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The role of RND drug efflux pump protein in *Staphylococcus aureus*

Abstract

The ubiquitous Gram-positive bacterium, *Staphylococcus aureus* causes a wide range of diseases in humans, including the fatal infections pneumonia and bacteremia. The rise of antibiotic resistance in *S. aureus* particularly, the community-acquired methicillin resistant *S. aureus* (CA-MRSA) is an ever-increasing concern for global health. Multidrug resistance is achieved through multiple mechanisms one of which is the use of efflux pump proteins. The RND efflux pump protein in *S. aureus* is one such contributor to antibiotic resistance. It is also known from Gram-negative bacteria, that this family of efflux pump can export various types virulence factors and quorum sensing peptides. *S. aureus* secretes several types of proteinaceous toxins (TSSTs, hemolysins, enterotoxins etc.,) and Agr quorum sensing peptides.

The Vediyappan lab has created a deleted mutant strain of *S. aureus* that lacks the RND efflux pump protein. In this project, we aim to characterize the differences in secreted protein profiles among the parent, mutant, and complemented strains. We were able to compare the three strains in both their late exponential and stationary growth phases, which allowed us to determine the role of this efflux pump. Since we noticed differences in total protein profiles, we next compared the strains' secreted proteolytic activity on a SDS-PAGE gel using casein as substrate. We observed reduced level of secreted caseinolytic activity in mutant at late exponential phase. In addition, we analyzed the strains' susceptibility to hydrogen peroxide by finding their minimum inhibitory concentrations (MIC). The mutant strain appears to lack staphyloxanthin, a carotenoid that is known to protect *S. aureus* from oxidative stress. We have found that the mutant strain is more susceptible to hydrogen peroxide compared to the parent and complemented strains suggesting that it is defective in the expression of staphyloxanthin. We plan to identify the differences in secreted proteins among the three strains by mass spectrometry and confirm the results. All these results indicate the important role of this efflux pump in *S. aureus* virulence and may need to verify in animal models.