RyhB is involved in the Fur-regulated capsular polysaccharide biosynthesis and biofilm formation in *Klebsiella pneumoniae* CG43

Yi-Ming Hong, Chia Chi, Jing-Ciao Lin, Chien-Chen Wu, Ching-Ting Lin*

School of Chinese Medicine, China Medical University, Taichung, Taiwan. Republic of China

Backgrounds:
RyhB is a small non-coding RNA that controls gene expression at post-transcriptional level. In *Escherichia coli*, the *f*erric *u*ptake *r*epressor (Fur) represses the transcription of *ryhB* to mediate its downstream gene expression. However, biological role of RyhB in *Klebsiella pneumoniae* remains largely unknown. We have previously showed that Fur regulated the capsular polysaccharide (CPS) biosynthesis and iron acquisition in *K. pneumoniae* CG43. Besides, bioinformatic analysis revealed putative Fur and RcsAB binding sequences in the promoter region of *ryhB*. In this study, function and regulation of *ryhB* in *K. pneumoniae* CG43 was characterized.

Methods:
The effect of *fur*- or *rcsB*-deletion in *K. pneumoniae* CG43 on the promoter activity of *ryhB* was assessed by LacZ reporter assay. The direct binding of Fur or RcsB to P_{ryhB} was detected by Fur titration assay or electrophoretic mobility shift assay. Deletion of *ryhB* in *K. pneumoniae* CG43 wild type, Δ*fur*, and Δ*rcsB* strains were respectively constructed, and then the mutants were subjected to various phenotypic analyses. RyhB regulation on the expression of its target genes were analyzed by quantitative real-time PCR and two-dimensional SDS-PAGE.

Results:
Deletion of *fur* or *rcsB* in *K. pneumoniae* activated the promoter activity of *ryhB*. Direct binding of Fur or RcsB to P_{ryhB} could also be observed. Bacterial iron acquisition, CPS biosynthesis, biofilm forming activity, and resistance to acid or oxidative stress were affected by the deletion of *fur*. However, the increased CPS biosynthesis and decreased biofilm forming activity in the Δ*fur* strain were partially restored by the *ryhB*-deletion. Transcription of the biosynthesis and regulatory genes of CPS were analyzed in Δ*fur* and Δ*fur*Δ*ryhB* strains to clarify the regulatory role of *ryhB* on the CPS biosynthesis. Furthermore, comparative proteomic analysis between the Δ*fur* and Δ*fur*Δ*ryhB* strains was performed to identify the RyhB-regulated genes in the Fur regulon.

Conclusion:

Both Fur and RcsB were showed to directly repress the transcription of *ryhB*. Besides iron acquisition and CPS biosynthesis, Fur also affected the bacterial biofilm forming activity and resistance to acid or oxidative stress in *K. pneumoniae*. RyhB was found to involve in the Fur-regulated CPS biosynthesis and biofilm formation.