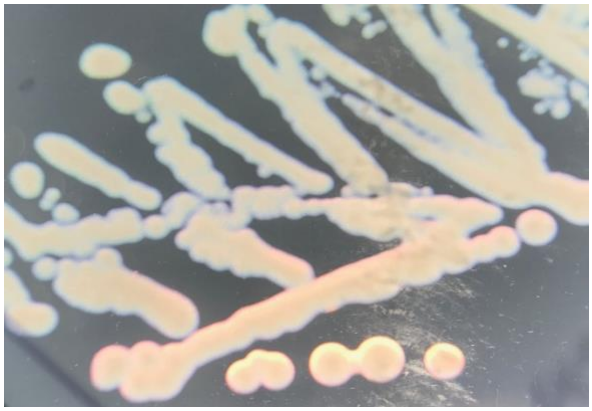

Figure 7d | Visualization of phenotypic switching with *hfq*-2 Q8A overexpression vector. LSO 5075 transformed with pWH1266 *hfq*-2 Q8A did not exhibit O to T switching.

Discussion

This paper reports the role of Hfq in regulating *ABUW_1645* expression and phenotypic switching in *Acinetobacter baumannii*. *A. baumannii* was shown to interconvert between two phenotypes, virulent opaque (O) and avirulent translucent (T).[7,29] The TetR transcriptional regulator *ABUW_1645* demonstrated a 60-fold increase in the T variant,[7] and previously, Hfq has been shown to positively regulate O to T switching. The purpose of this study was to analyze the role of Hfq in *ABUW_1645* expression, and to clarify the role of the multiple binding domains of Hfq in regulating *ABUW_1645* expression and phenotypic switching.

hfq was overexpressed in the 17978 *1645-lacZ* reporter strain and the low switching opaque variant, LSO 5075. Overexpression of *hfq* in the reporter strain caused an increase in *ABUW_1645* expression when visualized on X-gal plates. However, β-galactosidase assays from liquid culture did not show a significant difference in *ABUW_1645* expression. These data indicate that the cellular environment is important to consider when examining the effects of Hfq on *ABUW_1645* expression. It is unclear why assays from liquid culture did not demonstrate the same effects in expression as shown on plates. One plausible explanation is the increased cell density seen on plates. Another explanation is that the cultures were not assayed under growth conditions that exemplified the greatest possible expression. Still, the increased expression of *ABUW_1645* visualized on X-gal plates establishes the importance of Hfq in regulating *ABUW_1645* expression. Overexpression of *hfq* in LSO 5075 caused O to T switching. This confirms previous experiments showing that *hfq* positively regulates O to T switching.

Genomic DNA from an AB5075 *hfq::Tc* deletion mutant was transformed into the 17978 *1645-lacZ* reporter strain. β-galactosidase assays were performed to measure the relative expression of *ABUW_1645* in *hfq::Tc* deletion mutants. No significant difference in expression was seen in deletion mutants compared to the wild-type reporter strain. This result implies that Hfq is not required to reach baseline levels of *ABUW_1645* expression. Prior experiments have shown that *hfq::Tc* deletion mutants still switch from O to T, although the frequency is greatly reduced. This implies that, although Hfq positively regulates O to T switching, there may be other mechanisms governing the switch. It is possible that these same mechanisms also regulate the expression of *ABUW_1645*, which is why expression was still evident in the *hfq::Tc* deletion mutant. Future research could include analyzing what causes increased *ABUW_1645* expression and O to T switching in the absence of Hfq.

Site-directed mutagenesis of the Hfq distal face and proximal face was used to assess the importance of mRNA and sRNA binding for *ABUW_1645* expression and phenotypic switching. Hfq harboring the distal face mutation, F25A, was overexpressed in the 17978 *1645-lacZ* reporter strain and LSO 5075. Overexpression in the reporter strain yielded equal amounts of blue color in empty vector control and distal face mutation colonies when plated on X-gal. This shows that Hfq possessing the distal face mutation does not increase *ABUW_1645* expression. Overexpression of *hfq*-2 F25A in LSO 5075 did not induce O to T switching. This demonstrates that Hfq containing the distal face mutation also lost its activity in regards to regulating phenotypic switching. These experiments were repeated for Hfq harboring the proximal face mutation, Q8A, and the same results were shown. From these results, we see that both mRNA and sRNA binding are essential for Hfq to positively regulate *ABUW_1645* expression and

phenotypic switching. Understanding how Hfq regulates these processes gives insight into new treatment options for *A. baumannii* infections. The findings from this study suggest that targeting Hfq could effectively secure *A. baumannii* in the T state, which would decrease antibiotic resistance and reduce infection.

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