ABSTRACT

Small non protein-coding RNAs (npcRNAs) have emerged as the key regulators of cellular pathways involved in bacterial pathophysiology. The function of npcRNAs is mediated by Hfq, the RNA chaperon that facilitates the binding of npcRNAs to their target mRNAs. The main objective of this study was to identify novel npcRNAs involved in the virulence and pathogenicity of *Klebsiella pneumoniae*, a Gram-negative bacterium linked to various infectious diseases in human. Three different approaches were adopted to identify the novel npcRNA candidates from the genome of *K. pneumoniae* and to validitate their expression in three growth phases and five different stress conditions. In first approach, the sequences of known npcRNAs from *E. coli, S. typhimurium* and *S. typhi* were subjected to comparative genomic analysis against *K. pneumoniae* KCTC 2242 genome using the Blastn tool, leading to the identification of 72 novel *K. pneumoniae* npcRNAs. Out of these 72 npcRNAs, expression of five representative npcRNAs in three growth phases was verified by Northern blotting and their function was predicted based on the role of respective target mRNA. In second approach, 160 possible npcRNAs candidates lacking ORF with no hit in Rfam and unannotated in Blastn analysis were identified by transcriptome analysis of *K. pneumoniae*. Expression of five (5) representative npcRNAs in three growth phases was verified by Northern blotting and their functions was predicted based on the role of respective target mRNA. In third approach, a binding between *in-vitro* purified *K. pneumoniae* Hfq protein and total RNA isolated from *K. pneumoniae* cultivated in three growth phases and five different stress conditions was performed and the identity of Hfq bound RNAs was determined by Illumina sequencing. Twenty eight (28) novel npcRNAs were idetified through this approach. From these 28 novel npcRNAs, expression of eight (8) representative Hfq bound RNAs in three growth phases and five different stress condition was determined by Northern blotting and their function was predicted based on the predicted target mRNA. The dynamic expression of several island-encoded npcRNAs in three growth phases and

five different stress conditions suggested that these npcRNAs might be involved in the regulation of virulence and pathogenicity of *K. pneumoniae*. The identification of novel npRNAs and characterization of their predicted targets may contribute to better understanding of the molecular mechanisms underlying the pathogenicity of *K. pneumoniae*. It may also help in the design and development of highly sensitive biosensors for the diagnosis of diseases caused by *K. pneumoniae*.