Fig. S3

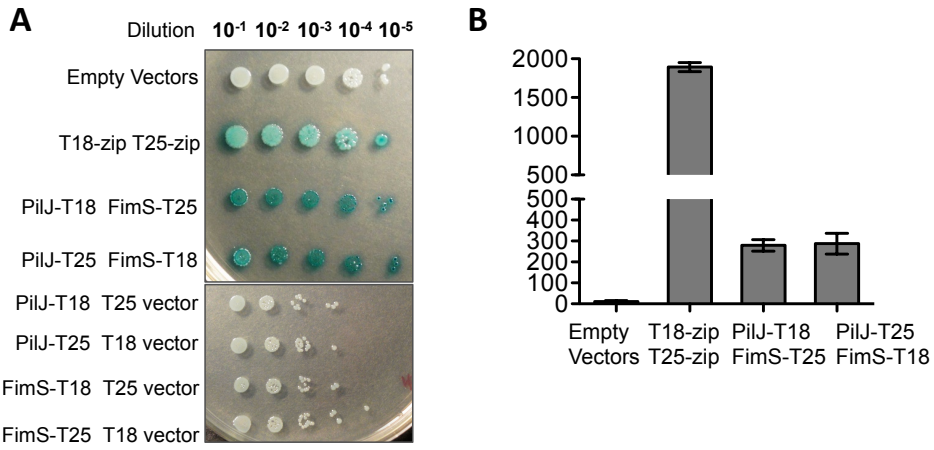

Fig. S3. **Detection of *in vivo* interaction between PilJ and FimS by a BACTH analysis**. Interaction between proteins was assessed using the bacterial adenylate cyclase two-hybrid (BACTH) assay. The PilJ, FimS, PilX, PilW, and PilY1 proteins were fused to either the T25 or the T18 fragment of the *Bordetella pertussis* adenylate cyclase, and co-expressed in *E. coli* strain BTH101 to systematically test for all pairwise interactions among these proteins (see the Supplemental Material for the complete list of tested pairs). **(A)** *E. coli* cells co-transformed with the recombinant plasmids (indicated on the left) were serial diluted 10-fold, spotted on LB agar containing Cb, Kan, and X-gal, and incubated for 40 h at 30 °C. The degradation of X-gal (blue color) indicates positive protein interaction. The positive control is cells with leucine zipper vectors (T18-zip and T25-zip). The negative controls are cells co-transformed with the two empty plasmids, as well as each protein of interest against the empty T18 or T25 plasmids. **(B)** Shown are β-galactosidase activities in transformants grown in LB broth supplemented with Cb and Kan overnight. The data represent three independent experiments with three biological replicates each, and values are reported as mean± SEM.