2 PRESENT STUDY

Small regulatory RNAs are strongly emerging as regulators of bacterial pathogenesis and virulence factors in a number of organisms. *Pseudomonas aeruginosa* causes acute and chronic infections in individuals with compromised immunity and in mechanically ventilated patients. It is one of the major contributors of pathogenesis in cystic fibrosis lungs. Its ubiquitous presence, genetic flexibility to survive in varied environments, inherent and acquired resistance to antibiotics, and multiple virulence factors make it a challenging pathogen. The advent of techniques like RNA-seq and tiling microarrays has enabled discovery of a burgeoning number of sRNAs in *P. aeruginosa*. However, only a few of these sRNAs have been studied for their role in bacterial life cycle and their involvement in regulating pathogenesis of this microbe. Therefore, the present study aims at the *in silico* analysis of the reported but uncharacterized sRNAs in *Pseudomonas* for prediction of pathogenicity related mRNA targets, selection of a few of such sRNAs, and characterization of their roles in regulating the predicted pathogenicity factors under the influence of altered levels of sRNAs.

Objectives of the study

1. Detailed bioinformatics analysis of the selected ncRNAs to determine their secondary structure and putative mRNA targets
2. Construction of overexpression and disruption strains of the above ncRNAs
3. Analysis of influence of overexpression and disruption of these ncRNAs on expression of target genes as obtained from bioinformatic analysis
4. Study of specific ncRNA/mRNA target interactions

The present study is aimed at the functional characterization of two selected sRNAs namely, PhrD and P18 for their involvement in regulation of pathogenicity of *P. aeruginosa*. The sRNAs were bioinformatically analysed to determine their putative mRNA targets. RhlR, the quorum sensing regulator of virulence genes was selected as putative target of PhrD, while alkaline protease secretion protein E and protease IV, major virulence factors involved in establishing acute infections, served as the targets of P18. Effect of the altered levels of these sRNAs on the selected targets was analyzed by means of transcriptional assays, *in vivo* RNA interaction studies on reporter gene fusions and physiological assays.