

IscR regulation of the capsular polysaccharide biosynthesis and iron-acquisition systems in *Klebsiella pneumoniae* CG43

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Klebsiella pneumoniae (Kp) is the predominant pathogen isolated from liver abscesses of diabetic patients in Asian countries. Capsular polysaccharide (CPS) is probably considered the major determinants of pathogenesis in Kp. Recently, we found the iron concentration and ferric uptake regulator (Fur) in Kp is critical for modulating CPS biosynthesis, iron-acquisition system, type 3 fimbriae, and biofilm formation. Like Fur, IscR in *Escherichia coli* has been shown to play a key iron regulator for controlling the Fe-S biosynthesis in response to cellular iron levels. IscR contains a Fe-S cluster that can be reversibly oxidized to regulate transcription depending on the specific target promoter. In this study, the role of IscR in control of virulence factor expression in Kp was identified. A deletion effect of *iscR* in wild type (WT) and Δfur strains was generated to evaluate the role of IscR in mediating CPS biosynthesis and iron acquisition systems. In addition, the complement plasmids carrying the WT and cysteine triple mutant of IscR were also generated to observe the role of Fe-S cluster in IscR regulation. Deletion of *iscR* caused an apparently reduction in *cps* mRNA expression, CPS biosynthesis, even serum resistance as compared to WT strain, and only the WT-IscR plasmid could complement the *iscR* deletion effect. The result indicates that holo-IscR plays a transcriptional activator for CPS biosynthesis. By electric mobility shift assay (EMSA), IscR could binds directly to the predicted type 1 IscR binding site in P_{galF} , but not in P_{wzi} and P_{manC} . Furthermore, the deletion of *iscR* in Δfur reduced the formation of the orange halo in Chrome azurol S (CAS) assay as compared to Δfur strain, since no apparent effect was found in WT and $\Delta iscR$ strain. However, introduction of the cysteine triple mutant of IscR plasmid into $\Delta iscR \Delta fur$ strain could increase the siderophore production in Kp. The result indicates that apo-IscR participates in Fur regulon to activate the iron-acquisition system. Quantitative real-time PCR (qRT-PCR) and EMSA were demonstrated that the gene expression of three of the eight putative iron-acquisition systems, *fhuA*, *iucA*, and *sitA*, were directly activated by apo-IscR. Take together, we has identified the role of the Fe-S cluster in IscR regulation in CPS biosynthesis and iron-acquisition systems in Kp.