Abstract

The type three secretion system (T3SS) was originally considered to be inactive in *B. pertussis*, but it was later described to be active in recent clinical isolates. Later, T3SS activity was observed also in highly passaged reference strain Tohama I upon contact with the host or under nutrient limiting conditions. In our study, we showed that not only recent clinical isolates but also a low-passage historic strain of *B. pertussis* are T3SS proficient. Using the THP-1 human monocytic cell line, we further showed that the expression of T3SS is reduced in the intracellular environment due to transition of *B. pertussis* cells to avirulent phase. On the contrary, we demonstrated that the contact with blood components, which may be for example available on the surface of the damaged respiratory epithelium, represents an important cue for *B. pertussis* and is also an important prerequisite for T3SS functionality. Additionally, our analysis of blood-responsive regulon revealed the novel T3SS chaperone BP2265, a homologue of CesT chaperone in *E. coli*, which connects the T3SS secretion with other cellular functions. Similarly to CesT, belonging to class IA chaperones, we confirmed that BP2265 is involved in regulation of metabolism, virulence and biofilm formation. Furthermore, we demonstrated that BP2265 specifically binds the BtrA anti-sigma factor and that it is further required for functional T3SS secretion. Using *in silico* structural modeling, we confirmed the formation of presumed complex between BP2265 dimer and BtrA characteristic for class IA T3SS chaperone-cargo pair. Thus, we suggest that BP2265 represents a unique chaperone allowing for expression of T3SS genes by export of an anti-sigma factor, the mechanism otherwise known in regulation of bacterial flagellum assembly.