CONCLUSIONS

- Bioinformatics analyses of the small RNA PhrD indicated the quorum sensing regulator RhlR as a potential target whereas P18 sRNA appeared to regulate the alkaline protease secretion protein E and protease IV mRNAs.

- *phrD* over expression influenced *rhlR* expression positively by 6-fold. Disruption of the sRNA reduced the *rhlR* levels to 0.2-fold which were restored by complementation with *phrD*.

- *rhlR::lacZ* reporter fusions and transcriptional assays indicated increased expression of *RhlR* by ~ 2-fold in WT as compared to the *phrD* disruption background in LB and phosphate deficient condition. This increase was however masked in N-limited condition owing to the influence of ppGpp on *rhlR* expression under this condition.

- The increase in *β*-galactosidase activity of *rhlR::lacZ* fusion in the presence of PhrD was seen when an intact interaction region was present on *RhlR*, and substitution of this region with a scrambled interaction sequence did not facilitate this increase in expression by PhrD.

- The exclusive effect of PhrD on RhlR regulation was also demonstrated in the heterologous host *E. coli* in the presence of PhrD over expression plasmid. This result proved that this interaction does not require any *P. aeruginosa* specific proteins. However, the *Pseudomonas* background facilitated better expression of *rhlR* than in *E. coli*, probably due to better recognition of P3-*rhlR* promoter by *Pseudomonas* sigma factors or involvement of additional *Pseudomonas* proteins.

- Over expression of PhrD increased the production of RhlR-regulated biosurfactant rhamnolipid and pyocyanin pigment by 2.5 and 4-fold respectively.

- Proteomic profiling of the extracellular proteins of altered strains of PhrD indicated several proteins to be differentially expressed.

- Another sRNA P18 when disrupted reduced the levels of protease IV and alkaline protease in transcriptional and phenotypic assays. However the over expression of P18 also negatively influenced the target gene expression. LasR, the transcriptional regulator of several proteases, showed extensive base pairing with P18 in an *in silico* analysis. Thus, the complex effect of P18 on the expression of the proteases could be mediated via LasR transcriptional regulator and downstream effectors.