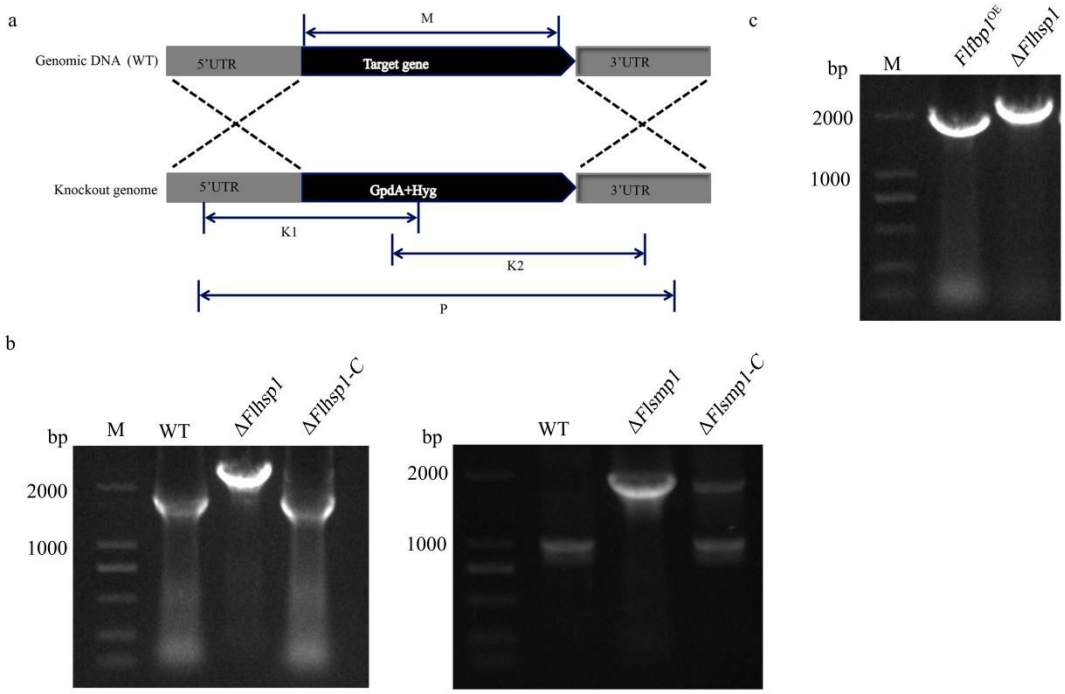

Fig. S13. Construction and identification of single-gene knockout mutants of *Flhsp1* and *Flsmp1*, and *Flhsp1* knockout mutant in the *Flfbp1*^{OE} background in *F. lateritium*. F1617. (a) Strategy for targeted gene knockout and complementation based on homologous recombination. Knockout strategy: The coding region of the target gene was replaced with a selection cassette containing the constitutive strong promoter GpdAand the hygromycin phosphotransferase resistance gene (HYG), to drive resistance gene expression for screening of positive transformants and achieve gene knockout. Complementation strategy: Homozygous knockout mutants confirmed by genotyping were used as recipient strains. By PEG-mediated protoplast transformation, a complementation construct harboring the full-length ORF of the target gene fused to its native 1.5–2.0 kb promoter was introduced to obtain complemented strains. Specific diagnostic PCR primers (K1, K2, P, M) were used to verify the correctness of both homologous recombination and complementation events. (b) Agarose gel electrophoresis of PCR-based genotyping of WT, Δ*Flhsp1*, Δ*Flsmp1* knockout mutants, and their corresponding complemented strains. M: DNA molecular weight marker (bp). (c) PCR-based genotyping of *Flfbp1*^{OE} and the Δ*Flhsp1* knockout mutant constructed in this background. M: DNA molecular weight marker (bp).