BfvR, an AraC-family regulator, controls biofilm formation and pH6 antigen production in opposite ways in *Yersinia pestis* biovar Microtus

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Short running title: BfvR controls biofilm and virulence

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Abstract

Biofilm formation is critical for blocking flea foregut and hence for transmission of *Y. pestis* by flea biting. In this study, we identified the regulatory role of the AraC-family transcriptional regulator BfvR (YPO1737 in strain CO92) in biofilm formation and virulence of *Yersinia pestis* biovar Microtus. Crystal violet staining, *Caenorhabditis elegans* biofilm assay, colony morphology assay, intracellular c-di-GMP concentration determination, and BALB/c mice challenge were employed to reveal that BfvR enhanced *Y. pestis* biofilm formation while repressed its virulence in mice. Further molecular biological assays demonstrated that BfvR directly stimulated the expression of *hmsHFRS*, *waaAE-coaD*, and *hmsCDE*, which, in turn, affected the production of exopolysaccharide, LPS and c-di-GMP, respectively. In addition, BfvR directly and indirectly repressed *psaABC* and *psaEF* transcription, respectively. We concluded that the modulation of biofilm- and virulence-related genes by BfvR led to increased biofilm formation and reduced virulence of *Y. pestis* biovar Microtus.

Keywords: *Yersinia pestis*; BfvR; biofilm; virulence; transcriptional regulation.

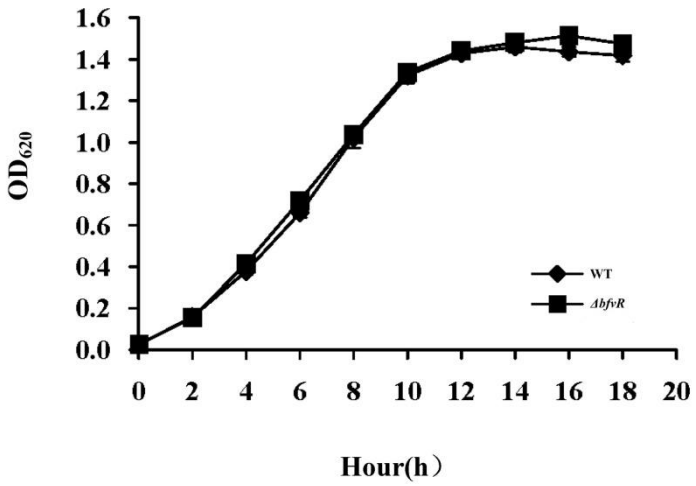

FIGURE S1. Bacterial growth curves. Overnight cell cultures at an OD620 value of approximately 1.0 were diluted 1:20 into 18 ml of LB medium with a pH value of 6.0. Bacteria were then grown at 26 ℃ with shaking at 230 rpm, and the OD620 values were monitored at 3-h intervals until the cell cultures reached the stationary growth phase. Three individual cultures (i.e., three biological replicates) were determined for each strain.

OD 620: Optical density at 620 nm; WT: Wild-type.

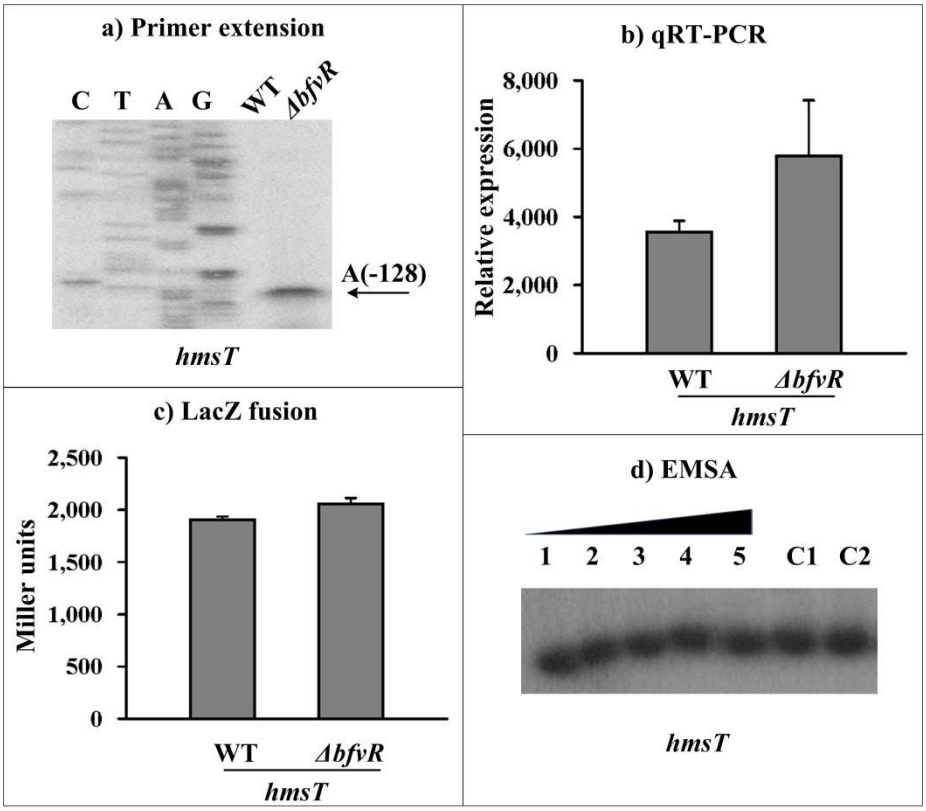

FIGURE S2. Regulation of *hmsT* by BfvR. See FIGURE 2 for the annotations of primer extension (**a**), quantitative RT-PCR (**b**), LacZ fusion (**c**), and EMSA (**d**). The promoter-proximal region was amplified from -410 to +42.

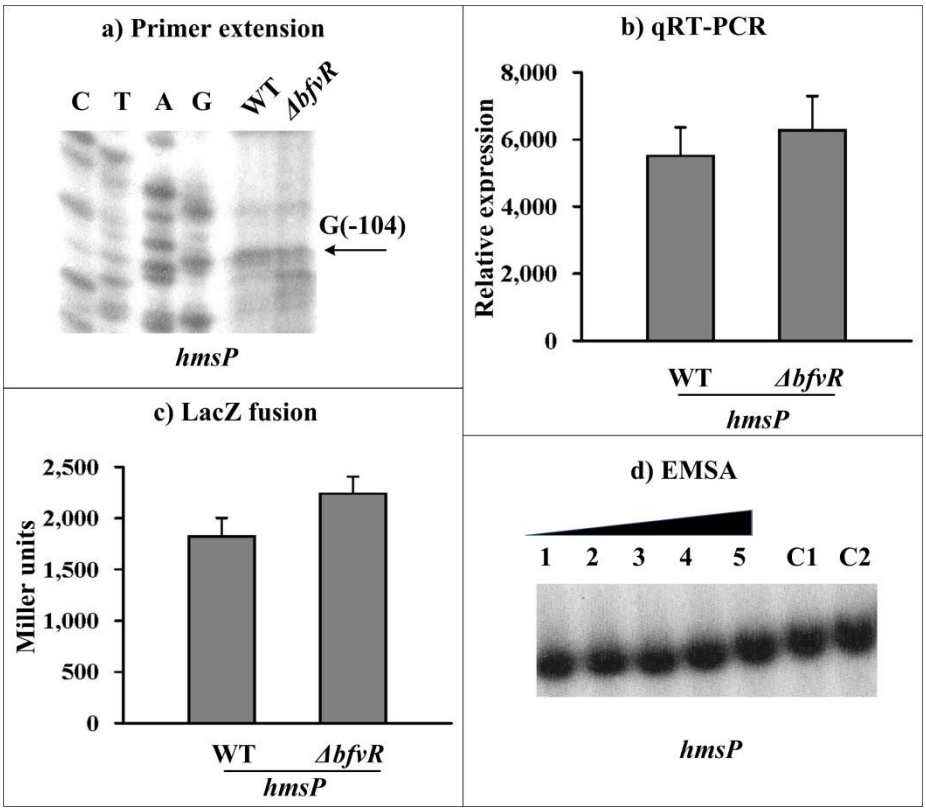

FIGURE S3. Regulation of *hmsP* by BfvR. See FIGURE 2 for the annotations of primer extension (**a**), quantitative RT-PCR (**b**), LacZ fusion (**c**), and EMSA (**d**). The promoter-proximal region was amplified from -659 to +65.

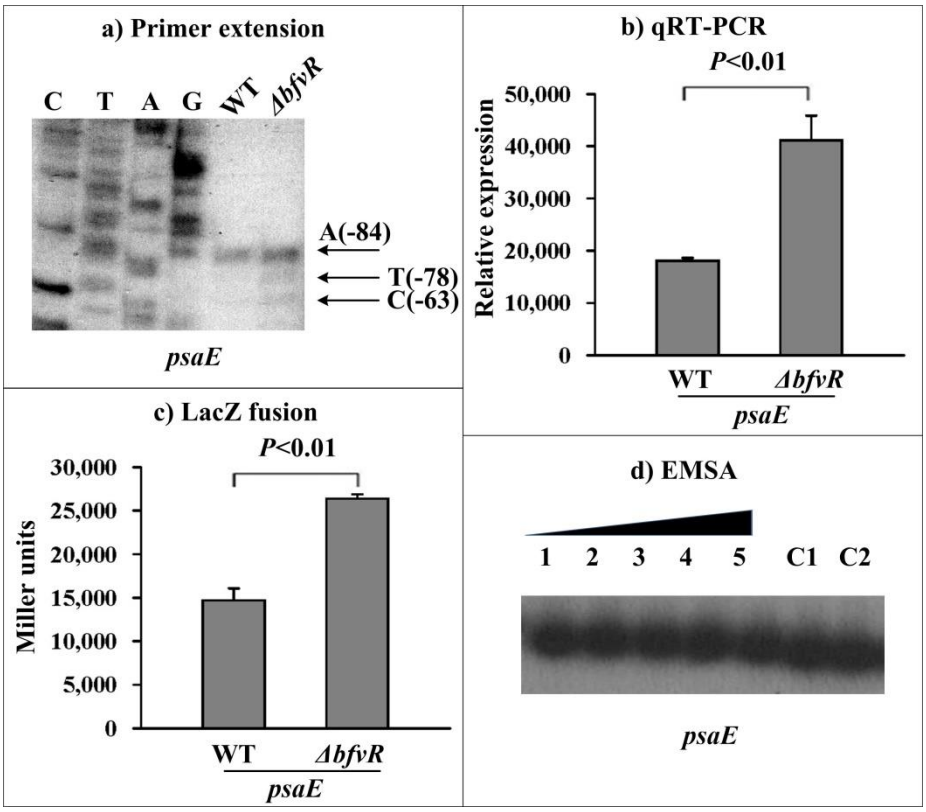

FIGURE S4. Regulation of *psaE* by BfvR. See FIGURE 2 for the annotations of primer extension (**a**), quantitative RT-PCR (**b**), LacZ fusion (**c**), and EMSA (**d**). The promoter-proximal region was amplified from -563 to +93.