5. SUMMARY

Staphylococcus aureus is a causative agent of multiple infections including bacteremia, infective endocarditis, osteomyelitis, septic arthritis, prosthetic device infections, and pneumonia. *S. aureus* secretes several virulence factors, which include surface proteins, exotoxins, invasive enzymes, *etc.*, for adhesion, invasion, and modulation of the host immune system. Regulation of these virulence factors includes complex multifactorial mechanisms mediated by two component systems (TCS), transcriptional regulators, sigma factors, RNA binding proteins and sRNAs as key regulatory molecules. This work aims at unraveling the regulatory function of sRNA SprX. SprX is a key virulence regulator and was previously reported to be involved in the enhancement of pathogenicity by modulating the hemolysin (hldδ) and clumping factor B (*clfB*). This study describes the regulation by SprX, of expression of multiple autolysins, which play an essential role in cell wall metabolism and function as important virulence factors that facilitate adhesion, internalization, and immune evasion during *S. aureus* colonization and pathogenesis.

A differential proteomic approach using two-dimensional gel electrophoresis with liquid chromatography-mass spectrometry was employed for the identification of potential targets of SprX using an overexpression strain. A significant down-regulation of IsaA proteins was observed under *sprX* overexpression posing IsaA as a potential new target of SprX. IsaA is a profoundly antigenic surface virulence protein with lytic transglycosylase activity that negatively regulates the clumping of cells. Knockdown of SprX resulted in 0.1- fold decrease in *isaA* transcripts in qRT-PCRs. Yet on the other hand overexpression of SprX did not show converse influence, but resulted in the marginal decrease in *isaA* transcripts. This could be a resultant effect of negative regulation of *isaA* by RNAIII, which in turn is positively regulated by SprX. *In silico* analysis by IntaRNA indicated base pair interaction between 3’ end of *isaA* mRNA and SprX that was further confirmed using gel mobility shift assay which revealed a weak complex formation. This interaction at the 3' coding region of *isaA* mRNA might influence the degradation and stability of *isaA* mRNA.

The effect of altered levels of SprX on IsaA and additional autolysins was further investigated in cell wall autolysis assay. A significant reduction of major autolysin AtlA and its processed intermediates was observed in the *sprX* knockdown strain. The implications and influence of reduced levels of autolysins on cell aggregation phenotype

was further studied using SEM and phase contrast microscopy, as well as Triton X-100 lysis assay. High degree of cell aggregation and increased resistance to Triton X-100 induced lysis was demonstrated by *sprX* knockdown strain.

The influence of SprX, observed simultaneously on multiple autolysins, implicated the involvement of SprX in the regulation a common regulator of autolysins. WalR, a common regulator of autolysins was freshly identified as a new target of SprX by IntaRNA. WalR is the response regulator protein of WalKR TCS and is involved in the regulation of cell wall metabolism by positively regulating the expression of autolysins IsaA, AtlA, and LytM in *S. aureus*. A 0.1 to 0.2- fold reduction in *isaA, atlA* and *lytM* transcripts in qRT-PCRs corroborated the above notion of WalR as a target of SprX. Additionally, the strong interaction of SprX with 5' coding region of *walR* mRNA was demonstrated using gel mobility shift assay. The specificity of SprX-*walR* mRNA interaction was validated by unlabeled (cold) SprX and nonspecific competitor sRNA PhrD from *P. aeruginosa*.

Autolysins play an essential role in peptidoglycan dynamics and pathogenesis. SprX positively regulates the autolysin regulator WalR by direct base pair interaction with mRNA of WalR that directly influence expression of downstream genes *atlA, isaA and lytM*. Each of these genes is additionally and differentially regulated by *agr* through RNAIII, SarA, Rot, and SrrA. These multiple layers of regulation enable the fine tuning of the expression of several virulence determinants where in SprX adds another layer of regulation by coordinating the expression of genes involved in cell wall metabolism and virulence. The versatile adaptability of *S. aureus* mediated by multiple virulence determinants enhances its survival through distinct stages of infection. In essence, the sRNA SprX regulates cell wall hydrolyzing autolysins, in addition to its involvement in the expression of other virulence factors of *S. aureus*. The finding presented in this work signifies the importance of SprX in fine tuning of pathogenicity regulation in *S. aureus*.