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### Role of Type IV pilin genes in virulence of *Francisella tularensis* subspecies *holarctica* and *tularensis*

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The biogenesis and regulation of type IV pilus (Tfp) is complex and can involve up to 40 genes. Analysis and comparisons of genome sequences of different *Francisella tularensis* subspecies has revealed the presence of genes encoding a putative Tfp. This analysis has also revealed interesting differences between the subspecies. Two of the pilin genes and the gene encoding the PilT ATPase are non-functional in subsp. *holarctica* (type B). In addition *pilA*, that we previously have shown to be required for mouse virulence, is lacking in the type B vaccine strain LVS.

**Aims:** The aims were to study the role of Tfp encoding genes in the human pathogenic subsp. *holarctica* and *tularensis* (type A).

**Methods:** Tfp encoding genes in a virulent human type B isolate and the type A strain SchuS4 were targeted by mutagenesis and evaluated for their potential role in virulence. The different mutants were assessed for virulence in single strain infections as well as in competition with the isogenic wild-type strain.

**Results:** Of the pilin genes only PilA was found to be required for mouse virulence in the type B strain. In addition, we could also establish that two pseudopilins FTT1621-22 contributed to virulence. We also verified that PilA, as well as the inner membrane protein PilC and the PilQ secretin are required for full virulence of the type A strain SchuS4. Interestingly PilT was found not to be required for mouse virulence of SchuS4.

**Conclusions:** We have confirmed that PilA is essential for mouse virulence in both type A and type B strains and that PilC and PilQ, postulated to be required for assembly of Tfp, also are required for full virulence in SchuS4. PilT on the other hand, which is only functional in type A strains and postulated to have a role in pilus retraction, was not required for mouse virulence. Further evaluation of the importance of these genes in other infection models, more relevant for human infections, is needed to establish the role of Tfp genes in the pathogenesis of tularemia.