ABSTRACT

Pseudomonas aeruginosa is equipped with a battery of virulence factors that allow it to survive in host and cause severe infections. The expression of these virulence factors is regulated by several global regulators, two component systems and small regulatory non-coding RNAs (ncRNAs) which are engaged in a complex cross-talk. Several ncRNAs have been reported in *P. aeruginosa* out of which only a few, *viz.* RsmY/Z, PrrF1/2, CrcZ, PhrS, have been characterized for their role in general metabolism and regulation of pathogenicity.

A number of reported but uncharacterized ncRNAs in *P. aeruginosa* strain PAO1 were analyzed for their putative targets using *in silico* programs. Out of these, two ncRNAs *viz.* PhrD and P18 were selected for further studies as they displayed assayable pathogenicity factors and major transcriptional regulators as potential targets. Selected sRNAs were cloned and over expressed from an *E. coli-Pseudomonas* shuttle vector. Further, their disruption constructs were made by insertion of a gentamicin resistance gene followed by replacement of chromosomal copy by homologous recombination. These constructs were confirmed by PCRs and expression studies.

The Hfq dependent sRNA PhrD expressed in Luria broth, phosphate limited and nitrogen limited conditions. PhrD positively regulated the expression of *rhlR*, a transcriptional regulator of quorum sensing in *Pseudomonas* in LB and phosphate limited conditions but did not have a marked effect under N-limited conditions as observed in transcriptional analysis and *β*-galactosidase assays. This could be due to the marked influence of ppGpp and stringent response in the increased expression of *rhlR* that masks the PhrD effect. Over expression of PhrD increased the production of RhlR-regulated biosurfactant rhamnolipid and pyocyanin pigment by 2.5 and 4-fold respectively. Direct and sequence specific interaction between PhrD and *RhlR* was confirmed by *rhlR*::*lacZ* reporter fusions in PAO1 and in the heterologous host *E. coli*, in the presence of PhrD over expression plasmids. Scrambling of sequences in the predicted PhrD interaction region of *RhlR* abolished the positive effect of PhrD, thus proving the involvement of the region in the afore said regulation of *RhlR*.

The sRNA P18 was predicted to base pair with alkaline protease secretion protein E and protease IV mRNAs. Disruption of P18 lead to a significant decrease in the secretion protein E and protease IV levels as observed by transcriptional and phenotypic assays, which was partially restored on P18 over expression. The disruption reduced the activities of alkaline protease and protease IV by 40% and 60% respectively. An *in silico* RNA-RNA interaction prediction showed extensive base pairing between P18 and *LasR* mRNA, which is a quorum sensing transcriptional regulator for the above studied proteases. The influence of P18 on the expression of alkaline protease and protease IV could be mediated via LasR.

Overall, this work establishes the positive influence of PhrD and P18 sRNAs in regulating the pathogenicity factors of *Pseudomonas* and their role in assisting this bacterium to tune in to the environmental and host induced stimuli.