RES-182

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Phospholipid transporter MlaFEDCB regulates Klebsiella pneumoniae virulence by modulating fimbriae synthesis and stress-adaptive growth

Klebsiella pneumoniae is a key opportunistic pathogen, and its emerging hyper-virulent strains pose a growing public health threat. An association exists between the phospholipid transporter MlaFEDCB and bacterial virulence; however, its regulatory role and underlying mechanisms remain elusive. Herein, we focused on *K. pneumoniae* virulence regulation via *mlaFEDCB* under in vitro and in vivo conditions.

Homology analysis showed that *mlaFEDCB* gene cluster is highly conservative among gram-negative bacterial strains and is contiguously arranged and co-transcribed within the genome. Experiment involving murine intraperitoneal infection revealed that mice infected with KP-Δ*mlaFEDCB* strain showed substantially prolonged survival (Figure 1; $P = 0.0005$).

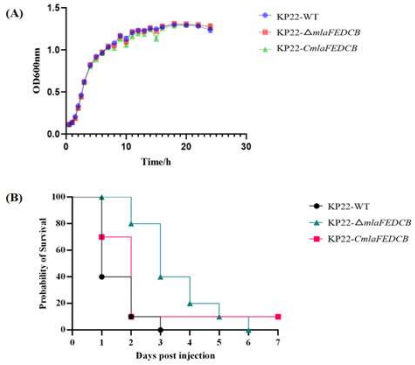

Figure 1A Growth curves of the wild-type strain WT-KP22, *mlaFEDCB* knockout strain KP22-Δ*mlaFEDCB*, and complemented strain KP22-C*mlaFEDCB*. Figure 1B Growth curve of *Klebsiella pneumoniae* in mouse intraperitoneal infection model (KP22 vs KP22Δ*mlaFEDCB*: $P = 0.0005$; KP22-Δ*mlaFEDCB* vs KP22-CmlaFEDCB : $P = 0.0079$).

Furthermore, transcriptomic analysis showed altered expression of virulence-associated genes, especially *fimH* and *fimD*, which are involved in fimbrial structure and host cell adherence (Figure 2). Scanning electron and transmission electron microscopy showed that the WT-KP strain demonstrated a complex fibrous fimbrial network, with several long, thin structures interwoven with those of neighboring bacteria, whereas the KP-Δ*mlaFEDCB* strain showed markedly fewer fimbriae and lacked the fimbrial network (Figure 3).

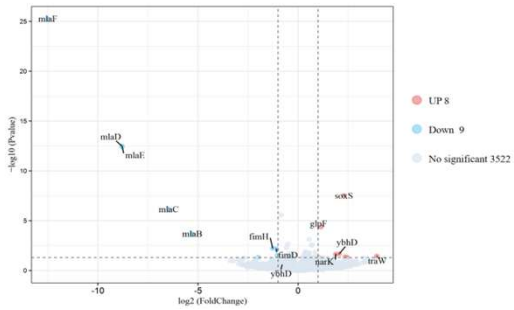

Figure 2 Volcano plot of differentially expressed genes from transcriptome sequencing between the KP22 and the *mlaFEDCB* knockout strains (KP22 vs KP22Δ*mlaFEDCB)*.

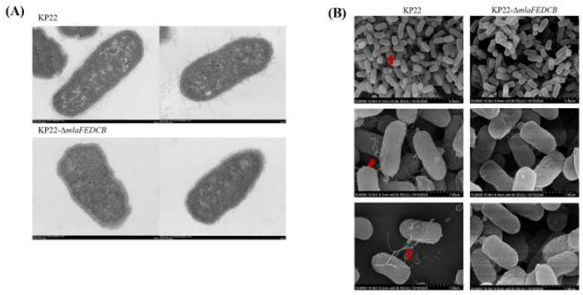

Figure 3A Transmission electron microscopy (TEM) of the wild-type and *mlaFEDCB* knockout strains.
Figure 3B Scanning electron microscopy (SEM) of the wild-type and *mlaFEDCB* knockout strains.

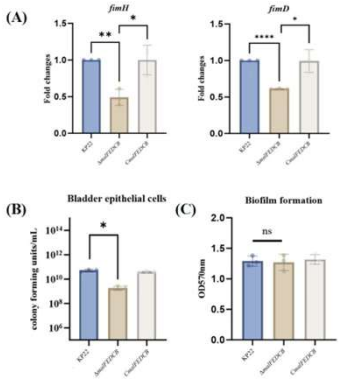

Figure 4A Transcription levels of *fimD* and *fimH* genes in the wild-type, *mlaFEDCB* knockout, and complemented strains (*: $P < 0.05$; **: $P < 0.01$; ****: $P < 0.0001$) ; Figure 4B Adhesion assay of the KP22, *mlaFEDCB* knockout mutant and complemented strain to the bladder epithelial cells (*: $P < 0.05$);Figure 4C Biofilm formation ability of the wild-type strain, *mlaFEDCB* knockout mutant, and complemented strain (not statistically significant)

Furthermore, bladder epithelial cells adhesion assay showed an apparent reduction in the KP-Δ*mlaFEDCB* strain compared to the WT-KP strain (Figure 4). The growth of the KP-Δ*mlaFEDCB* strain was significantly compromised in comparison to the wild-type strain under stress conditions (Figure 5).

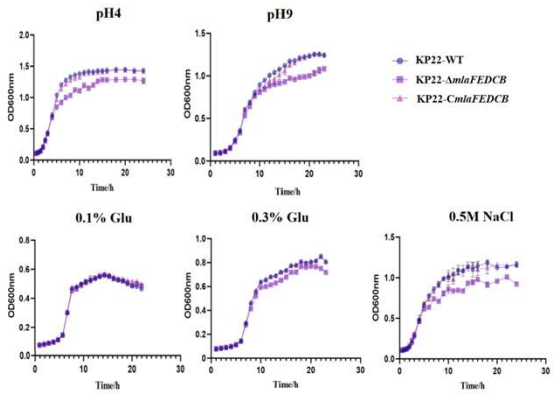

Figure 5 Survival Curves of the wild-type strain, *mlaFEDCB* knockout mutant, and complemented strain under different stress conditions

Thus, *mlaFEDCB* gene cluster increases the adhesion, invasion, and environmental adaptability of *K. pneumoniae* by modulating virulence-related gene expression, pilus synthesis, and growth under stress conditions.