MrkI Inversely Regulates the Expression of Type 1 and Type 3 Fimbriae in *Klebsiella pneumoniae* CG43

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ABSTRACT

Type 3 fimbriae play a crucial role in *Klebsiella pneumoniae* biofilm formation; nevertheless, how the type 3 fimbrial operon is regulated is largely unknown. We have found that deletion of *mrkI*, a LuxR-type regulatory gene, from *K. pneumoniae* CG43 not only abolished the production of MrkA, the major pilin of type 3 fimbriae, but also reduced the biofilm-forming activity. The following quantitative RT-PCR and promoter-reporter assays of *mrkA* verified that MrkI regulated type 3 fimbriae expression at the transcriptional level. Electrophoretic mobility shift assay analysis revealed that MrkI bound the *mrkA* promoter in the presence of the phosphodonor acetyl-phosphate. Furthermore, MrkI-mediated type 3 fimbriae expression was obviously impaired when the aspartate residue (D56), a putative phosphorylation residue of MrkI, was substituted by alanine. On the other hand, the Δ*mrkI* strain exhibited an activation of type 1 fimbriae expression resulting from the OFF-to-ON inversion of the switch region *fimS*. This inversion is likely due to MrkI regulation on the expressional level of the recombinases FimB and FimE. MrkI may also exert an additional regulation because an MrkI-*fimS* binding complex was demonstrated in the presence of acetyl-phosphate. Thus, our results show that a signaling phosphorelay is probably involved in the MrkI-dependent regulation of type 1 and type 3 fimbriae expressions.

RESULTS

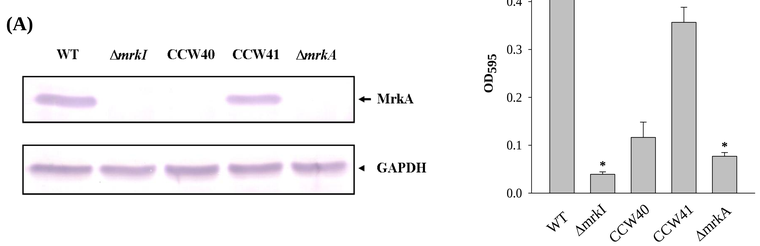

Fig. 1. Deletion of *mrkI* decreases the expression of type 3 fimbriae and biofilm formation. (A) *K. pneumoniae* CG43S3 (WT, wild-type), Δ*mrkI*, the *mrkI*-complement strain CCW41, and the control strain CCW40 were grown overnight at 37°C with agitation in LB broth. Bacterial total protein, approximately 5 μg per lane, was separated by SDS-PAGE and then subjected to western blot analysis against MrkA antiserum. The MrkA protein is indicated by an arrow. GAPDH (arrowhead) was probed as a protein loading control. (B) Quantification of the *K. pneumoniae* biofilm forming activity. The results are shown as averages of triplicate samples. Error bars indicate standard deviations. *, $P < 0.001$ compared with the WT strain. #, $P < 0.001$ compared with the CCW40 strain.

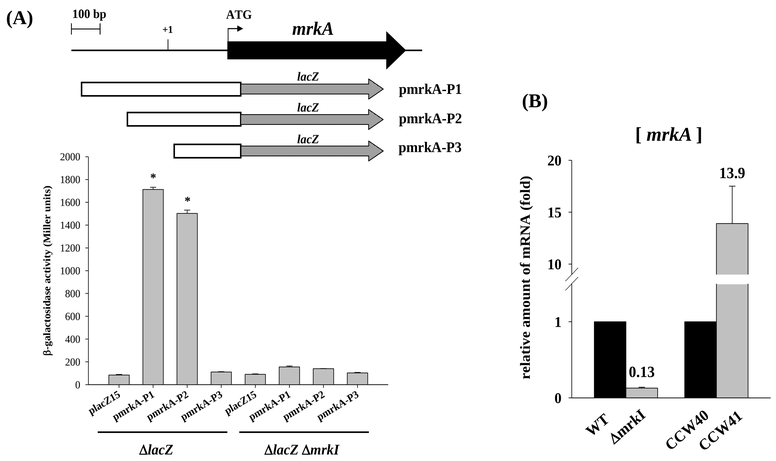

Fig. 2. Deletion of *mrkI* decreases transcription of *mrkA*. (A) The β-galactosidase activities of *K. pneumoniae* CG43S3Δ*lacZ* and its isogenic *mrkI*-deletion mutant (Δ*lacZ*Δ*mrkI*) carrying each of the reporter plasmids pmrkA-P1, pmrkA-P2, and pmrkA-P3 were determined from log-phased cultures grown in LB broth. (B) qRT-PCR analysis of the effect of *mrkI* deletion on the expression of *mrkA*.

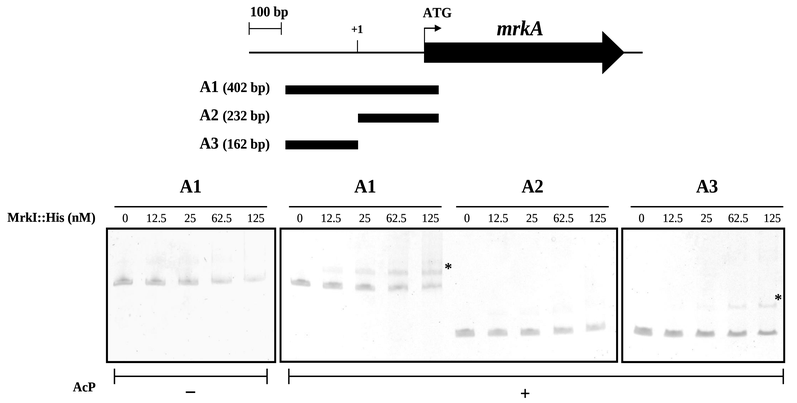

Fig. 3. EMSA of the recombinant MrkI and the *mrkA* promoter. (A) The *mrkA* region and the position of the promoter are outlined. (B) Increasing amounts of the MrkI::His_6 were incubated with different lengths of P_{mrkA} regions (A1, A2, or A3) in the presence or absence of 50 mM acetyl-phosphate (AcP), as indicated in the margin, for the assay. The DNA-protein complex is indicated by an asterisk.

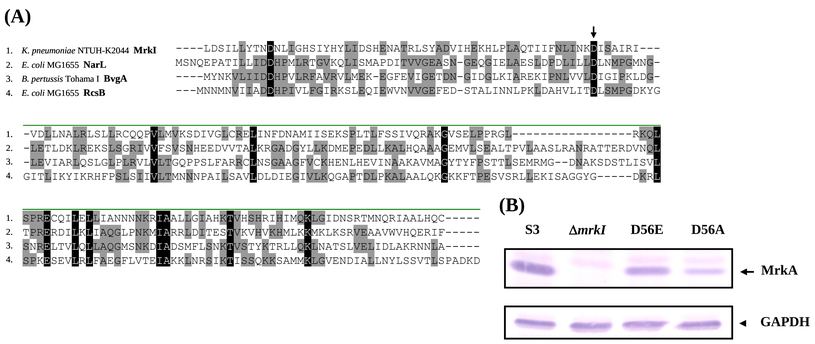

Fig. 4. MrkI is probably a response regulator activated by phosphorylation. (A) Sequences of MrkI and LuxR-type transcriptional regulators NarL, BvgA, and RcsB were aligned using Vector NTI software. The conserved aspartate (D56) residue of MrkI as a putative target site for phosphorylation is indicated by an arrow. (B) D56 is important for MrkI functionality. *K. pneumoniae* CG43S3 (WT, wild-type), the Δ*mrkI* strain, and the mutant strains expressing $MrkI_{D56E}$ (D56E) or $MrkI_{D56A}$ (D56A) were grown overnight at 37°C with agitation in LB broth. Bacterial total protein, approximately 5 μg per lane, was separated by SDS-PAGE and then subjected to western blot analysis using MrkA antiserum. The MrkA protein is indicated by an arrow. GAPDH (arrowhead) was probed as a protein loading control.

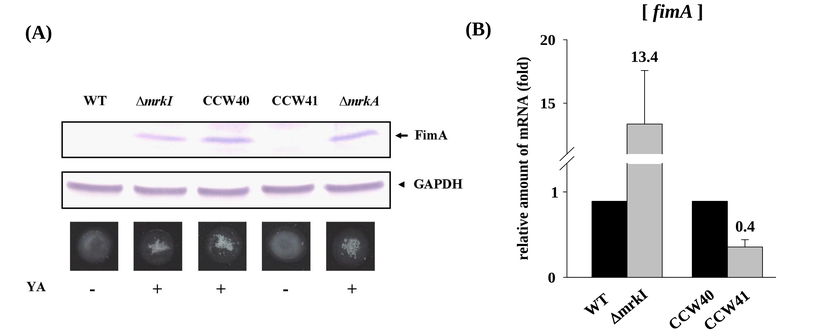

Fig. 5. Deletion of *mrkI* activates the expression of type 1 fimbriae. (A) *K. pneumoniae* strains were grown overnight at 37°C with agitation in LB broth. Bacterial total protein, approximately 5 μg per lane, was separated by SDS-PAGE and then subjected to western blot analysis against FimA antiserum (upper panel). The FimA protein is indicated by an arrow. GAPDH (arrowhead) was probed as a protein loading control. Yeast agglutinating activities of the tested strains are also shown in the lower panel. (B) qRT-PCR analysis of the effect of *mrkI* deletion on the expression of *fimA*.

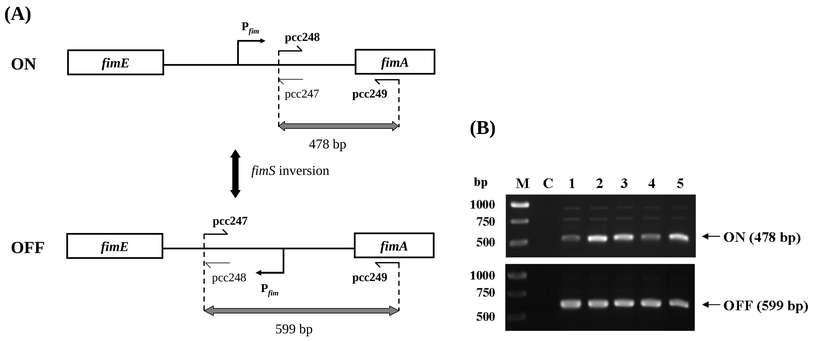

Fig. 6. Deletion of *mrkI* affects the phase variation of type 1 fimbriae. (A) PCR-based detection for assessing the inversion of the *fim* promoter. Location of the primers (pcc247 or pcc248 along with pcc249) and the size of the PCR amplicons in the ON or OFF phase are indicated. (B) PCR detection of the ON and OFF orientations of *fimS*. Bacterial chromosomal DNA (10 ng) purified from the tested strains was used as template for PCR detection of the ON phase (the upper panel) and the OFF phase (the lower panel). M, DNA molecular size markers; C, a nontemplate PCR control; lane 1, *K. pneumoniae* CG43S3; 2, Δ*mrkI*; 3, CCW40; 4, CCW41; 5, Δ*mrkA*.

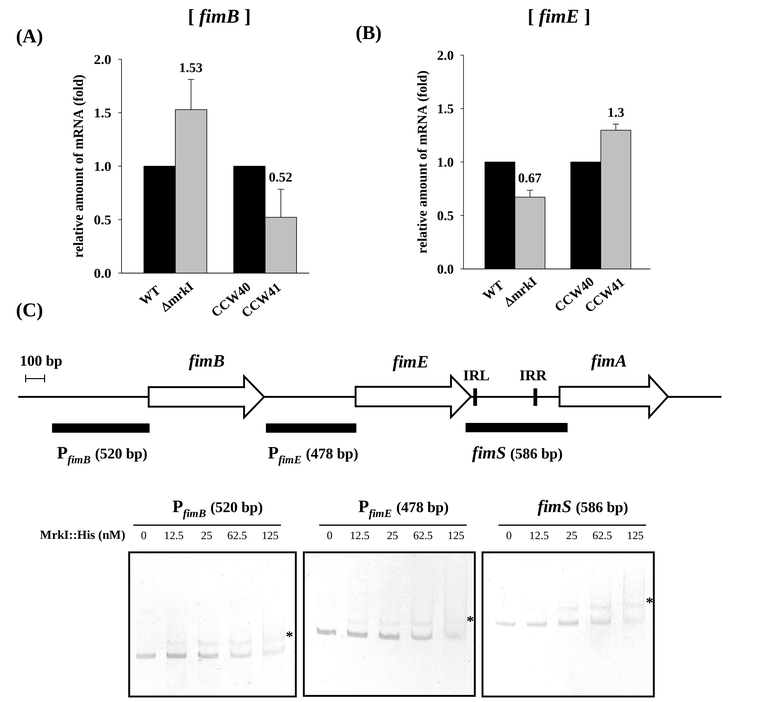

Fig. 7. Regulation of the expression of *fimB* and *fimE* by MrkI. qRT-PCR analysis of the effect of *mrkI* deletion on the expressions of *fimB* (A) and *fimE* (B). (C) MrkI binding to the *fim* regulatory DNA regions. The upper panel outlines the *fimB*, *fimE*, and *fimA* regions. Increasing amounts of MrkI::His_6, as indicated in the upper panel, were incubated with P_{fimB}, P_{fimE}, or *fimS* in the presence of 50 mM acetyl-phosphate for the assay. The DNA-protein complex is indicated by an asterisk.

SUMMARY

- ◎ **MrkI controls biofilm formation in *K. pneumoniae* by activating type 3 fimbriae expression.**
- ◎ **MrkI represses the expression of type 1 fimbriae by modulation of the phase variation.**
- ◎ **DNA binding activity of MrkI is affected by phosphorylation of its D56 residue.**