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

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**Backgrounds:**
RyhB is a small non-coding RNA that controls gene expression at post-transcriptional level. In *Escherichia coli*, the ferric uptake repressor (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<sub>*ryhB*</sub> was detected by Fur titration assay or electrophoretic mobility shift assay. Deletion of *ryhB* in *K. pneumoniae* CG43 wild type, Δfur, and ΔrcsB 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<sub>*ryhB*</sub> 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 Δfur strain were partially restored by the *ryhB*-deletion. Transcription of the biosynthesis and regulatory genes of CPS were analyzed in Δfur and ΔfurΔryhB strains to clarify the regulatory role of *ryhB* on the CPS biosynthesis. Furthermore, comparative proteomic analysis between the Δfur and ΔfurΔryhB 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.