



**Figure S1. (A) Schemes of markerless gene knock-out procedures in *B. thuringiensis* BMB171.** This figure is taken from the previous study in our laboratory (Zheng et al., 2015). **(B) Screening process of several *pde* genes deletion mutants.** Four candidate genes (*RS02850*, *RS03240*, *RS18570* and *RS19795*) with high transcription and high PDE activity *in vitro* were selected to construct different mutants in our study. Using cell motility as the screening criteria, two single *pde* gene deletion mutants  $\Delta$ RS19795 and

$\Delta RS03240$  were constructed in the first step, and then  $RS02850$  was selected to construct the two double *pde* genes deletion mutants  $\Delta RS19795\Delta RS02850$  and  $\Delta RS03240\Delta RS02850$ . Subsequently,  $RS18570$  was deleted from  $\Delta RS03240\Delta RS02850$  to obtain a relative high c-di-GMP concentration mutant  $\Delta RS03240\Delta RS02850\Delta RS18570$ . The names of  $\Delta 1pde$ ,  $\Delta 2pde$  and  $\Delta 3pde$  will be used to refer to the single *pde* gene deletion mutant  $\Delta RS03240$ , the double *pde* genes deletion mutant  $\Delta RS03240\Delta RS02850$  and the triple *pde* genes deletion mutant  $\Delta RS03240\Delta RS02850\Delta RS18570$ , respectively.