The Characterization of Fusidic Acid-selected *fusA* Mutants of *faiR* Deletion Mutants of *Staphylococcus aureus*

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Abstract

This project analyzed a gene (faiR) in Staphylococcus aureus, which is thought to be a regulator within an efflux pump operon that had been previously found in the laboratory. The characterization focused primarily on fusidic acid resistance expression. Initially fusidic acid–resistant mutants were selected from both a parent strain and a mutant missing faiR culture that had the gene of interest inactivated. The data suggests that faiR and perhaps the faiRAB operon, has no impact on fusidic acid resistance expression. Furthermore, screening for the effects of faiR deletion on susceptibility to other antibiotics demonstrated that this gene and possibly the faiRAB operon do not play a role in intrinsic antimicrobial resistance in S. aureus.

Keywords: *Staphylococcus aureus*, fusidic acid, efflux pump

Introduction

Staphylococcus aureus is a Gram-positive human opportunistic pathogen that harbors numerous virulence factors and can readily acquire horizontally-transmitted genes that allows the organism to impede the effects of antibiotics (Lowy 2003). One of the more-well known examples of antibiotic-resistant S. aureus is methicillin-resistant S. aureus (MRSA) (Lowy 2003) and in 2011, MRSA led to 80,000 infections and to 11,000 deaths within the United States (Center for Disease Control 2003).

Fusidic acid is a steroid antibiotic that was clinically introduced in the 1960s to treat S. aureus infections that is currently used widely throughout Europe and soon to be licensed for use in the United States (Moellering et al. 2011), (Howden and Grayson 2006). Fusidic acid inhibits protein synthesis by binding to the ternary complex of elongation factor G (EF-G), guanosine diphosphate (GDP), and the ribosome (Tanaka et al. 1968), (Cundliffe 1972). Therefore, fusidic acid causes the ribosome to stall on the mRNA strand and inhibits translation (Collignon and Turnidge 1999).

Resistance to fusidic acid by S. aureus is mediated by two distinct mechanisms. The first is through mutations that occur within the gene that codes for EF-G, fusA, and the second is due to the acquisition of fusB or fusB homologues, which have been given names such as fusC and fusD (Nagaev et al. 2001), (O'Brien et al. 2002), (O'Neill et al. 2007). These mutations and acquired genes are thought to lead to a reduction in the binding affinity between EF-G and fusidic acid and as a result negate the effects of the antibiotic (Nagaev et al. 2001). fusA mutants of S. aureus can be easily isolated in the laboratory from S. aureus populations following fusidic acid selection, as seen in this experiment.

Our laboratory previously discovered two genes that were induced by growth in the presence of fusidic acid (Delgado et al. 2008)and are here named as fusidic acid induced A and B (faiA and faiB). Both genes are thought to code for a bipartite multidrug efflux pump. Efflux pumps are important to bacterial survival in the presence of actively efflux antibiotics as the

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outside of the cell (Van Bambeke et al. 2000). faiA encodes a single membrane spanning protein of 215 amino acid bases that is a member of the resistance-nodulation-division (RND) family of transport proteins (Lomovskaya and Lewis 1992). faiB encodes a protein that is a homologue to the S. aureus major facilitator superfamily (MFS) proteins LmrS and MdeA, which are thought to efflux fusidic acid (Floyd et al. 2010), (Huang et al. 2004). Upstream of faiA lies the divergently encoded tetR homologue, faiR, that we hypothesize that FaiR controls the transcription of faiAB.

In order to have adaptable survival to an unstable environment with variables constantly changing and shifting, bacteria have adaptive responses triggered by regulatory proteins that, in response to environmental signals, altar transcription (Ramos et al. 2005). One large family of these regulators is TetR, known to be a regulator of the tet genes, which confer resistance to tetracycline (Ramos et al. 2005). One known TetR homologue in S. aureus is QacR, which is responsible for the regulation of the multidrug efflux pump gene, qacA (Grkovic et al. 1998). Another known TetR in Staphylococcus aureus, which is associated with regulation of the ica locus, which is involved with the production of biofilm, is IcaR (Jefferson et al. 2003). Another S. aureus TetR homologue, FaiR is hypothesized to be the regulator of its adjacent genes faiAB, which codes for a putative antimicrobial efflux pump.

In this project we will determine if the deletion of faiR will affect the level of fusidic acid resistance expressed by a fusidic acid-selected fusA mutant of strain JE2ΔfaiR previously constructed in our laboratory. We hypothesized that if faiR acts as a negative regulator of faiAB expression, and faiAB supports fusidic acid resistance, that the JE2ΔfaiR-fusA mutants would express a higher level of fusidic acid resistance, compared to JE2fusA mutants.

Methods

S. aureus strain JE2 (Fey et al. 2013) (USA300) is a community-associated MRSA strain, cured of all plasmids and was utilized for this study. We first selected for JE2 and JE2ΔfaiR fusidic acid-resistant fusA mutants. To do so we started by making 2 mL overnight cultures of both strains. We then prepared Muller Hinton agar (MHA) plates which contained 2 µg/ml fusidic acid and inoculated these plates with 100 µL of undiluted overnight culture and incubated these plates overnight at 37°C. We then picked 3 isolated colonies for each strain, JE2 and JE2ΔfaiR. These colonies chosen were then passaged twice on drug free MHA and incubated overnight at 37°C. We then used minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) techniques to determine the levels of resistance/susceptibility to fusidic acid expressed by JE2 and JE2□faiR, and the fusidic acid-selected JE2fusA and JE2□faiR-fusA mutants. To do this we first created overnight cultures of each strain. We then diluted JE2, JE2ΔfaiR, and the fusidic acid-selected JE2fusA and JE2ΔfaiR-fusA mutants to an OD580nm of 0.01. These diluted cultures were then put in test tubes with concentrations of fusidic acid from 0 µg/mL -1024 mg/ml in successive two-fold changes in concentration. The test tubes were then incubated statically overnight at 37°C. On the following day the MICs were determined and the MIC tube and tubes containing higher fusidic acid concentrations were plated onto drug free MHA and grown overnight at 37°C. The MBC was the concentration of fusidic acid where no growth was observed on the MHA plate following overnight growth

We then used Kirby Bauer methods to test for any possible changes in susceptibility/resistance to antibiotics, including fusidic acid. To do this, 2 mL MHB overnight cultures of JE2, JE2ΔfaiR, and the fusidic acid-selected JE2fusA, and JE2ΔfaiR-fusA mutants were prepared. These cultures were then diluted to an OD580nm of 0.01. The diluted cultures were then plated onto large petri dish plates of MHA. Arrangements of antimicrobial assay disks were placed evenly and the plates were then incubated overnight at 37°C and the zones of growth inhibition were determined.

Table 1 - This table shows both the MIC and MBC results for resistance/susceptibility to fusidic acid.

Strain	MIC	MBC
JE2	2 µg/mL	8 µg/mL
JE2-FA-R-1	128 µg/mL	1024 µg/mL
JE2-FA-R-2	128 µg/mL	1024 µg/mL
JE2-FA-R-3	128 µg/mL	1024 µg/mL
JE2Δ*faiR*	2 µg/mL	8 µg/mL
JE2Δ*faiR*-FA-R-1	128 µg/mL	1024 µg/mL
JE2Δ*faiR*-FA-R-2	128 µg/mL	1024 µg/mL
JE2Δ*faiR*-FA-R-3	128 µg/mL	1024 µg/mL

Table 2 – This table shows the Kirby Bauer results.

Strain	SPT	LEV	TGC	RD	CAZ	FD	VA	TE	C
JE2	I	R	S	S	R	S	S	S	S
JE2-FA-R-1	I	R	S	S	R	R	S	S	S
JE2-FA-R-2	I	R	S	S	R	R	S	S	S
JE2-FA-R-3	I	R	S	S	R	R	S	S	S
JE2ΔfaiR	I	R	S	S	R	S	S	S	S
JE2ΔfaiR-FA-R-1	I	R	S	S	R	R	S	S	S
JE2ΔfaiR-FA-R-2	I	R	S	S	R	R	S	S	S
JE2ΔfaiR-FA-R-3	I	R	S	S	R	R	S	S	S

Results

Within this experiment, fusidic acid-resistant mutants of JE2 and JE2ΔfaiR were successfully isolated with 2 µg/mL fusidic acid selection process. This can be confirmed using the MIC and MBC data, which showed that each of the fusidic acid selected strains had an MIC of 128 µg/mL and an MBC that grew over the highest fusidic acid concentration, which was 1024 µg/mL (Table 1).

The MIC and MBC data demonstrates that faiR deletion does not have a dramatic effect on the level of fusidic acid resistance expressed by JE2 and JE2ΔfaiR and their fusidic acid-selected fusA mutants. There were also no significant change differences amongst the strains investigated in the zones of inhibition observed in the Kirby Bauer data (Table 2).

Discussion

S. aureus fusidic acid resistance has been well described in the literature. Since JE2 and JE2ΔfaiR are derived from a strain that has been cured of all plasmids, the fusidic acid-resistant mutants of these strains are most likely a result of fusA mutations (Nagaev et al. 2001), (Chopra 1976) and not fusB acquisition (O'Brien et al. 2002). This is important because it can give possible insight as to the properties of the faiRAB operon not having an impact on the fusidic acid resistance. Clearly, from both the MIC and the MBC data given, the deletion of faiR does not have an impact on fusidic acid resistance levels or susceptibility to the antibiotics represented in the Kirby Bauer data. These results can lead to understanding more about the operon. It is possible as well that faiR does not regulate faiAB. Overall though, we would conclude that that FaiA and FaiB do not represent an antimicrobial efflux pump.

Nonetheless, because faiAB is induced by fusidic acid (Delgado et al. 2008) and because fusidic acid is a steroid antibiotic, it is likely that the inducer for the operon in nature is a steroid. It also remains possible the FaiR binds steroids. The conclusion of this work suggests that faiR and possibly faiAB (if faiR controls this locus) play no role in the intrinsic antimicrobial resistance mechanism in S. aureus.

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