ABSTRACT

The pathogenesis of *Staphylococcus aureus*, from local infections to systemic dissemination, is mediated by a battery of virulence factors, which include secreted toxins, capsule, extra cellular enzymes and several surface proteins that promote host adhesion and colonization. The expression of these virulence factors is regulated by intricate mechanisms, which include transcriptional factors, two component systems, regulatory proteins and small RNAs (sRNAs) as key regulatory molecules. Several ncRNAs have been reported in *S. aureus*, out of which only a few, *viz.* RsaA, RsaC, RsaD, RsaE, SprC, SprD, SprX, SSR42, ArtR, and Teg41 have been functionally characterized for their role in regulation of virulence and general metabolism.

The sRNA RsaF, from the group of 11 novel small RNAs (RsaA-K), was investigated for its putative targets using *in silico* programs TargetRNA, RNAPredator, CopraRNA and IntaRNA. Pathogenicity genes hyaluronate lyase (HysA) and serine protease like protein D (SplD) were identified as targets of RsaF and were analyzed for its regulation by employing *S. aureus* strains with overexpression and disruption of *rsaF*. The subsequent observations on the altered regulation of target genes by RsaF in real-time PCRs and physiological assays have elucidated the functional significance of this sRNA.

Disruption of RsaF resulted in down regulation of *hysA* transcripts by 0.2 to 0.0002 fold, and hyaluronate lyase activity by 0.2-0.1 fold and in turn, increased biofilm formation when supplemented with hyaluronic acid, which corroborates with the previous reports (Ibberson *et al.*, 2016) of increased biofilm formation observed in *hysA* mutants. Conversely, overexpression of *rsaF* resulted in a 2 to 4 fold increase in *hysA* mRNA levels and hyaluronidase activity. HysA has been characterized as a spreading factor of *S. aureus* and sRNA RsaF adds another layer of regulation to HysA expression. *S. aureus* strain with disruption of *rsaF* exhibited down regulation of *splD* transcripts by 0.8 to 0.005 fold, and reduced activity of multiple proteases in zymography.

Both *hysA* and *splD* mRNAs demonstrated an increased stability in $RsaF^+$ strains. *In silico* RNA-RNA interaction indicated a direct base pairing of RsaF with *hysA* and *splD* mRNAs, which was established in electrophoretic mobility shift assays. Interaction with RsaF imparted stability to *hysA/splD* mRNAs, possibly by the formation mRNA-sRNA duplexes, thus positively influencing their expression.

Overall, this work characterizes the functional significance of sRNA RsaF of *S. aureus* and demonstrates a positive regulatory role of RsaF in the expression of the virulence factors HysA and SplD.