



**Fig. 12.** (A) Analysis of the deletion effect of phgS or phgM on the activity of  $P_{fimB}$ ,  $P_{fimE}$ ,  $P_{S-mrkA}$ , and  $P_{L-mrkA}$ . A significant difference (P < 0.001) was marked by a star. (B) The purified recombinant PhgS protein resolved on SDS-12.5% polyacrylamide gel by electrophoresis is shown on the left. M: Marker. Lane 1: E. coli NovaBlue (DE3)/pET-PhgS induced with IPTG, lysed by sonication, separated by centrifugation, and then denatured with 6 M urea. Lane 2: the purified PhgS protein. The EMSA assessment for the DNA binding activity of the recombinant PhgS is shown on the right. The amounts of protein used are indicated on top of each lane. Excess amount of unlabelled P<sub>S-mrkA</sub> DNA (lane 4) was added into the reaction mixture as competitor for the binding. The DNA and protein complexes formed is indicated as C and the free forms is indicated as F. (C) Quantitative determination of the mRNA by Limiting-dilution RT-PCR analysis. The measurement and the primer pairs used for amplification of mrkA (Up) and 23S rRNA (down) have been described in the Materials and Methods. The PCR products were electrophoresed on 1 % agarose gels. The amounts of the cDNA used are as following: undiluted cDNA (lane 1), 1/4 dilution (lane 2), 1/16 dilution (lane 3), 1/64 dilution (lane 4). (D) Western blotting analysis of the expression of type 3 fimbriae. Total proteins isolated from the bacteria were resolved on SDS-12.5% polyacrylamide gel by electrophoresis (left), and the gel transferred onto PVDF and the expression of type 3 fimbriae recognized with anti-MrkA antibody (right). M: marker. Lane 1: K. pneumoniae CG43S3; 2: K. pneumoniae CG43S3phgS; 3: K. pneumoniae CG43S3phgM.