TITLE: SIGMA FACTOR RPOE AND FUR COREGULATE THE EXPRESSION OF STRESS RESISTANCE GENES IN *KLEBSIELLA PNEUMONIAE*

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ABSTRACT:
Klebsiella pneumoniae is considered an opportunistic pathogen responsible for infections in the gastrointestinal, respiratory and genitourinary tracts. During the infection, the bacterial cell surface can undergoes alterations, because during this process the pathogens face a hostile environment that includes changes in the temperature, nutrients scarcity and the presence of antimicrobial agents. In these adverse conditions, regulatory proteins are recruited to active the expression of genes involved in the maintenance of the bacteria cell envelope integrity. Fur is among the most important bacterial transcriptional regulators and modulates the expression of innumerous virulence genes. Fur complexes with ferrous iron and blocks transcription by binding to specific DNA sequences, named Fur boxes, located at the promoter region of the target genes. On the other hand, Sigma factors are dissociable subunits of RNA polymerase that provide promoter recognition and transcription initiation in bacteria. The Sigma factor RpoE binds on specific –10 and –35 recognition sites upstream from the transcription start site and coordinates the expression of genes involved in bacterial survival under stressful conditions. This study aimed to identify genes coregulated by Fur and Sigma factor RpoE in *K. pneumoniae*. Bioinformatics analyzes were employed to identify putative RpoE-binding sites and Fur boxes on the promoter region of target genes. The *rpoE* and *fur* genes from *K. pneumoniae* were PCR amplified and cloned into bacterial expression vector, and the Fur and RpoE proteins were purified by affinity chromatography. Fur and RpoE proteins were then used on Gel Shift analysis to validate the putative binding sites. Quantitative Real Time PCR were performed to investigate the expression pattern of the target genes in *K. pneumoniae* cells submitted to stressful conditions. Putative Fur boxes and RpoE-binding sites were found on the promoter region of *lpp* and *htrA* genes. Gel Shift analysis confirmed the binding of Fur and RpoE on these binding sites. The expression of *lpp* and *htrA* genes were induced during iron deprivation and in *K. pneumoniae* cells submitted to stress conditions. The identification and characterization of genes that belong to both Fur and RpoE regulons will contribute to a better understanding of the role of these regulators in the *K. pneumoniae* pathogenicity.

Keywords: *Klebsiella pneumoniae*; Transcription Factors; Sigma Factor, Stress Physiological.
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