Francisella tularensis: unravelling the secrets of an intracellular pathogen

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Francisella tularensis has been recognized as the causative agent of tularemia for almost a century. Since its discovery in 1911, it has been shown to infect a wide range of hosts, including humans. As early as the 1920s it was suggested to be an intracellular pathogen, but it has proven to be an enigmatic organism, whose interaction with the host has been difficult to elucidate, and we still have a very limited understanding of the molecular mechanisms of virulence. However, the recent availability of genome sequence data and molecular tools has allowed us to start to understand the molecular basis of F. tularensis pathogenicity, and will facilitate the development of a vaccine to protect against infection.

Francisella tularensis

Francisella tularensis is a small pleomorphic Gram-negative cocccobacillus. It was first isolated in 1911 from ground squirrels found dying of a plague-like illness in Tulare County, CA, USA (McCoy & Chapin, 1912). Initially called Bacterium tularense, it was eventually allocated to a new genus named Francisella in honour of the man who pioneered research on the organism, Dr Edward Francis (Dorofe'ev, 1947). Modern taxonomic methods have assigned Francisella to the γ-subclass of Proteobacteria (Forsman et al., 1994). Sequencing of 16S genes, and more recently whole genome analysis, indicates that the closest relatives are endosymbionts, such as Wolbachia persica (Forsman et al., 1994; Keim et al., 2007). The taxonomic status of Francisella is supported by an unusual fatty acid composition and the high lipid content of the cell wall (Hood, 1977). Three species are currently recognized: F. tularensis, Francisella philomiragia and Francisella novicida, although it has been suggested that F. novicida should be reclassified as a subspecies of F. tularensis, and this classification has been adopted in many publications, including Bergey's Manual of Systemic Bacteriology (Sjostedt, 2005). Three F. tularensis subspecies are recognized, tularensis, holarctica and mediasiatica, which differ in geographical distribution and virulence (Table 1). The subspecies tularensis has been divided into two clades, A.I and A.II (Farlow et al., 2005; Johansson et al., 2004), based on geographical distribution, disease outcome and transmission routes. However, recent isolation of Francisella-like organisms from fish and human infections indicates that the genus may be more diverse and widespread than previously assumed (Nylund et al., 2006; Ostland et al., 2006; Whipp et al., 2003).

F. tularensis is a fastidious organism that requires enriched medium for growth, such as cysteine glucose blood agar or an enriched cysteine-supplemented chocolate agar (Ellis et al., 2002). A heavy inoculum will yield visible growth in 18 h at 37 °C, but the appearance of individual colonies may require 2 to 4 days of incubation. On chocolate agar, colonies are 2 to 4 mm in size, greenish-white, round, smooth and slightly mucoid, whilst on media containing whole blood there is usually a small zone of α-haemolysis surrounding colonies. F. tularensis does not grow well in liquid medium even when the medium is supplemented with cysteine. Fully virulent strains of F. tularensis must be handled at containment level 3 (Titball et al., 2007). In the USA, F. tularensis is listed as a 'select agent' by the Centers for Disease Control and Prevention.

Tularemia

Tularemia is a zoonotic infection caused by F. tularensis (Ellis et al., 2002; Sjostedt, 2007). It circulates in populations of rodents and lagomorphs, and outbreaks in humans often parallel outbreaks in animal populations. However, it is not clear whether these animal species are the true reservoir of the bacterium in the environment. A wide range of arthropod vectors have been implicated in the transmission of the pathogen causing tularemia between mammalian hosts, including mosquitoes, ticks and deer flies (Boyce, 1975; Mörner, 1992). These vectors can also transmit the pathogen to man. In addition, F. tularensis can be acquired by contact with, or ingestion of, contaminated material, including food and water, and by inhalation of infectious particles. Rural populations, and especially those individuals who spend periods of time in endemic areas, such as farmers, hunters, walkers and forest workers, are most at risk of contracting tularemia (Levesque et al., 1995; Syrjala et al., 1985). Outbreaks associated with contaminated water supplies can involve large numbers of cases, but usually the incidence of the
Table 1. Virulence and distribution of Francisella

<table>
<thead>
<tr>
<th>Francisella species</th>
<th>Subspecies</th>
<th>Relative virulence</th>
<th>Region</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. novicida</td>
<td></td>
<td>Low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. philomiragia</td>
<td></td>
<td>Low</td>
<td>Central and Eastern USA, California</td>
<td>Rarely isolated</td>
</tr>
<tr>
<td>F. tularensis</td>
<td>tularensis A.I</td>
<td>Very high</td>
<td>Northern hemisphere</td>
<td>Rarely isolated</td>
</tr>
<tr>
<td></td>
<td>tularensis A.II</td>
<td>Moderate: lower than subsp. holarctica (Staples et al., 2006)</td>
<td>Western USA</td>
<td>Distribution matches that of vectors Amblyomma americanum and Dermacentor variabilis (Farlow et al., 2005)</td>
</tr>
<tr>
<td>holarctica</td>
<td>High</td>
<td></td>
<td>Northern hemisphere, widespread</td>
<td></td>
</tr>
<tr>
<td>mediasiatica</td>
<td>High</td>
<td></td>
<td>Central Asia</td>
<td></td>
</tr>
</tbody>
</table>

The type and severity of the disease depends on strain, dose, and route of infection (Ellis et al., 2002). F. tularensis subspecies tularensis and holarctica cause the majority of reported cases, with subspecies tularensis causing the more severe disease of the two. Although tularemia can be a severely debilitating or even fatal disease, especially when caused by F. tularensis subspecies tularensis, many cases of disease caused by lower-virulence strains are undiagnosed due to the non-specific nature of the symptoms. The incubation period is 3–5 days normally (range 1–21 days), and patients develop flu-like symptoms that may be protracted and relapsing if untreated (Evans et al., 1985).

Infection through the skin results in ulceroglandular tularemia. This is the most common presentation of the disease and can arise following the bite of an infected vector or through direct contact with the flesh of infected animal (Ohara et al., 1991). A lesion develops at the site of infection, often a single papule that develops into an ulcer surrounded by a zone of inflammation (Oyston et al., 2004). The ulcer is relatively painless and heals within a week. Where no ulcer is reported, this is termed glandular tularemia. Within a few days, the patient develops fever, chills, malaise, headaches and a sore throat. The local draining lymph nodes become enlarged and painful, like a bubo. Lymphadenopathy can take a significant period to resolve, even with treatment, and without treatment suppuration occurs in approximately 30% of cases (Helvaci et al., 2000; Kavanaugh, 1935). Less commonly, infection can occur through the conjunctiva. This is termed ocuglandular tularemia and arises following direct contamination of the eye (Steinemann et al., 1999). The patient develops conjunctivitis in the infected eye, swollen eyelids and a purulent secretion. Untreated, the infection can spread to the local lymph nodes, similarly to ulceroglandular tularemia. Ingestion of infected meat or water can result in oropharyngeal or gastrointestinal tularemia (Stewart, 1996; Tarnvik et al., 1996). Ulcers, pharyngitis, and swollen cervical lymph nodes develop, and a yellow-white pseudomembrane may be seen in oropharyngeal tularemia. Gastrointestinal tularemia can range from a mild but persistent diarrhoea to an acute fatal disease with extensive ulceration of the bowel, depending on the size of the infecting dose.

Inhalation of F. tularensis results in respiratory or pneumonic tularemia. Respiratory tularemia has been reported in farmers following activities such as making hay, where infectious dusts can be generated (Stewart, 1996; Syrjala et al., 1985). Other high-risk activities in endemic areas are lawn-mowing and brush cutting (Feldman et al., 2001; Matyas et al., 2007). For example in Martha’s Vineyard, MA, USA, the majority of investigated cases were respiratory, in landscapers undertaking these types of work (Matyas et al., 2007). Pneumonia can also arise following haematogenous spread in other forms of tularemia. Symptoms can be variable and depend on the virulence of the strain involved. Infection with the most highly virulent strains can have a case fatality rate of up to 30% if untreated, but antibiotic therapy reduces this to around 2% (Dennis et al., 2001). Presentation can range from a mild pneumonia to an acute infection with high fever, malaise, chills, cough, delirium and pulse-temperature dissociation. Radiological examination may reveal par enchymal infiltrates, most commonly in one lobe, and hilar lymphadenopathy may be present (Tarnvik & Chu, 2007).
biological weapon by various nations, including the reported production of antibiotic-resistant strains (Dennis et al., 2001; Oyston et al., 2004). Since the events of September 2001 and the subsequent anthrax attacks on the USA, concern about the potential misuse of this organism has increased (Oyston et al., 2004). The associated increase in funding, particularly in the USA, has resulted in an influx of researchers working on the pathogen.

Tularaemia responds well to antibiotic therapy. As described above, the mortality rate of the more acute forms of the disease is reduced significantly if the patient receives suitable antibiotics. Historically aminoglycosides have been the drugs of choice. Although clinically effective, streptomycin is rarely used now due to problems of ototoxicity and nephrotoxicity (Titball et al., 2007). Similarly, although chloramphenicol has been used historically for treatment, it would be unlikely to be used as a first choice due to the possibility of irreversible effects on haematopoiesis (Titball et al., 2007). Gentamicin is a suitable alternative aminoglycoside, and has been used for treatment of pneumonic tularaemia on Martha’s Vineyard (Matyas et al., 2007). Due to the requirement for parenteral dosing and monitoring of serum levels, aminoglycosides are now only used for the most serious cases. The tetracyclines have been associated with high relapse rates on withdrawal (Dennis et al., 2001). Doxycycline is effective in the treatment of tularaemia, but should be avoided for use in young children due to possible effects on developing teeth (Tarnvik & Chu, 2007). Ciprofloxacin has been shown to be highly effective in oral therapy, and can be considered the current drug of choice for uncomplicated tularaemia (Johansson et al., 2001; Syrjala et al., 1991). It has shown to be effective in treating tularaemia in children, and may be suitable for use in pregnant women.

No licensed vaccine is available for prophylaxis of tularaemia. Killed cell preparations were reactogenic and of dubious efficacy (Foshay et al., 1942; Foshay, 1950), although studies in humans indicated that immunization with these vaccines reduced the number of infections and considerably modified the course of the disease (Foshay et al., 1942; Kadull et al., 1950). The identification of the antigens responsible for induction of a protective response has been an aim of research for 50 years. To date, the only protective antigen identified is LPS. In humans the predominant antibody response is to LPS. However, animal studies have shown that while immunization with LPS isolated from a live vaccine strain (LVS) induces protection against F. tularensis strains of low virulence, it is less effective at providing protection against strains of F. tularensis subspecies tularensis (Conlan et al., 2002; Fulop et al., 1995, 2001), and LPS isolated from strain SCHU S4 was similarly unable to protect immunized mice against homologous challenge (Prior et al., 2003). To date, many immunogenic proteins have been identified (Titball & Petrosino, 2007), but none capable of inducing a protective immune response. This is probably a reflection that antibody is not sufficient to protect against tularaemia and a T-cell memory response must be induced for a vaccine to be protective (Tarnvik, 1989). A LVS was developed in the 1950s, and used extensively to vaccinate at-risk workers under ‘investigational new drug’ status, which resulted in a significant decrease in laboratory-acquired infections (Burke, 1977). The LVS vaccine provided good protection against an airborne challenge with 10 infectious doses of a virulent strain of F. tularensis subspecies tularensis, but only partial protection against 100 infectious doses and poor protection against 1000 infectious doses (McCrum, 1961). Although LVS appears to be effective, there have been problems with the strain, such as reversion to virulence, mixed colony morphology and variable immunogenicity, and thus the LVS strain has failed to achieve licensing for human use (Oyston et al., 2004). The development of a candidate vaccine suitable for licensing is currently the focus of much research.

A facultative intracellular pathogen

F. tularensis can invade and multiply in a range of cell types (Anthony et al., 1991; Ben Nast et al., 2006; Buddingh & Womack, 1941; Councilman & Strong, 1921; Francis, 1927; Lindemann et al., 2007; Shepard, 1959), but in vivo its primary target appears to be the macrophage (Fortier et al., 1994). Based on homology searches of the genome, F. tularensis does not produce any classical virulence factors, such as an exotoxin. Instead, the virulence of F. tularensis appears to stem from its ability to proliferate to large numbers within various host tissues and organs, thereby disrupting their normal functions and inducing a significant host inflammatory response that itself appears to contribute to the disease. The components of the organism that induce the inflammatory response are unknown, although LPS does not appear to be responsible (Ancuta et al., 1996; Sandstrom et al., 1992). However, differences in response observed between murine and human immune cells following exposure to LPS may indicate that the contribution of LPS to the inflammatory response may vary depending on the host (Rahhal et al., 2007). The innate response to Francisella has been shown to be mediated by interaction of bacterial components with TLR2 (Malik et al., 2006). Two lipoproteins, TUL4 and FTT1103, have been identified that interact with TLR2, which may be responsible for proinflammatory cytokine induction during infection (Thakran et al., 2007). A host protein, host matrix metalloprotein 9, has also been implicated in influencing development of morbidity following infection with Francisella (Malik et al., 2007). Host matrix metalloprotein 9, a member of the host matrix metalloproteinases family, is released by neutrophils and activated macrophages, and plays an important role in modulating leukocyte recruitment. Mice defective in production of the host matrix metalloprotein 9 were able to resolve infection, even with highly virulent subspecies tularensis strains, while normal expression rendered mice more susceptible to infection, with higher bacterial burdens...
The acid phosphatase AcpA has been shown to play a key role in intracellular survival of Francisella, by inhibiting the respiratory burst generated by macrophages (Reilly et al., 1996). Deletion of acpA resulted in a mutant that was more susceptible than wild-type to killing by human macrophages and had decreased phosphatase activity associated with membrane fractions (Mohapatra et al., 2007). Additionally, the mutant showed a decreased ability to escape from the phagosome (Mohapatra et al., 2007). Several theories have been proposed to explain this observation (Mohapatra et al., 2007), one possibility being membrane disruption by phospholipase activity, which has been reported for AcpA in addition to its acid phosphatase activity (Reilly et al., 1996). Multiple acid phosphatases have been identified in the genome sequence of Francisella, but the contribution of each to the observed inhibition of the respiratory burst has not been determined. Francisella has also been shown to enter neutrophils without triggering the respiratory burst, by an unknown mechanism that inhibits NADPH oxidase assembly (McCaffrey & Allen, 2006), and then escapes the phagosome to survive in the neutrophil cytoplasm. Thus Francisella belongs to a very small group of microbial pathogens able to avoid killing by neutrophils, and the importance of this observation to pathogenesis has yet to be determined.

The intracellular life cycle of F. tularensis is complex, and the genes involved with all stages have yet to be elucidated. The large Francisella pathogenicity island (FPI) was originally identified when inactivation of genes by random transposon mutagenesis resulted in an intracellular growth defect (Gray et al., 2002). For this reason the region was initially named the intracellular growth locus. The FPI contains 19 genes, including iglABCD and pdpABCD, which have been shown to be essential for virulence (Nano et al., 2004). While F. novicida contains only a single copy of the FPI, subspecies tularensis and holarctica both possess two copies (Larsson et al., 2005). This may be one reason for the lower virulence of F. novicida. The functions of the proteins encoded by the FPI are currently the focus of much research. Bioinformatic analysis revealed the FPI to encode a putative type VI secretion system, similar to the systems involved in virulence of Pseudomonas aeruginosa (Mougous et al., 2006) and Vibrio cholerae (Pukatzki et al., 2006). The effectors secreted by the type VI secretion apparatus have yet to be determined. However, the IgABC proteins have received much attention previously due to their contribution to intracellular pathogenesis. IgIA and IgIB appear to associate in the bacterial cytoplasm (de Bruin et al., 2007) and are suggested to be involved in secretion. IgIC is a 23 kDa protein with no homologues identified to date. Francisella-induced apoptosis is dependent on IgIC (Lai et al., 2004), and IgIC appears to downregulate Toll-like receptor signalling to modulate the immune response (Telepnev et al., 2003). Genes within the FPI are regulated by MsgA, which shows high similarity to SspA of Escherichia coli, a stringent response transcriptional regulator. Similarly to SspA, MsgA interacts with
RNA polymerase, but whereas SspA expression is induced at stationary phase MglA expression is maximal during lag and exponential phases of growth (Baron & Nano, 1999; Brotcke et al., 2006). MglA expression is also increased during the early stages of macrophage infection, as would be predicted for a regulator of the genes of the intracellular growth locus (Baron & Nano, 1999). Inactivation of MglA results in a significant intracellular growth defect and attenuation (Baron & Nano, 1998; Lauriano et al., 2004). Transcriptional analysis indicated that a wide range of genes is regulated by MglA, including many outside of the FPI (Brotcke et al., 2006), such as the protease PepO and a putative β-glucosidase BgLX.

In addition to the putative type VI secretion system carried by the FPI, Francisella has also been shown to secrete virulence factors by a type II secretion system. The type II secretion involves genes also involved in expression of type IV pili (Hager et al., 2006). These surface structures have been shown to play an important role in virulence for a range of Gram-negative pathogens (Craig et al., 2004). Several genes in the pili operons show extensive homology with type II secretion genes, and some pathogens, such as P. aeruginosa, may possess multiple type II secretion systems in addition to type IV pili (Stover et al., 2000). Deletion of genes involved in type IV pili biosynthesis is attenuating for subspecies holarctica (Forslund et al., 2006). Spontaneous recombination between direct repeats in the genome of a subspecies holarctica strain designated FSC074 led to the loss of pilA from the pilAEV cluster, resulting in loss of pili production and attenuation in mice challenged by the subcutaneous route of infection (Forslund et al., 2006). Inactivation of pil genes has different effects on virulence depending on the gene inactivated: subspecies holarctica pilC or pilQ mutants retain virulence, while the isogenic pilA mutant is attenuated (Forsberg & Guina, 2007; Forslund et al., 2006). Unexpectedly, a F. novicida pilC mutant was more virulent than wild-type when tested in mice (Hager et al., 2006). The increased virulence appeared to be due to the effect of the mutation not on type IV pilus expression, but rather on the secretion of effector proteins. In contrast to the more virulent subspecies that do not appear to secrete proteins (Lee et al., 2006) in vitro, F. novicida secretes at least seven proteins detectable in culture supernatants, including chitinases, chitin binding protein, PepO and BgLX (Hager et al., 2006). The increased virulence of the F. novicida pilC mutant was linked to a decrease in PepO secretion (Hager et al., 2006). PepO has homology with proteins involved with cleavage of proendothelin. The endothelin thus produced is a potent vasoconstrictor. Thus PepO secretion would result in localized vasoconstriction and therefore limit dissemination of F. novicida from the local site of infection, but abrogating PepO secretion by mutating the pil genes of the secretion machinery would increase dissemination, and thus virulence. The genome sequences of subspecies holarctica and tularensis revealed that pepO has been mutated so it is no longer expressed by these strains, and this appears to have been a key step in the evolution to high virulence in mammalian hosts.

A putative type I secretion system has been proposed for F. tularensis, although this remains to be proven. F. tularensis possesses two orthologues of the E. coli TolC protein, TolC and FtlC (Gil et al., 2006). In E. coli, TolC is the outer membrane channel component used by type I secretion systems and multidrug efflux pumps. Inactivation of tolC and ftlC increased sensitivity to a range of toxic compounds, indicating a role in efflux, but only the tolC mutant was attenuated (Gil et al., 2006). This may reflect differences in substrates between TolC and FtlC efflux pumps, or it may indicate that F. tularensis possesses a type I secretion system, even though the other components of type I secretion have not been identified by homology as yet.

Iron acquisition is recognized as an important virulence trait of many intracellular pathogens, but little was known until recently about how Francisella acquires iron from the mammalian host. Transcriptional analysis of bacteria grown in media with low iron concentrations revealed an iron-responsive operon involved in siderophore production (Deng et al., 2006; Milne et al., 2007). Inactivation of a gene involved in siderophore production resulted in an intracellular growth defect in F. novicida, but not in the subspecies holarctica strain LVS (Deng et al., 2006). However, the siderophore production of the wild-type LVS strain was significantly reduced compared to that of F. novicida, which suggests that mutations elsewhere in the genome were affecting siderophore activity in LVS. The iron-regulated operon also included the siderophore receptor required for uptake of the siderophore–iron complex (Milne et al., 2007). Inactivation of the gene encoding the receptor resulted in attenuation of F. novicida in mice, indicating that iron acquisition is a virulence trait in Francisella similarly to other intracellular pathogens.

A significant proportion of F. tularensis genes are annotated as hypothetical proteins with unknown function, and elucidation of their roles will help in understanding this enigmatic pathogen. Several whole genome sequences are now available for different strains and subspecies of F. tularensis (Table 2). Comparison of the subspecies tularensis strain SCHU S4 with subspecies holarctica strains LVS and OSU18 revealed that, despite high nucleotide identity between the strains, significant rearrangements were observed between the two subspecies (Petrosino et al., 2006). There were 51 syntenic blocks rearranged between SCHU S4 and OSU18 genomes. The rearrangements were due to homologous recombination between the numerous copies of insertion sequence elements around the genome, and multiple copies of rRNA sequences. Despite the repeated DNA sequences being present in subspecies holarctica, no rearrangements were found between the minimally passaged isolate OSU18 and LVS, a highly passaged and attenuated strain (Petrosino et al., 2006). The reason for this genomic stability in subspecies holarctica is not known. Detailed
comparison of the two subspecies *holarctica* strains revealed that LVS and OSU18 are very nearly identical. Considering that LVS was derived in Russia from a European subspecies *holarctica* strain and OSU18 was isolated over three decades later in Oklahoma, USA, this high level of identity is striking, but typing has suggested that subspecies *holarctica* strains show little genetic diversity worldwide (Johansson et al., 2004). It appears, therefore, that the relative attenuation of LVS is due to small sequence variations, insertions and deletions. Comparison of the LVS with the genomes of minimally passaged *holarctica* strains identified, for example, mutations in genes encoding the type IV pilus cluster discussed above, a tyrosine phosphoprotein, a multi-drug efflux pump and a glycosyltransferase (Petrosino et al., 2006; Rohmer et al., 2006). Individually these may not be responsible for attenuation of LVS, but could each contribute subtly and in part to the phenotype.

Comparison of virulent and attenuated strains identified another locus of interest. An empirically attenuated mutant of SCHU S4 possessed a deletion spanning two adjacent genes, *FTT0918* and *FTT0919*, resulting in a novel ORF that encodes a hybrid protein consisting of the N terminus of the protein encoded by the former gene and the C terminus of the protein encoded by the latter gene (Twine et al., 2005). The attenuated subspecies *holarctica* strain LVS possesses a similar deletion. Isogenic deletion of *FTT0918*, but not *FTT0919*, severely attenuated SCHU S4. Combined restoration of *FTT0918* and *pilA* genes in LVS by complementation resulted in a fully virulent phenotype, indicating that these two mutations contribute significantly to the observed attenuation of LVS. The function of *FTT0918* is not known. However, although it has no homology to any other protein in the database, it was identified as being one member of a protein family along with *FTT0919*, *FTT0025*, *FTT0267* and *FTT0602* (Larsson et al., 2005).

With the availability of genome sequence data, the main impediment to understanding the molecular basis of *Francisella* virulence was the paucity of molecular tools with which to manipulate the pathogen, particularly for global mutagenesis approaches. However, shuttle plasmids (Bina et al., 2006; LoVullo et al., 2006; Maier et al., 2004; Rasko et al., 2007), transposons (Gallagher et al., 2007; Kawula et al., 2004; Maier et al., 2006; Weiss et al., 2007) and allelic replacement methodologies (Golovliov et al., 2003; Lauriano et al., 2003; Twine et al., 2005) have all recently been developed. Global mutagenesis of *F. tularensis* subspecies *tularensis* strain SCHU S4 (Qin & Mann, 2006), subspecies *holarctica* strain LVS (Maier et al., 2007; Su et al., 2007) and *F. novicida* (Gallagher et al., 2007; Tempel et al., 2006; Weiss et al., 2007) has resulted in the identification of genes essential for growth in vitro (Gallagher et al., 2007), for intracellular survival in hepatic cells (Qin & Mann, 2006) or macrophages (Maier et al., 2007; Tempel et al., 2006) and in vivo (Su et al., 2007; Weiss et al., 2007). The genes identified as important to the intracellular pathogenesis of *Francisella* could be targets for novel antimicrobial development (Gallagher et al., 2007) or a novel attenuated vaccine. In addition these studies have

<table>
<thead>
<tr>
<th>Sequenced strain</th>
<th>Length (nt)</th>
<th>Sequencing centre</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. tularensis</em> subsp. <em>tularensis</em> SCHU S4</td>
<td>1 892 819</td>
<td>Swedish Defence Research Agency</td>
<td>Fully virulent, A.I clade, laboratory passaged</td>
</tr>
<tr>
<td><em>F. tularensis</em> subsp. <em>holarctica</em> OSU18</td>
<td>1 895 727</td>
<td>Baylor College of Medicine</td>
<td>Minimally passaged</td>
</tr>
<tr>
<td><em>F. tularensis</em> subsp. <em>tularensis</em> FSC198</td>
<td>1 892 616</td>
<td>University of Birmingham</td>
<td>European isolate</td>
</tr>
<tr>
<td><em>F. tularensis</em> subsp. <em>holarctica</em> LVS</td>
<td>1 895 994</td>
<td>Lawrence Livermore National Laboratory</td>
<td>Attenuated, highly passaged</td>
</tr>
<tr>
<td><em>F. novicida</em> U112</td>
<td>1 910 031</td>
<td>University of Washington</td>
<td></td>
</tr>
<tr>
<td><em>F. tularensis</em> subsp. <em>holarctica</em> FTA</td>
<td>1 890 909</td>
<td>US Department of Energy Joint Genome Institute</td>
<td></td>
</tr>
<tr>
<td><em>F. tularensis</em> subsp. <em>tularensis</em> WY96-3418</td>
<td>1 898 476</td>
<td>Translational Genomics Research Institute</td>
<td>A.II clade, CDC collection, isolated from a finger wound in 1996 (Beckstrom-Sternberg et al., 2007)</td>
</tr>
</tbody>
</table>

Unfinished genomes

*F. tularensis* subsp. *holarctica* FSC200 | 1 790 358 | University of Washington | Minimally passaged European isolate |
*F. tularensis* subsp. *tularensis* FSC33 | 1 844 205 | Broad Institute Genome Sequencing Platform | Fully virulent strain, isolated from a squirrel in Georgia, USA (Johansson et al., 2000) |

*F. novicida* GA99-3548 | 1 845 491 | Broad Institute Genome Sequencing Platform | |
*F. novicida* GA99-3549 | 1 897 440 | Broad Institute Genome Sequencing Platform | |

CDC, Centers for Disease Control and Prevention.
provided information about the mechanisms of pathogenesis employed by the organism. For example, one of these global mutagenesis studies identified that bacteria injected subcutaneously passed through a bottleneck before systemic dissemination occurred (Weiss et al., 2007). This bottleneck was not apparent when the mice were challenged by the intranasal route (Su et al., 2007) or intraperitoneally (Weiss et al., 2007). Similar bottlenecks have been reported for enteric pathogens invading from the gut (Mecsas et al., 2001). The nature of the bottleneck is not known, and shows that we still have much to learn about the life of the pathogen in vivo.

**Conclusion**

Until recently, our understanding of the molecular mechanisms of virulence of *Francisella* was significantly impaired by a lack of molecular tools and information. As indicated above, multiple genome sequences are becoming available, but low levels of homology between genes and those of other pathogens have resulted in as many questions being raised as answered. New molecular tools are being developed, and our ability to manipulate the pathogen is improving. Several studies now report global approaches to identifying genes involved in virulence and particularly genes involved in intracellular multiplication. As such, we are getting closer to addressing key issues such as the need for an effective licensable vaccine. It appears we are still some way from understanding the pathogenesis of the organism however.

**References**


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