

ABSTRACT

One of the rising interest in bacterial RNA research is due to the enormous discovery of non-protein-coding RNAs (npcRNAs) with diverse regulatory roles in fundamental cellular processes and adaptive responses to environmental changes/ stress conditions, thus affecting virulence. This work reports two experimental analyses of small npcRNA expression patterns in pathogenic bacteria i) *Staphylococcus aureus* that is responsible for many human diseases ranging from minor skin diseases to life-threatening infections and ii) Gram-negative *Shigella flexneri* which is the leading bacterial causes of diarrhea and dysentery in humans. To elucidate the role of npcRNAs in *S. aureus* adaptation to environmental changes and stress conditions, the expression profile of 8 *S. aureus* npcRNAs were analyzed under various stress conditions by Northern blot. The expression of many pathogenicity island-encoded *S. aureus* npcRNAs were associated with exponential growth phase and stress conditions, indicating possible function in virulence. Moreover, *S. aureus* small-colony variant (SCV)-phenotype specific Sau-66 was highly expressed during cold shock treatment. RNA sequences and secondary structures of these npcRNAs were highly conserved among strains of *S. aureus* showing its importance in *S. aureus* regulatory networks. *In-silico* target mRNA predictions showed Sau-02 and Sau-63 could bind to ribosomal binding site (RBS) of their respective target mRNAs and prevent the formation of ribosomal initiation complexes. Sau-31 and Sau-66 target the open reading frame (ORF) of their target mRNAs in an antisense mechanism. Despite the blooming data on bacterial npcRNAs, no studies have been reported on *S. flexneri* npcRNAs. Thus, comparative analysis of known Gram-negative bacterial npcRNAs was performed against *S. flexneri* genome. Totally, 142 of the Gram-negative bacterial npcRNAs have conserved in *S. flexneri* with remarkable similarity at homologous regions. Out of these

142 predicted npcRNA candidates, 5 were selected and their expression was verified by Northern blot analyses. Interestingly, all npcRNAs showed expression and with stage-specific regulation during *S. flexneri* growth. Both sequences and secondary structures of these npcRNAs were conserved among Gram-negative bacteria. *In-silico* target predictions revealed that most of the npcRNAs negatively regulate its target mRNA by binding either to the RBS or in an antisense mechanism. Positive regulation of target mRNA was observed for SflR-8 which binds to the 5' - UTR of *sun* mRNA to unmask the RBS. Collectively, this study reveals the possible role of npcRNAs in *S. aureus* adaptation process in response to environmental changes or stress conditions and possible 142 npcRNA candidates in *S. flexneri*. This study also reveals the probable mechanisms by which these npcRNAs control its target gene expression.