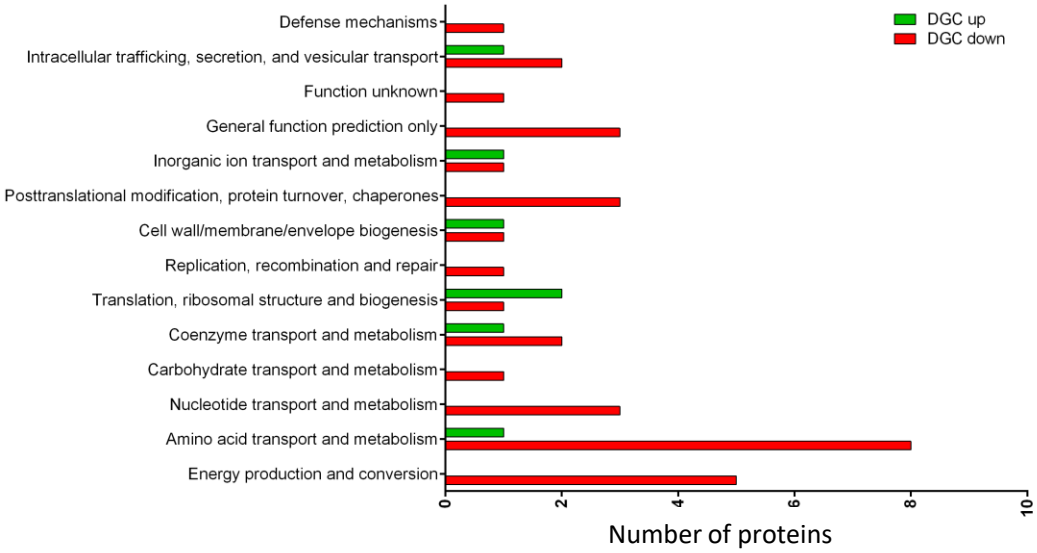
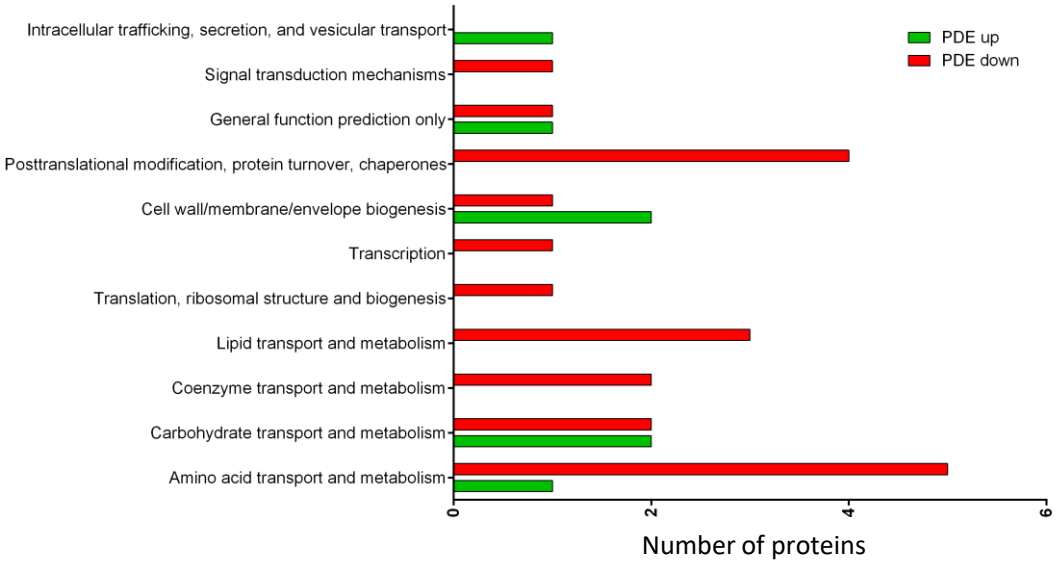
**Cyclic di-GMP regulates type three secretion system and virulence in *Bordetella bronchiseptica.***

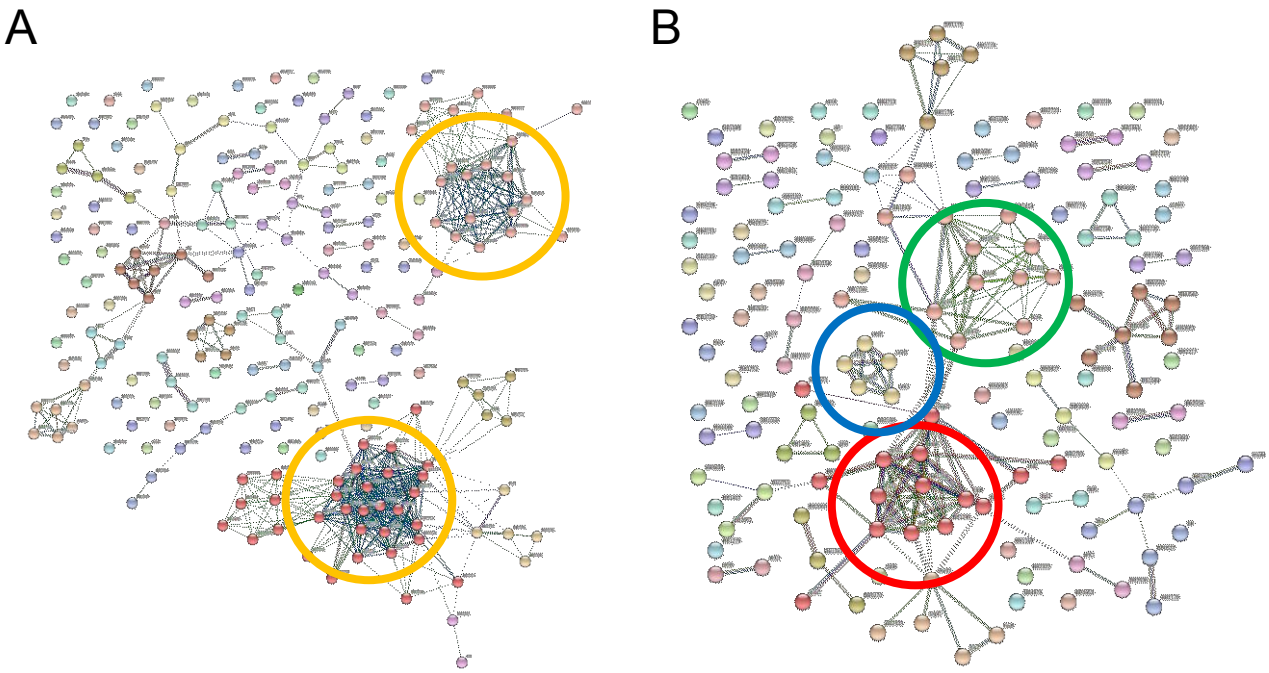
Gutierrez et al. 2021.

Supplemental Figures.

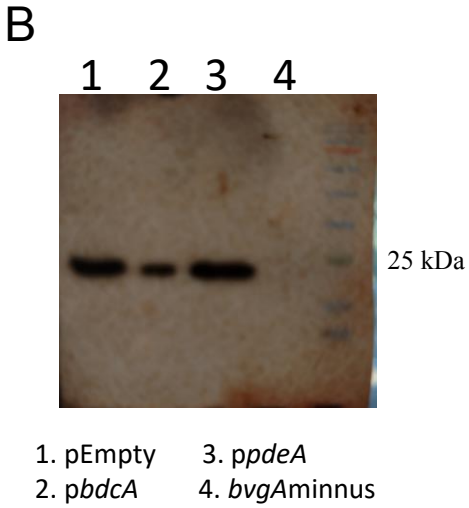


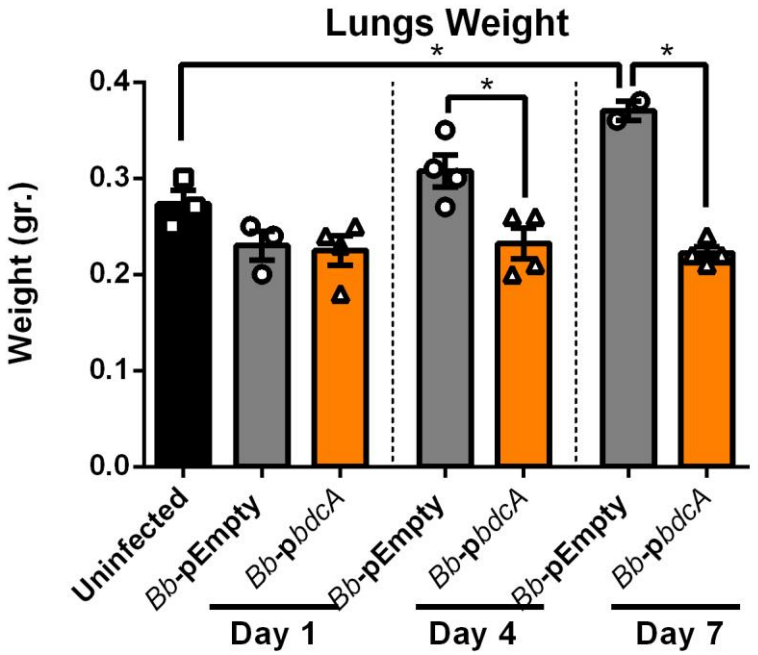

**Supplemental Figure 1.** Clusters of Orthologous Groups of proteins found in the shotgun proteomic approach. Proteins identified in Bb-p*bdcA* (A) or in Bb-p*pdeA* (B) and differentially expressed (up regulated in green and down regulated in red).



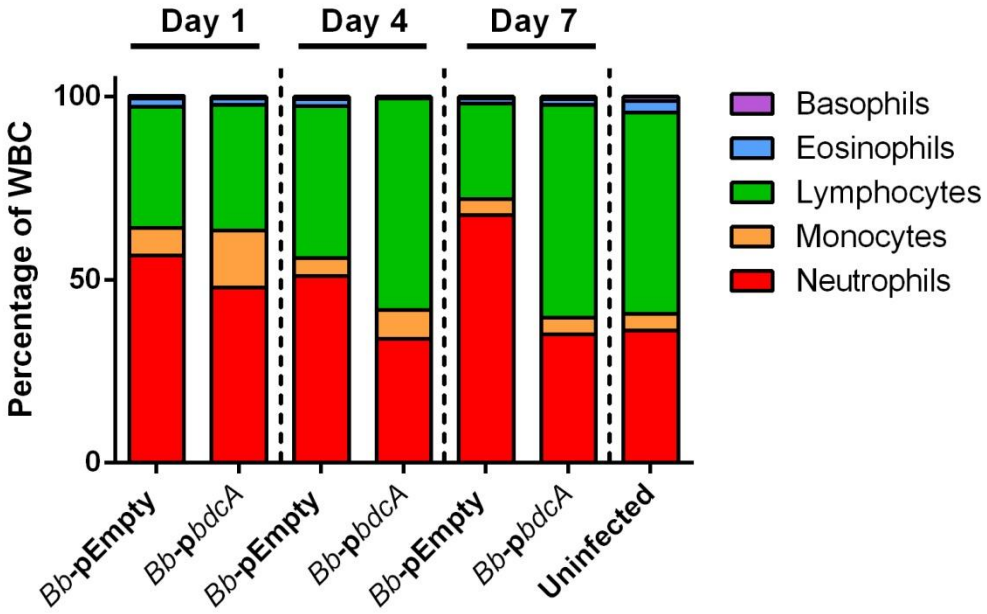

**Supplemental Figure 2.** Identified sequences with statistically different fold changes (A or B positive or negative fold changes respectively) were analyzed by STRING software to predict interactions. Circled in yellow, red, blue or green are shown phage-, TTSS-, nitrogen- and stress-related genes, respectively.



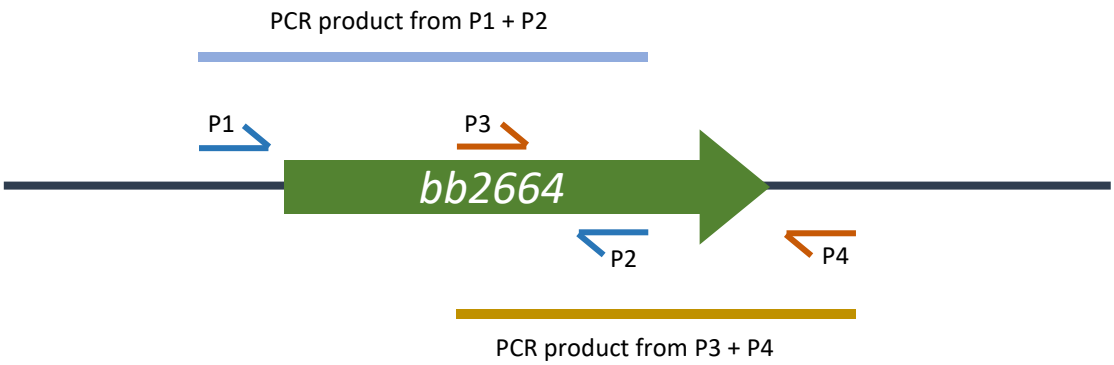

**Supplemental Figure 3.** Western blot with anti-Bsp22 antibodies shown in Figure 4B superimposed to acrylamide transferred gel to determinate protein molecular mass with the protein marker *Blue Plus* IV Protein Marker (10 -180 kDa) (TransGen Biotech Co).




**Supplemental Figure 4.** Lungs weight (gr) was recorded immediately after the lungs were aseptically removed from mice. Each symbol represents data from one mouse. Group comparisons were analyzed by one-way ANOVA followed by a Tukey's multiple-comparison test. Results are shown as mean ± SEM values, *p ≤ 0.05.




**Supplemental Figure 5.** Relative percentage of whole blood cell type in peripheral blood. Different types of whole blood cells (WCB) (neutrophils, monocytes, lymphocytes, eosinophils and basophils) were detected using a hematology analyzer at days 1, 4 and 7 post-infection. Results are shown as percentages of WBC.




**Supplemental Figure 6.** Scheme of the strategy to clone the entire *bb2664* gen. 759 bp and 853 bp oligonucleotides with overlapping sequences were amplified and are indicated in the scheme. Both PCR products were mixed with pMQ72 plasmid and recombination was allowed with the yeast cloning system.