ABSTRACT

Small non-protein coding RNAs (npcRNAs) have emerged as the key regulators of cellular pathways involved in bacterial pathophysiology. Most of the npcRNAs in Gram negative bacteria exhibit their functions mediated by Hfq, an RNA chaperon that facilitates the binding of npcRNAs to their target mRNAs. The main objective of this study was to identify novel npcRNAs related to the pathogenicity of *Proteus mirabilis*, Gram-negative bacterium linked to various infectious diseases in human causing mainly urinary tract infection (UTI). Four different approaches were adopted to identify the novel npcRNA candidates in *P. mirabilis* and studied the expression profile of some of these novel npcRNAs during various growth conditions. In the first approach, computational npcRNA prediction tool 'nocoRNAc' was employed to predict putative npcRNA candidates in *P. mirabilis*. A total of 683 putative npcRNA candidates were predicted by this tool from the whole genome of *P. mirabilis*. In the second approach, the sequences of known npcRNAs from *E. coli*, *S. typhimurium* and *S. typhi* were subjected to comparative genomic analysis against *P. mirabilis* HI4320 genome using the NCBI BLASTn tool, leading to the identification of 14 novel npcRNAs. Out of these 14 npcRNAs, the expression of 6 representative npcRNAs in various growth conditions was verified by northern blot and their regulatory function was predicted based on the role of their respective target mRNAs. In the third approach, total transcriptome of *P. mirabilis* was analyzed to find 140 possible npcRNA candidates that were not annotated in NCBI and Rfam databases. Expression of 8 representative npcRNAs in various growth conditions was validated by northern blot and their regulatory functions was predicted based on the role of their respective target mRNAs. In the fourth approach, a binding experiment between *in-vitro* purified *P. mirabilis* global RNA chaperone protein, Hfq and total RNA isolated from *P. mirabilis* was performed, and the bound transcriptome

was sequenced using high-throughput sequencing. A total of 114 more novel and Hfq bound npcRNA candidates were identified from the Hfq bound transcriptome. Expression of 7 representative Hfq bound npcRNAs in various growth condition was validated by northern blot and their regulatory function was predicted based on respective target mRNAs and their functions. The dynamic expression of these npcRNAs in various growth conditions suggested that they might be involved in the regulation of virulence and pathogenicity of *P. mirabilis*. All 114 Hfq bound npcRNAs were screened for conservation across different bacterial species and one npcRNA candidate hfqpm_nv50 was validated for its specificity by monoplex Polymerase Chain Reaction (PCR). The detection limit of hfqpm_nv50 amplification on agarose gel electrophoresis was determined as 0.649pg of genomic DNA/µl. The identification of novel npcRNAs and characterization of their predicted targets may contribute to better understanding of the molecular mechanisms underlying the pathogenicity of *P. mirabilis* and contribute to a new paradigm of antimicrobial therapy for *P. mirabilis*. The identified species specific Hfq bound npcRNA can be used as a biomarker to develop biosensors for rapid and accurate detection of *P. mirabilis* in clinical samples.