2 PRESENT STUDY

Small regulatory RNAs (sRNAs) have emerged as the central regulators of bacterial pathogenesis affecting the expression of many virulence factors which include hemolysins, enterotoxin, leukocidin, coagulase, hyaluronate lyase, staphylokinase, proteases, and surface proteins that help in recognizing adhesive matrix molecules to promote colonization and invasion. A number of sRNAs have been identified in *S. aureus* but only few have been studied for their role in bacterial life cycle and their involvement in regulating pathogenesis.

Small RNAs RsaA, RsaC, RsaD, and RsaE from the group of 11 novel small RNAs (RsaA-K) have shown significant impact on virulence by modulating the expression levels of target mRNAs through various networks. The small RNA RsaF of *S. aureus* Newman was characterized for the first time in this study for its functional mechanism and significance, in the involvement of regulation of pathogenicity genes *hysA* and *splD* in the clinical isolate *S. aureus* Newman.

The present study is focused on the functional characterization of the sRNA RsaF, a highly conserved and σ^B dependent sRNA in *S. aureus*. *In silico* analysis by various programs like TargetRNA, IntaRNA, RNAPredator, and CopraRNA identified potential virulence factors hyaluronate lyase (HysA) and serine protease like protein (SplD) as the targets of RsaF. Hyaluronate lyase (HL), encoded by *hysA*, is a potent virulence factor and acts as a potential nutrient scavenger by degrading hyaluronic acid (HA), a major component of the extracellular matrix of the human and animal connective tissues. SplD acts on several olfactory receptors, which are trans-membrane proteins expressed in nares, the primary colonization niche of *S. aureus*.

This study investigates the involvement of sRNA RsaF, in the regulation of pathogenicity genes *hysA* and *splD*, by employing *S. aureus* strains with disruption and overexpression of *rsaF*.

OBJECTIVES OF THE STUDY

1. Analysis of RsaF expression in *S. aureus* strain with overexpression and disruption of *rsaF*.
2. Analysis of the influence of altered RsaF levels on the transcription of the selected targets and associated physiology.
3. Study of specific interactions of RsaF with the selected target mRNAs.
4. Assessment of the stability of target mRNA under the influence of differentially expressed RsaF.

S. aureus strains with overexpression and disruption of *rsaF* were generated and the effect of altered levels of RsaF on the *hysA* and *splD* was analyzed by transcriptional analyses , physiological assays, zymographic analysis, and RNA stability. Interaction of *hysA/splD* mRNAs with RsaF was studied by *in vitro* RNA-RNA interactions. The findings elucidated a positive regulatory role for small RNA RsaF that modulates the expression of the secreted virulence factors, HysA and SplD.