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Francisella tularensis metabolism and its relation to virulence

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Francisella tularensis is a Gram-negative bacterium capable of causing the zoonotic disease tularaemia in a large number of mammalian species and in arthropods. *F. tularensis* is a facultative intracellular bacterium that infects and replicates *in vivo* mainly inside macrophages. During its systemic dissemination, *F. tularensis* must cope with very different life conditions (such as survival in different target organs or tissues and/or survival in the blood stream…) and may thus encounter a broad variety of carbon substrates, nitrogen, phosphor, and sulfur sources, as well as very low concentrations of essential ions. The development of recent genome-wide genetic screens have led to the identification of hundreds of genes participating to variable extents to *Francisella* virulence. Remarkably, an important proportion of the genes identified are related to metabolic and nutritional functions. However, the relationship between nutrition and the *in vivo* life cycle of *F. tularensis* is yet poorly understood. In this review, we will address the importance of metabolism and nutrition for *F. tularensis* pathogenesis, focusing specifically on amino acid and carbohydrate requirements.

Keywords: *Francisella tularensis*, pathogenesis, metabolism

INTRACELLULAR PATHOGENS AND METABOLIC REQUIREMENTS

The *in vivo* metabolism of pathogenic bacteria constitutes an important, and yet insufficiently studied, aspect of host–pathogen interactions. Metabolic pathways comprise: (i) degradative pathways (catabolism) of organic molecules, processes generally accompanied by the production of energy; and (ii) biosynthetic pathways (anabolism) that uses energy to build-up molecules. Both metabolic pathways require the sequential action of dedicated enzymes whose expression and activity may be tightly regulated in response to environmental changes. In order to survive and efficiently replicate in host cells, intracellular pathogens must adapt their metabolism to the available nutrients and physical conditions (including pH, oxygen availability, osmotic pressure, etc.). Metabolism is tightly associated with nutritional capacities, which involve nutrient availability and dedicated nutrient uptake systems. Indeed, pathogenic bacteria use the host organism as a macronutrient system that comprises many different specialized microenvironments. In particular, bacteria capable of systemic dissemination like *Francisella tularensis* have to cope with very different life conditions (such as survival in different target organs or tissues and/or survival in the blood stream, etc.) and may thus encounter a broad variety of carbon substrates, nitrogen, phosphor, and sulfur sources, as well as very low concentrations of essential ions such as magnesium, manganese, and iron (Eisenreich et al., 2010).

Many intracellular bacteria reside in a vesicular compartment (e.g., *Salmonella, Legionella, Brucella, Mycobacteria,* etc.). These bacteria encounter stressful conditions in these membrane-bound vacuoles (low pH, free-radicals, nutrient deprivation, antimicrobial compounds, etc.) and have therefore developed efficient defense mechanisms. For example, *S. enterica* is able to survive and replicate for extended periods in *Salmonella*-containing vacuoles (SVCs) of infected cells (preferentially macrophages) and thus can cause chronic infections. Notably, *S. enterica*, seems to undergo only limited replication cycles in SVCs. Although the nutritional content of SVCs is still poorly defined, transcriptomic and proteomic analyses have suggested that ions such as magnesium, manganese, and iron, could be limited in the SCV. Furthermore, *in vivo* studies have indicated that sugars, fatty acids, and acetate, could be used as carbon sources (García-del Portillo et al., 2008). Other intracellular bacteria, like *Francisella, Listeria, Shigella*, and *Rickettsia*, have chosen the cytosol as a replication niche (Casadevall, 2008). The host cell cytosol is generally viewed as a more permissive milieu than the phagosomal compartment.

In this review, we shall try to understand the importance of metabolism and nutrition for *F. tularensis* pathogenesis, focusing specifically on amino acid and carbohydrate requirements.

F. TULARENSIS NUTRITIONAL REQUIREMENTS

Francisella tularensis is a Gram-negative bacterium capable of causing the zoonotic disease tularaemia in a large number of mammalian species and in arthropods such as ticks, flies, and mosquitoes (Keim et al., 2007). It is a highly infectious bacterium that can be transmitted to humans in numerous ways, including contact with infected animals, inhalation, ingestion of contaminated water or food, or insect bites (Sjostedt, 2007). Four different subspecies (subsp.) of *F. tularensis* are generally recognized that differ in virulence and geographic distribution. These are four designated subsps. *tularensis* (type A), *holarctica* (type B), *novicida*, and *mediasiatica*, respectively. However, the classification of *novicida* as a subspecies is still a matter of debate (Huber et al., 2010; Johansson et al., 2010). *F. tularensis* subsp. *tularensis* is the most virulent subspecies causing a severe disease in humans, whereas *F. tularensis* subsp. *holarctica* causes a similar disease but of less severity (McLendon et al., 2006). Because of its high infectivity and lethality, *F. tularensis* is considered a potential bioterrorism agent (Oyston and Griffiths, 2009).

Francisella tularensis is a facultative intracellular bacterium that infects and replicates *in vivo* mainly inside macrophages, but which can also infect and survive in a variety of non-phagocytic mammalian cells such as hepatocytes, endothelial cells, epithelial cells, and fibroblasts (Santic et al., 2010). Remarkably, *F. tularensis* is also one of the rare bacteria that can survive within neutrophils (McCaffrey and Allen, 2006). *F. tularensis* subsp. *holarctica* live vaccine strain (LVS) has been shown to inhibit the respiratory burst by preventing NADPH oxidase assembly at the phagosomal membrane (McCaffrey and Allen, 2006). Attempts to identify LVS genes that affect neutrophil function have only led to the selection of uracil auxotrophs (*carA*, *carB*, and *pyrB*) whose intracellular growth defect are most likely pleiotropic.

Francisella tularensis strains, including highly virulent species, have also been reported to survive and multiply in amebae in the environment (Abd et al., 2003; El-Etr et al., 2009), suggesting a potential link between ameba–*Francisella* interactions and environmental persistence. The specific nutritional requirements of *F. tularensis sensu lato* in the ameba have not yet been studied.

The recent availability of complete genome sequences and the development of numerous genome-scale genetic methods have led to the identification of hundreds of genes participating to variable extents to *Francisella* virulence (Ahlund et al., 2010; Akimana et al., 2010; Asare and Abu Kwaik, 2010; Asare et al., 2010; Lai et al., 2010; Meibom and Charbit, 2010, and references therein; Moule et al., 2010; Peng and Monack, 2010). However, the specific contribution of only a limited number of these genes is currently understood at the molecular level. Although an important proportion of the genes identified are related to metabolic and nutritional functions, the relationship between nutrition and the *in vivo* life cycle of *F. tularensis* is yet poorly understood.

F. TULARENSIS BIOSYNTHETIC PATHWAYS AND VIRULENCE

Let us first consider the general relationship between the presence or absence of a biosynthetic pathway and its associated nutritional requirement. By definition, in a synthetic (or minimal) medium, a prototrophic (heterotrophic) facultative intracellular bacterium is able to synthesize all its components from the carbohydrate source provided. When one or several genes of a biosynthetic pathway are missing, or have been inactivated, supplementation of the medium by the substrate is required for growth (the bacterium is auxotroph for this substrate). In infected cells, two outcomes exist when a biosynthetic pathway is impaired in a mutant strain: (i) if the mutant bacteria require supplementation of the medium by the cognate substrate for intracellular growth, it is deduced that the intracellular milieu is depleted (or limited) for that substrate; (ii) alternatively, if the mutant bacteria grow like the wild-type strain, this is deduced the intracellular milieu is replete in that substrate (and implies that the bacterium is able to take-up enough substrate for growth).

A chemically defined growth medium was developed to support the growth of *F. tularensis,* which includes 13 amino acids (Traub et al., 1955; Nagle et al., 1960). Later, Chamberlain optimized the concentrations of the different components (amino acids, vitamins, ions) of this medium that is still widely used (Chamberlain, 1965). *F. tularensis* strains Schu S4 (subsp. *tularensis*) and LVS (subsp. *holarctica*) both require cysteine for growth, most likely due to a non-functional pathway for sulfate assimilation (Larsson et al., 2005). The absolute requirement for growth of the other 12 amino acids contained in the medium has not been experimentally confirmed. Functional biosynthetic pathways have been identified in the Schu S4 genome for the seven non-essential amino acids (alanine, asparagine, glutamate, glycine, glutamine, phenylalanine, and tryptophan). Evidence was also found in the Schu S4 genome for biosynthetic pathways for 8 of the 13 amino acids that are supplied in the synthetic medium (see **Table 1**; Larsson et al., 2005). However, of these eight, the biosynthetic pathways for isoleucine, valine, and threonine are predicted to have missing steps and therefore to be non-functional (due to presence of pseudogenes encoding enzymes catalyzing the missing steps in the pathways). Altogether, the pathways for arginine, histidine, lysine, tyrosine, methionine, cysteine, threonine, valine, and isoleucine biosynthesis seem to be incomplete or absent. *F. tularensis* subsp. *tularensis* strain Schu S4 is hence auxotroph for these amino acids. It remains to be determined whether one or more of the other four amino acids for which pathways were predicted to be present, but supplied by the synthetic medium (i.e., serine, aspartate, leucine, and proline), are absolutely required for growth.

Aromatic amino acid biosynthetic pathways

The shikimate pathway (**Figure 1**) is the common pathway for the biosynthesis of chorismate, which is the precursor for the generation of aromatic amino acids, *para*-aminobenzoic acid (*p*ABA,

Table 1 | Nutritional requirements of *F. tularensis*.

Amino acids	Biosynthetic pathway predicted	Pathway score*
REQUIRED FOR GROWTH IN SYNTHETIC BROTH		
Arginine	No	–
Histidine	No	–
Lysine	No	–
Methionine	No	–
Tyrosine	No	–
Cysteine (incomplete/required)	Yes	5/3/3
Isoleucine (incomplete/required)	Yes	5/4/1
Threonine (incomplete/required)	Yes	2/1/0
Valine (incomplete/required)	Yes	4/3/2
Serine (required ?)	Yes	3/2/2
Aspartic acid (required ?)	Yes	1/1/1
Leucine (required ?)	Yes	4/2/0
Proline (required ?)	Yes	4/2/1
NOT REQUIRED FOR GROWTH IN SYNTHETIC BROTH		
Alanine	Yes	3/2/2
Asparagine	Yes	2/1/0
Glutamate	Yes	1/1/1, 1/1/1, 1/1/1
Glutamine	Yes	2/2/1
Glycine	Yes	2/1/1, 1/1/1
Phenylalanine	Yes	3/2/2, 4/2/2, 3/2/2
Tryptophan	Yes	5/5/0

In the F. tularensis subsp. tularensis Schu S4 genome. Each predicted pathway, P, was assigned a score X/Y/Z, according to Larsson et al. (2005). P consists of X reactions; enzymes for Y reactions were identified in the genome; and Z of the Y reactions are used in other predicted pathways.

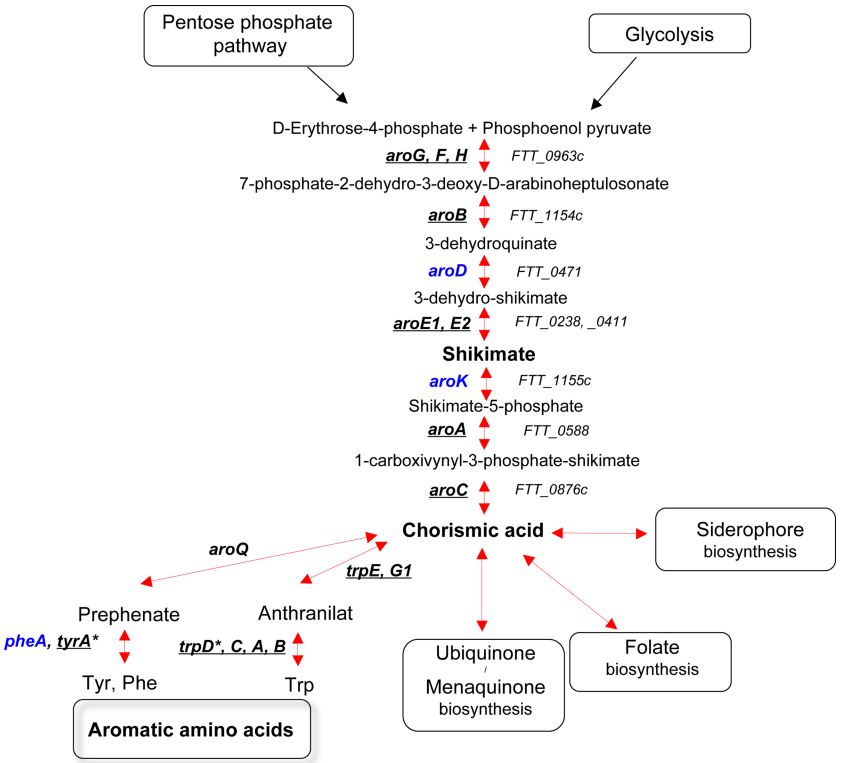

FIGURE 1 | The shikimate pathway. Genes that have been identified in genetic screens (*in vivo* or *in vitro*) are underlined. Genes that have not been hit in any screen are in blue. TrpD*, the gene is absent in the Schu S4 and LVS strains but present in the subsp. *novicida* (FTA_2078). TyrA*, the gene is absent in the Schu S4 strain but present in both LVS (FTL_048) and subsp. *novicida* (FTN_0055).

folate biosynthesis), 2,3-dihydroxybenzoic acid (DHB, biosynthesis of siderophores), ubiquinone, and menaquinone (Bentley, 1990; **Figure 1**). Notably, *F. tularensis sensu lato* has been shown to express a siderophore under iron limiting conditions. This siderophore, structurally similar to rhizoferrin, promotes the growth of both LVS and Schu S4 strains under iron limitation. The siderophore locus, designated *fsl* in *F. tularensis* subsp. *tularensis* (Schu S4) and *F. tularensis* subsp. *holarctica* (LVS), or *fig* in *F. tularensis* subsp. *novicida*, is involved in both synthesis and uptake of the siderophore (Sullivan et al., 2006; Kiss et al., 2008; Ramakrishnan et al., 2008; Crosa et al., 2009).

Bruce Stocker's pioneer work on the genetics of *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) has demonstrated the crucial importance of the aromatic biosynthetic pathway for bacterial virulence. Mutations in different genes coding for the biosynthesis of aromatic amino acids have been used to reduce the virulence of *Salmonella* strains and for immunization of various animal species (Chatfield et al., 1994, 1995). The attenuation of *aro* mutants of *S. typhimurium* is thought to be due to the inability of the bacterium to generate *p*ABA and DHB from chorismate (Hoiseth and Stocker, 1981). Remarkably, *aroA* and *aroD* mutants of *S. enterica* serovar Typhi were also successfully tested as a vaccine against human typhoid (Tacket and Levine, 2007).

All of the necessary genes encoding the chorismate pathway enzymes are present in the genome of *F. tularensis* Schu S4 (i.e., *aroG, aroB, aroD, aroE, aroK, aroA*, and *aroC*). The genes (*pabA* and *pabB*) encoding the two components of *para*-aminobenzoate synthase have also been identified in the *F. tularensis* Schu S4 genome as well as the gene encoding FolP, FolC, and FolA, involved in folate biosynthesis. In recent screens, we and others (Su et al., 2007; Alkhuder et al., 2009) have selected mutants in gene *FTL_1240* (*aroG*), encoding DAHP synthase. This is the first enzyme of the aromatic amino acid biosynthetic pathway, which converts erythro-4-phosphate and phosphoenolpyruvate (PEP) to 3-deoxy-D-arabino-heptuosonate-7-phosphate. We have shown that an *aroG* mutant of LVS had only a slightly reduced intracellular growth capacity, in both J774 cells and in bone marrow-derived macrophages (BMM). Still, in the mouse model, the mutant strain was very severely attenuated. Of interest, wild-type *Escherichia coli* has been shown to produce three feedback inhibitor-sensitive DAHP synthase isoenzymes: a tyrosine-sensitive, a phenylalanine-sensitive, and a tryptophan-sensitive enzyme (encoded by genes genes *aroF, aroG*, and *aroH*, respectively). Hence, the functionality of a biosynthetic pathway does not only depend on the presence of intact genes but may also rely on the amount and activity of the enzymes.

This interplay between the availability of an amino acid and the activity of its cognate biosynthetic pathway might exist for other types of nutrients that the bacterium can either biosynthesize or acquire from its growth medium.

Additional genes of the *F. tularensis* aromatic amino acid biosynthetic pathway have been identified in genome-wide screens: *aroA, aroB, aroC, aroE1, tyrA, trpA, trpB, trpC*, and *trpE*. The gene

aroA is responsible for the sixth step of the biosynthetic pathway (converting shikimate-3-phosphate and PEP to 5-enolpyruvyl-shikimate-3-phosphate). An *aroA* mutant of *F. tularensis* Schu S4 strain was selected in a screen of transposon insertion mutants performed in the human hepatic carcinoma cell line HepG2 (Qin and Mann, 2006). The same screen also led to the identification of purine and pyrimidine auxotrophs (see below).

An *aroB* mutant of *F. tularensis* subsp. *novicida* (*FTN_1135*), encoding a putative 3-dehydroquinate synthetase, has been very recently identified in a screen in human macrophages (Asare and Abu Kwaik, 2010). The mutant strain, which was also deficient for growth in *Drosophila melanogaster*-derived S2 cells, localized to the cytosol in macrophages, indicating a cytosolic growth defect.

The gene *aroC* encodes chorismate synthase, the seventh step of the aromatic biosynthetic pathway. It was identified in a screen of transposon insertion mutants performed in *F. tularensis* subsp. *novicida*, searching for genes required for pulmonary and systemic infection in mice (Kraemer et al., 2009). The gene *tyrA* (*FTN_0055*), which encodes the enzyme prephenate dehydrogenase converting chorismate to tyrosine, was also hit in this *in vivo* screen, as was *aroC*. Notably, the *tyrA* gene is absent in the Schu S4 strain but present in LVS (*FTL_0048*). *aroC* encodes the enzyme performing the last step of chorismic acid synthesis. The gene *aroE1,* which encodes shikimate-5-dehydrogenase, the fourth step of the biosynthetic pathway, was identified in two screens of transposon insertion mutants. One screen was performed in *F. tularensis* subsp. *holarctica* LVS searching for auxotrophic mutants unable to grow on chemically defined medium (Maier et al., 2006) and the other was a screen of *F. tularensis* subsp. *novicida* for mutants unable to replicate intracellularly in macrophages (Asare and Abu Kwaik, 2010). Finally, five genes (*trpA, trpB, trpC, trpE,* and *trpG1*) encoding enzymes involved in the conversion of chorismic acid to tryptophan were identified in three *in vivo* selections using *F. tularensis* subsp. *novicida* (Weiss et al., 2007; Kraemer et al., 2009; Peng and Monack, 2010).

Altogether, almost every gene in the aromatic amino acids biosynthetic pathway has been identified in mutant screens, highlighting the importance this pathway for *F. tularensis* virulence. Interestingly, several of the genes have also been found to be important for growth/virulence in a non-mammalian model, *in vivo* in *D. melanogaster* or *in vitro* in *D. melanogaster*-derived cells (Asare et al., 2010; Moule et al., 2010).

Purine and pyrimidine biosynthetic pathways

In silico analysis reveals that the *F. tularensis* genomes encode all the enzymes necessary for the *de novo* synthesis of purines and pyrimidines. The two pathways are virtually identical to those in *E. coli*.

Pur pathway. Several pur auxotrophic mutants of *F. tularensis* (**Figure 2**), selected from banks of mutants or genetically engineered, have already been obtained and tested for virulence (Gray et al., 2002; Qin and Mann, 2006; Tempel et al., 2006; Quarry et al., 2007; Titball et al., 2007; Weiss et al., 2007; Kadzhaev et al., 2009; Asare and Abu Kwaik, 2010; Asare et al., 2010; Peng and Monack, 2010). The pur mutants with single gene mutations showed variable degrees of attenuation *in vivo* and growth defects *in vitro*, suggesting that the step at which the pathway is inactivated may have a distinct impact

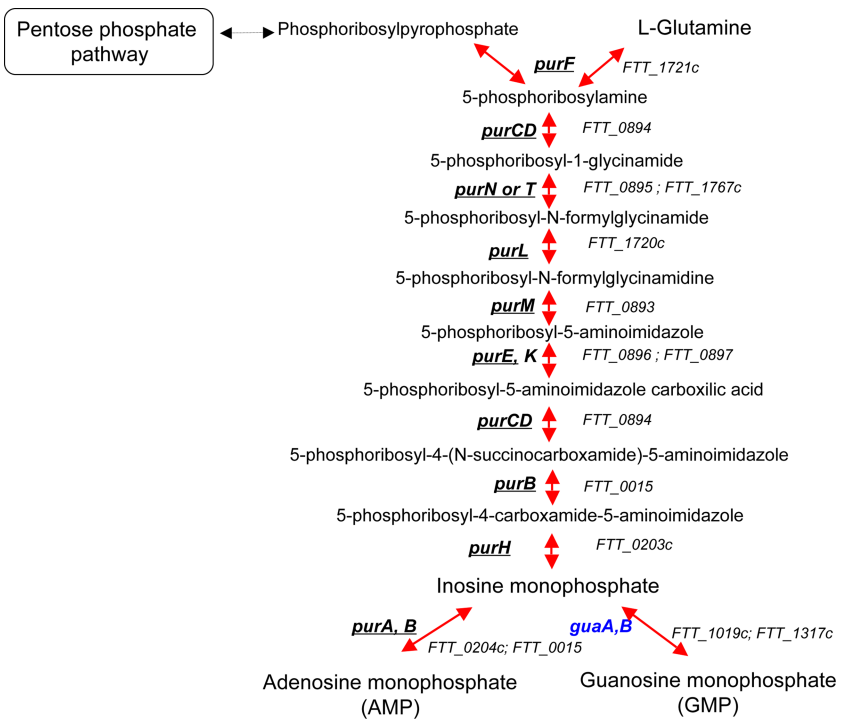

FIGURE 2 | The purine biosynthetic pathway. Genes that have been identified in genetic screens (*in vivo* or *in vitro*) are underlined. Genes that have not been hit in any screen are in blue.

on virulence. Remarkably, Pechous et al. showed that a triple mutant *ΔpurMCD* in both LVS (Pechous et al., 2006) and Schu S4 (Pechous et al., 2008) strains led to severe intracellular growth defects and strong attenuation in the mouse model. The fact that inability of these strains to synthesize purines *de novo* leads to a severe intracellular growth defect supports the notion that macrophages contain limiting concentrations of purines (Appelberg, 2006).

Pyr pathway. The pathway converting L-glutamine to uridine monophosphate (UMP) comprises six steps (**Figure 3**). Several mutants in this pathway have been identified in genetic screens (Qin and Mann, 2006; Weiss et al., 2007; Schulert et al., 2009; Asare and Abu Kwaik, 2010; Peng and Monack, 2010). Mutants unable to perform the first step, i.e., bacteria with mutations in genes *carA* or *carB* (encoding the two subunits of carbamoyl phosphate synthetase, converting L-glutamine to carbamoyl-P) were severely impaired in intramacrophage growth. However, conflicting results were reported regarding the phenotype of *pyrB* mutants. On one hand, a pyrB transposon insertion mutant of Schu S4 (Qin and Mann, 2006) showed reduced growth in HepG2 hepatocytes but normal growth in J774 cells, and was attenuated in mice and a similar mutant in subsp. *novicida* was shown to have a growth defect in human macrophages (Asare and Abu Kwaik, 2010). In contrast, another recent study (Kadzhaev et al., 2009) reports that a *pyrB* deletion mutant of Schu S4 is barely attenuated. In addition, mutants with transposon insertions in the *carA, carB*, and *pyrB* genes were selected upon screening of a bank of transposon mutants in LVS for mutants that failed to prevent the oxidative burst (Schulert et al., 2009). The three mutants appeared to grow normally in HepG2 and J774 cells but were killed by human monocytes and monocyte-derived macrophages. Transposon mutants of subsp. *novicida* with insertions in *pyrF* (encoding orotidine-5P decarboxylase, converting orotidine-5P to UMP) were identified in two *in vivo* screens (Weiss et al., 2007; Peng and Monack, 2010) and recently, a *pyrF* deletion mutant was generated in both *F. tularensis* subsp. *holarctica* (LVS) and *tularensis* (Schu S4). These *F. tularensis* Δ*pyrF* mutants were unable to replicate in primary human macrophages but retained full virulence in the mouse model (Horzempa et al., 2010).

These data clearly indicate that defects in the pyrimidine biosynthetic pathway affect *Francisella* virulence in a cell type- and strain-specific manner and suggest that *in vivo* the *pyr* pathway is not as important as the *pur* pathway for *F. tularensis* virulence.

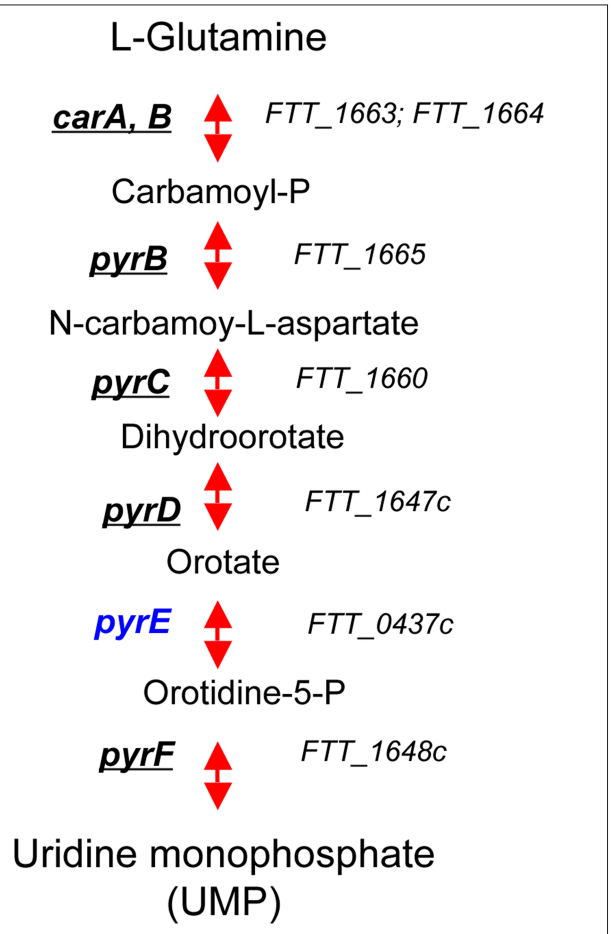

FIGURE 3 | The pyrimydine biosynthetic pathway. Genes that have been identified in genetic screens (*in vivo* or *in vitro*) are underlined. Genes that have not been hit in any screen are in blue.

F. TULARENSIS CARBON METABOLISM AND VIRULENCE

Each intracellular pathogen has adapted its intracellular metabolism to the nutrient supply of the host cell. Nevertheless, two bacterial species using the same host cell compartment (facing thus the same nutritional environment) may have quite different preferred carbon sources (and hence carbon metabolisms). Carbon catabolism provides the bacterial cell with energy and essential biosynthetic precursors such as glucose-6-phosphate (G6-P), fructose-6-phosphate (F6-P), 3-phosphoglycerate, PEP, and acetyl-CoA.

Glycolysis and gluconeogenesis pathways

Hexoses such as glucose are the preferred carbon and energy sources for many bacteria. The three best-characterized pathways of sugar catabolism in bacteria are glycolysis (**Figure 4**), the pentose phosphate pathway and the Entner–Doudoroff pathway. Each of these different pathways can be the preferred, or exclusive, carbon utilization pathway in a given pathogenic bacterial species. In the following we will discuss only genes encoding enzymes in glycolysis (and gluconeogenesis) and shown in **Figure 4** and not other carbohydrate metabolism pathways.

pckA. The enzyme PEP carboxykinase, encoded by the gene *pckA*, catalyzes the conversion of oxaloacetate to PEP. In *Mycobacterium bovis* BCG, a *pckA* mutant is attenuated both *in vitro* and *in vivo* (Liu et al., 2003), while this gene is dispensable in *Salmonella* (Tchawa Yimga et al., 2006). In *F. tularensis* subsp. *tularensis* Schu S4, Kadzhaev et al. (2009) observed no attenuation of a Δ*pckA* mutant in mice. The biological activity of this enzyme has not been experimentally established.

glpX. The glycolytic and gluconeogenic pathways comprise essentially the same set of enzymes that catalyze reversible reactions, except between F6-P and fructose-1,6-bisphosphate (F1,6-P2). The glycolytic reaction leading to the production of F1,6-P2 is catalyzed by phosphofructokinase, while the gluconeogenic reaction

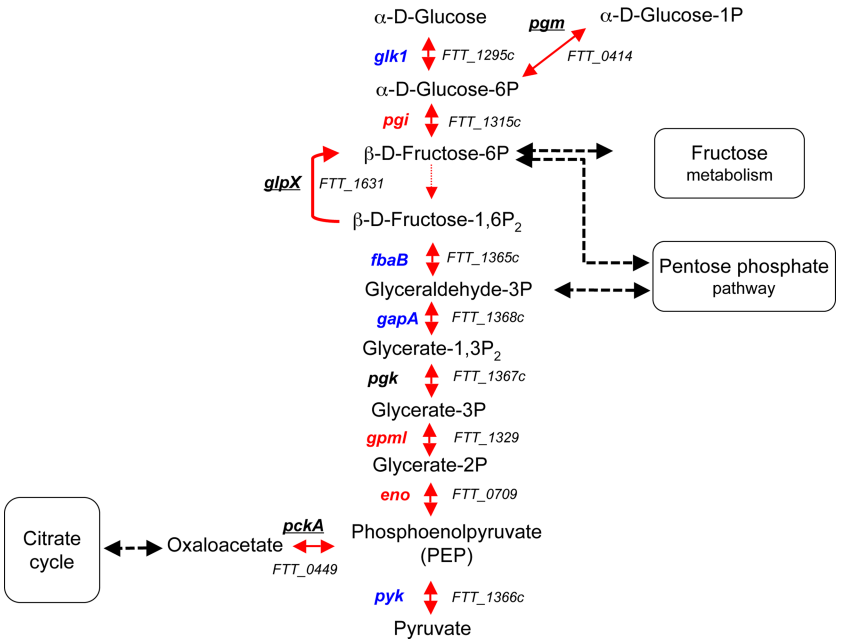

FIGURE 4 | The glycolytic and gluconeogenic pathways. GlpX is the only enzyme not promoting a reversible reaction. Genes that have been identified in genetic screens (*in vivo* or *in vitro*) are underlined. Genes that have not been hit in any screen are in blue. Genes predicted to be essential (according to Gallagher et al., 2007) are in red. The black dotted arrows indicate the connection between the glycolytic/gluconeogenic pathways and the pentose phosphate and fructose pathways. In each pathway, the gene name is indicated to the left of each reaction and the corresponding FTT number, to the right.

yielding F6P is catalyzed by fructose-1,6-bisphosphatase (FBP). The *F. tularensis* subsp. tularensis Schu S4 genome encodes apparently only a FBP (encoded by *glpX*) and lacks a *pfkA* gene encoding phosphofructokinase (Raghunathan et al., 2010). This suggests that *F. tularensis* uses the Embden–Meyerhof–Parnas pathway for gluconeogenesis rather than for glycolysis. However, the exact biological activity of the FBP enzyme remains to be experimentally established in *F. tularensis*. Mutants in the *glpX* gene have been repeatedly obtained in genetic screens, *in vivo* as well as *in vitro* (Maier et al., 2007; Su et al., 2007; Weiss et al., 2007; Kraemer et al., 2009; Peng and Monack, 2010). Moreover, a *glpX* deletion mutant of *F. tularensis* subsp. *tularensis* Schu S4 has been shown recently to be almost avirulent in the mouse model (Kadzhaev et al., 2009). Altogether, these observations strongly suggest that gluconeogenesis is critical for the full virulence of *F. tularensis*. At this stage, it cannot be excluded that the virulence defect of the *glpX* mutant could be due to another function of this enzyme, possibly regulatory. Supporting this hypothesis, none of the other genes involved in the conversion of glucose to PEP has been identified in previous genetic screens, but it is possible that some of these (*pgi, gpmI, eno*) are essential genes and therefore will not be found in screens (Gallagher et al., 2007). It remains to be determined by a systematic mutagenesis approach whether other enzymes in this pathway may also be involved in virulence.

pgm. The *pgm* gene encodes a predicted phosphoglucomutase, a glyconeogenic enzyme involved in the reversible conversion of glucose-1-phosphate (G1-P) to G6-P. A mutant in the *pgm* gene has been identified after an *in vivo* genetic screen in the *F. tularensis* subsp. *novicida* (Weiss et al., 2007).

The intracellular transcriptome of *F. tularensis* reveals that a number of genes involved in carbohydrate metabolism were up-regulated in BMM (Wehrly et al., 2009). In particular, genes of the glycolytic/gluconeogenic pathway have been found (*pgm, glk, fbaB,* and *pgk*; **Figure 4**), suggesting that this pathway is used during intracellular growth.

Very recently, a systems biology approach was applied to identify *F. tularensis* metabolic networks (Raghunathan et al., 2010). Integration of *in silico* metabolic reconstitutions and experimental data (including metabolic profiling and transcriptomic analyses) suggested that significant changes in carbohydrate metabolism occur during the intracellular growth phase. Gene expression profiling further supported the prediction that *F. tularensis* preferentially utilizes specific amino acids for energy and fatty acids as gluconeogenic substrates rather than relying on carbohydrate sources like glucose and fructose during infection.

NUTRIENT UPTAKE SYSTEMS OF *F. TULARENSIS*

Intracellular bacteria must possess dedicated nutrient uptake systems to capture their necessary host-derived nutrients. These systems must be particularly efficient for substrates available in limiting concentrations. The majority of the predicted transport systems present in *F. tularensis* are secondary carriers (**Table 2**). Secondary transporters encompass several major families, including: (i) the major facilitator superfamily (MFS, 31 proteins), predicted to participate in various functions including drug efflux, amino acids and sugar uptake; (ii) the amino acid-polyamine-organocation transporters (APC, 11 proteins); (iii) the hydroxy/aromatic amino acid permeases (HAAAP, 7 proteins); and (iv) the proton-dependent oligopeptide transporters (POT, 8 proteins).

Table 2 | The transport systems of *F. tularensis*.

ORF	Family ID[a]	Substrate	Experimental data[b]
ION CHANNELS			
FTT0685c	VIC	Potassium ion channel	Identified screen (Weiss et al., 2007)
FTT1775c	ClC	Chloride ion channel	–
FTT0475	MscS	Mechanosensitive channel	Identified screen (Asare et al., 2010)
FTT0992	MscS	Mechanosensitive channel	Identified screen (Asare et al., 2010)
FTT0133	MIP	Glycerol uptake	–
FTT1342	LIC	Glutamate-gated chloride channel	Identified screen (Asare and Abu Kwaik, 2010)
			Down-regulated in cells (Wehrly et al., 2009)
SECONDARY TRANSPORTERS			
FTT0006	MFS	Proline/betaine	–
FTT0026c	MFS	Drug efflux, Iron transport	Identified screen (Asare et al., 2010)
			Up-regulated in cells (Wehrly et al., 2009)
FTT0028c	MFS	Multidrug efflux, Iron transport	Identified screen (Weiss et al., 2007; Asare et al., 2010; Moule et al., 2010)
			Up-regulated in cells (Wehrly et al., 2009)
FTT0053	MFS	?	Identified screen (Maier et al., 2007; Weiss et al., 2007; Moule et al., 2010)
FTT0056c	MFS	?	Identified screen (Qin and Mann, 2006; Weiss et al., 2007; Moule et al., 2010; Peng and Monack, 2010)
FTT0070c	MFS	AmpG homolog	MglA regulated (Brotcke et al., 2006)
FTT0104c	MFS	?	Identified screen (Kraemer et al., 2009; Asare and Abu Kwaik, 2010)
FTT0127c	MFS	Multidrug efflux?	Identified screen (Kraemer et al., 2009)
FTT0129	MFS	?	Identified screen (Qin and Mann, 2006; Asare et al., 2010)
FTT0164c	MFS	Bicyclomycin efflux	Identified screen (Asare et al., 2010)
FTT0280c	MFS	Multidrug efflux?	–
FTT0442c	MFS	Bicyclomycin efflux	Down-regulated in cells (Wehrly et al., 2009)
FTT0444	MFS	Tetracycline efflux	Identified screen (Su et al., 2007)
FTT0488c	MFS	?	Identified screen (Asare et al., 2010)
			Down-regulated in cells (Wehrly et al., 2009)
FTT0671	MFS	?	–
FTT0708	MFS	?	Identified screen (Su et al., 2007; Asare et al., 2010)
			Up-regulated in cells (Wehrly et al., 2009)
FTT0719	MFS	Proline/betaine	Identified screen (Asare et al., 2010)
FTT0725c	MFS	Glycerol-3-phosphate	Identified screen (Asare and Abu Kwaik, 2010)
FTT0804	MFS	Proline/betaine	Down-regulated in cells (Wehrly et al., 2009)
FTT0931	MFS	Glucose/galactose	–
FTT0995	MFS	Proline/betaine	Identified screen (Asare et al., 2010; Moule et al., 2010)
FTT1148c	MFS	?	–
FTT1196c	MFS	Proline/betaine	Identified screen (Peng and Monack, 2010)
FTT1256	MFS	Multidrug efflux	Identified screen (Asare et al., 2010)
			Down-regulated in cells (Wehrly et al., 2009)
FTT1291	MFS	D-Galactonate	–
FTT1311	MFS	Multidrug efflux	Identified screen (Moule et al., 2010)
FTT1473c	MFS	Galactose/proton symport	Identified screen (Asare et al., 2010)
FTT1474c	MFS	Galactose/proton symport	Identified screen (Asare and Abu Kwaik, 2010)
FTT1683c	MFS	Multidrug efflux	–
FTT1727c	MFS	Chloramphenicol efflux	Down-regulated in cells (Wehrly et al., 2009)
FTT1783	MFS	Proline/betaine	Identified screen (Moule et al., 2010)
FTT0446	POT	Dipeptide/tripeptide/oligopeptide	Identified screen (Tempel et al., 2006)
			Down-regulated in cells (Wehrly et al., 2009)
FTT0572	POT	Dipeptide/tripeptide/oligopeptide	Up-regulated in cells (Wehrly et al., 2009)
FTT0651	POT	Dipeptide/tripeptide/oligopeptide	MglA-regulated (Brotcke et al., 2006)

(Continued)

Table 2 | Continued

ORF	Family ID[a]	Substrate	Experimental data[b]
FTT0686c	POT	Dipeptide/tripeptide/oligopeptide	Identified screen (Asare and Abu Kwaik, 2010) Up-regulated in cells (Wehrly et al., 2009)
FTT0953c	POT	Dipeptide/tripeptide/oligopeptide	Up-regulated cells (Wehrly et al., 2009)
FTT1005c	POT	Dipeptide/tripeptide/oligopeptide	Identified screen (Asare and Abu Kwaik, 2010; Moule et al., 2010)
FTT1233c	POT	Dipeptide/tripeptide/oligopeptide	–
FTT1253	POT	Dipeptide/tripeptide/oligopeptide	Identified screen (Kraemer et al., 2009) Up-regulated in cells (Wehrly et al., 2009)
FTT0219c	PiT	Phosphate	–
FTT1630c	SSS	Sodium ion/proline	Identified screen (Kraemer et al., 2009; Asare and Abu Kwaik, 2010)
FTT0598c	DAACS	Sodium ion/dicarboxylate	Up-regulated in cells (Wehrly et al., 2009)
FTT1337c	DAACS	C4-dicarboxylate	Identified screen (Asare et al., 2010)
FTT0849	BASS	Sodium ion/bile acid	Identified screen (Asare et al., 2010)
FTT0310	APC	Amino acid	MglA-regulated (Brotcke et al., 2006)
FTT0361c	APC	Glutamate/γ-aminobutyrate	Identified screen (Kraemer et al., 2009; Asare et al., 2010)
FTT0480c	APC	Glutamate/γ-aminobutyrate	Identified screen (Maier et al., 2007; Weiss et al., 2007; Kraemer et al., 2009; Peng and Monack, 2010)
FTT0881c	APC	Amino acid	Identified screen (Su et al., 2007)
FTT0968c	APC	Amino acid	Identified screen (Weiss et al., 2007; Su et al., 2007; Ahlund et al., 2010; Moule et al., 2010; Peng and Monack, 2010)
FTT0979c	APC	Amino acid	–
FTT1020c	APC	Amino acid	Identified screen (Moule et al., 2010)
FTT1149c	APC	Amino acid	Identified screen (Asare et al., 2010) Mutant constructed (Kadzhaev et al., 2009)
FTT1520c	APC	Glutamate/γ-aminobutyrate	–
FTT1633c	APC	Lysine	Identified screen (Weiss et al., 2007; Peng and Monack, 2010)
FTT1730c	APC	Amino acid	–
FTT1760	NhaA	Sodium ion/proton	Identified screen (Asare et al., 2010)
FTT0268	CPA1	Sodium ion/proton	Identified screen (Alkhuder et al., 2009; Kraemer et al., 2009)
FTT0604	CPA1	Sodium ion/proton	–
FTT1490	CPA1	Sodium ion/proton	Identified screen (Qin and Mann, 2006; Maier et al., 2007; Weiss et al., 2007; Moule et al., 2010)
FTT0669	CPA2	Potassium ion efflux	–
FTT1638	Trk	Potassium ion uptake	Identified screen (Alkhuder et al., 2010)
FTT0756	CDF	Cation efflux	Identified screen (Weiss et al., 2007; Asare and Abu Kwaik, 2010)
FTT0885	CDF	Cation efflux	–
FTT0827c	NCS2	Xanthine/uracil	–
FTT0115	CNT	Nucleosides	Identified screen (Moule et al., 2010)
FTT0116	CNT	Nucleosides	Identified screen (Moule et al., 2010)
FTT0712c	HAAAP	Serine	Identified screen (Weiss et al., 2007; Asare and Abu Kwaik, 2010)
FTT1126	HAAAP	Aromatic amino acid	–
FTT1502	HAAAP	Tyrosine	–
FTT1510c	HAAAP	Tyrosine	–
FTT1668	HAAAP	Serine	Identified screen (Asare and Abu Kwaik, 2010)
FTT1688	HAAAP	Tyrosine	Identified screen (Maier et al., 2007; Weiss et al., 2007; Asare et al., 2010)
FTT1732c	HAAAP	Tyrosine	Identified screen (Asare and Abu Kwaik, 2010)
FTT0853	ArsB	Arsenite (ArsB)	–
FTT1339c	SulP	Sulfate	–
FTT0105c	RND	Multidrug efflux	Identified screen (Su et al., 2007) Mutant constructed (Bina et al., 2008)
FTT1115c	RND	Protein-export (SecDF)	–

(Continued)

Table 2 | Continued

ORF	Family ID[a]	Substrate	Experimental data[b]
FTT1114c	RND	Protein-export (SecDF)	–
FTT1728	NhaD	Sodium ion/proton	Identified screen (Asare et al., 2010)
FTT0368c	MOP	Virulence factor MviN	Identified screen (Asare et al., 2010)
FTT1332	MOP	Polysaccharide	–
FTT1453c	MOP	O-antigen	–
FTT0157c	DMT	Choline uptake (LicB)	–
FTT0759	DMT	Drug/metabolite?	Identified screen (Su et al., 2007)
FTT1004c	DMT	Drug/metabolite?	Identified screen (Asare et al., 2010)
FTT1118c	DMT	Drug/metabolite?	Identified screen (Kraemer et al., 2009; Asare and Abu Kwaik, 2010)
FTT1399	DMT	Drug/metabolite?	Down-regulated in cells (Wehrly et al., 2009)
FTT1511	DMT	Drug/metabolite?	–
FTT1787c	LysE	Lysine efflux	–
FTT1431	RhtB	Threonine efflux	–
FTT0829c	AAE	Aspartate/alanine	Identified screen (Kraemer et al., 2009; Moule et al., 2010)
FTT0233c	Oxa1	OxaA homolog	Down-regulated in cells (Wehrly et al., 2009)
ATP-DEPENDENT			
FTT0109	ABC	Lipid A export	–
FTT0126	ABC	Oligopeptide	–
FTT0125	ABC	Oligopeptide	Mutant constructed, not attenuated (Kadzhaev et al., 2009) MglA-regulated (Brotcke et al., 2006)
FTT0175c	ABC	Nitrate/sulfonate/taurine	Identified screen (Asare et al., 2010) Up-regulated in cells (Wehrly et al., 2009)
FTT0265	ABC	Nitrate/sulfonate/taurine	–
FTT0266	ABC	Nitrate/sulfonate/taurine	Identified screen (Asare and Abu Kwaik, 2010)
FTT0209c	ABC	Manganese/zinc ion	Identified screen (Su et al., 2007; Asare et al., 2010) Up-regulated in cells (Wehrly et al., 2009)
FTT0208c	ABC	Manganese/zinc ion	Identified screen (Asare and Abu Kwaik, 2010)
FTT0207c	ABC	Manganese/zinc ion	–
FTT0405	ABC	Lipoprotein releasing	Identified screen (Weiss et al., 2007)
FTT0404	ABC	Lipoprotein releasing	Down-regulated in cells (Wehrly et al., 2009)
FTT0481	ABC	Putrescine	–
FTT0564	ABC	Putrescine	Identified screen (Kraemer et al., 2009)
FTT0563	ABC	Putrescine	–
FTT0562	ABC	Putrescine	Identified screen (Asare and Abu Kwaik, 2010)
FTT0728	ABC	Multidrug	–
FTT0729	ABC	Multidrug	–
FTT0793	ABC	Multidrug	Identified screen (Weiss et al., 2007; Asare and Abu Kwaik, 2010; Peng and Monack, 2010)
FTT0972	ABC	? (Fe–S assembly/SufBCD system)	–
FTT0971	ABC	? (Fe–S assembly/SufBCD system)	–
FTT0973	ABC	? (Fe–S assembly/SufBCD system)	Identified screen (Kraemer et al., 2009)
FTT1024c	ABC	? (YhbG)	–
FTT1125	ABC	D-Methionine	Identified screen (Maier et al., 2007; Su et al., 2007; Kraemer et al., 2009; Asare et al., 2010) Up-regulated in cells (Wehrly et al., 2009)
FTT1124	ABC	D-Methionine	Identified screen (Maier et al., 2007) Up-regulated in cells (Wehrly et al., 2009)

(Continued)

Table 2 | Continued

ORF	Family ID[a]	Substrate	Experimental data[b]
FTT1248	ABC	?	Identified screen (Asare et al., 2010)
FTT1247	ABC	?	–
FTT1608	ABC	Toluene tolerance	–
FTT1249	ABC	Toluene tolerance	Identified screen (Moule et al., 2010)
FTT1611	ABC	Toluene tolerance	Identified screen (Su et al., 2007; Asare et al., 2010)
FTT1609	ABC	Toluene tolerance	–
FTT1335	ABC	CydC/CydD homolog	–
FTT1336	ABC	CydC/CydD homolog	–
FTT1435c	ABC	Multidrug?	–
FTT1434c	ABC	Multidrug?	Identified screen
FTT1782c	ABC	?	Identified screen (Su et al., 2007; Weiss et al., 2007; Moule et al., 2010)
FTT0060	F-ATPase	Protons	–
FTT0064	F-ATPase	Protons	–
FTT0059	F-ATPase	Protons	–
FTT0063	F-ATPase	Protons	–
FTT0058	F-ATPase	Protons	–
FTT0062	F-ATPase	Protons	–
FTT0061	F-ATPase	Protons	Identified screen (Peng and Monack, 2010)
FTT0065	F-ATPase	Protons	Identified screen (Kraemer et al., 2009)
FTT1737c	P-ATPase	Potassium ion	Identified screen (Asare and Abu Kwaik, 2010; Moule et al., 2010)
FTT1738c	P-ATPase	Potassium ion	Identified screen (Asare et al., 2010)
FTT0120	IISP	SRP receptor FtsY	Identified screen (Moule et al., 2010)
FTT0964c	IISP	SRP protein Ffh	–
PTS			
FTT1280c	SSPTS	Nitrogen regulatory	–
UNCLASSIFIED			
FTT0668	PnuC	Nicotinamide mononucleotide	Identified screen (Asare et al., 2010; Moule et al., 2010)
			Up-regulated in cells (Wehrly et al., 2009)
FTT0707	PnuC	Nicotinamide mononucleotide	–
FTT1090	PnuC	Nicotinamide mononucleotide	MlgA-regulated (Brotcke et al., 2006)
FTT0249	FeoB	Ferrous ion	Identified screen (Su et al., 2007; Ahlund et al., 2010)

[a]Transport family names: MFS, major facilitator superfamily; VIC, voltage-gated ion channel superfamily; CIC, chloride channel family; MscS, small conductance mechanosensitive ion channel; MIP, major intrinsic protein family; LIC, ligand-gated ion channel; POT, proton-dependent oligopeptide transporter family; PiT, inorganic phosphate transporter family; SSS, solute:sodium symporter family; DAACS, dicarboxylate/amino acid:cation (Na+ or H+) symporter family; BASS, bile acid:Na+ symporter family; APC, amino acid-polyamine-organocation family; NhaA, NhaA Na+:H+ antiporter family; CPA1, monovalent cation:proton antiporter-1 family; Trk, K+ transporter family; CDF, cation diffusion facilitator family; NCS2, nucleobase:cation symporter-2 family; CNT, concentrative nucleoside transporter family; HAAAP, hydroxy/aromatic amino acid permease family; ArsB, arsenite–antimonite efflux family; SulP, sulfate permease family; RND, resistance-nodulation-cell division superfamily; NhaD, NhaD Na+:H+ antiporter family; MOP, multidrug/oligosaccharidyl-lipid/polysaccharide flippase superfamily; DMT, drug/metabolite transporter superfamily; LysE, L-lysine exporter family; RhtB, resistance to homoserine/threonine family; AAE, aspartate:alanine exchanger family; oxa1, cytochrome oxidase biogenesis family; ABC, ATP-binding cassette superfamily; F-ATPase, H+- or Na+-translocating F-type, V-type and A-type ATPase superfamily; P-ATPase, P-type ATPase superfamily; IISP, type II (General) secretory pathway family; SSPTS, sugar specific PTS; PnuC, nicotinamide mononucleotide (NMN) uptake permease family; FeoB, ferrous iron uptake family.

[b]For each putative transporter it is indicated if the encoding gene has been identified in a cell-based or in vivo screen, is regulated by the major virulence regulator MglA, or has a changed expression after entry into macrophages.

Several mutants in secondary transporters were identified in various genetic screens. For example, the gene *xasA*, encoding a predicted glutamate/γ-aminobutyrate transporter of the APC family (FTT_0480c, **Table 2**), has been identified in four different screens (Maier et al., 2007; Weiss et al., 2007; Kraemer et al., 2009; Peng and Monack, 2010), supporting a functional role in *F. tularensis* virulence. The gene *FTT_0708*, encoding a transporter of the MFS family has also been identified in an *in vivo* screen (Su et al., 2007), as were other transporters of this family.

The *F. tularensis* subsp. *tularensis* Schu S4 genome also encodes 15 complete transport ABC-type carriers, consisting of a membrane-spanning permease and an ATP-binding subunit (Atkins et al., 2006). These ABC transporters are predicted to participate in diverse functions, ranging from amino acid/peptide and ion uptake to multidrug efflux. Remarkably, mutants in one ABC transporter (FTT_1125) have been identified in several *in vitro* (Maier et al., 2007) and *in vivo* (Su et al., 2007; Kraemer et al., 2009) screens for attenuated mutants, indicating a direct contribution to *F. tularensis* virulence.

Supporting a role of transport systems in intracellular survival, transcriptional profiling of the *F. tularensis* subsp. *tularensis* Schu S4 strain in BMMs (Wehrly et al., 2009) revealed that genes encoding various transporters showed significantly altered expression (either up- or down-regulated) after host cell entry. Notably, five of the eight POT family members were up-regulated intracellularly. The amino acid identity between the POT family members does not exceed 47% (ranging from 22 to 31%, in most cases), suggesting that they might have distinct transport properties. Also, four of the POT transporters have been found in mutant screens (Tempel et al., 2006; Kraemer et al., 2009; Asare and Abu Kwaik, 2010), further indicating that these oligopeptide transporters and therefore amino acid metabolism is important during infection.

In addition, the *F. tularensis* subsp. *tularensis* Schu S4 genome encodes six putative ion channels but is devoid of any PEP-dependent phosphotransferase (PTS) system. At present, no biochemical data are available on any of the transporters present in *F. tularensis*.

One example of amino acid supply provided by the host cytosol

The cytosol of eukaryotic cells contains a high concentration (10 mM) of the tripeptide γ-glu-gly-cys named glutathione (in its reduced form, GSH; Alkhuder et al., 2009). GSH plays a pleiotropic and major role in mammalian cell homeostasis and GSH-deficiency has been associated with various severe diseases (Griffith, 1999; Wu et al., 2004; Franco et al., 2007). Biosynthesis of GSH is dependent on the availability of the amino acid precursors glutamate, glycine, and cysteine. The intracellular pool of cysteine is relatively small (0.10–0.25 mM) and cysteine is generally the limiting amino acid for GSH synthesis. The other two precursors, glycine and glutamate, are found in considerable higher intracellular concentration. As mentioned earlier, *F. tularensis* subspecies requires cysteine for growth. We have recently demonstrated that gene *FTL_0766* encodes a genuine γ-glutamyl transpeptidase (GGT) involved in the metabolism of γ-glutamyl-containing peptides. GGT allows the utilization of γ-glutamyl peptides as a source of cysteine during intracellular multiplication of the *F. tularensis* subsp. *holarctica* strain LVS, and is thus critical for its virulence (Alkhuder et al., 2009). This work represents the only direct experimental evidence of nutrient utilization by intracellular *F. tularensis*. The transporter of GSH and the molecular mechanism of crossing the bacterial envelope remain to be discovered.

STARVING THE INVADING BACTERIA AS A HOST CELL DEFENSE MECHANISM

The capacity of a macrophage to deprive intracellular pathogens of required nutrients (Appelberg, 2006) can be viewed as an intrinsic antimicrobial innate immune defense mechanism. Thus, microbial killing may not rely only on a toxic environment (such as low pH and oxidative stress in the phagosomal compartment) but also may result from the scarcity of nutrients in the cellular compartment it occupies (transiently or permanently). At any rate, one must keep in mind that the notion of a limiting concentration of nutrient may vary considerable from one intracellular pathogen to another.

Two distinct mechanisms of nutrient deprivation exist: (i) constitutive mechanisms, such as that mediated by the divalent cation transporter Nramp1, present in the membrane of endosomal compartment and participating to iron depletion (Cellier et al., 2007); and (ii) induced mechanisms, such as those triggered by the cytokine IFN-γ in activated macrophages, which may also affect iron availability (Mulero and Brock, 1999).

Notably, another pathway triggered by IFN-γ has been shown to play a role in the nutritional control of several intracellular pathogens (Taylor and Feng, 1991). Activation of the enzyme indoleamine 2,3-dioxygenase (ID) which degrades L-tryptophan by IFN-γ thus leads to tryptophan deprivation. Monack and co-workers very recently showed that tryptophan auxotrophs of *F. tularensis* subsp. *novicida* were severely affected in intracellular survival and multiplication and were attenuated in the mouse model (Peng and Monack, 2010). Interestingly, tryptophan metabolism appeared to be important only for bacterial colonization of the lungs, suggesting an organ specificity of this metabolic need. The authors found that this antimicrobial starvation mechanism mediated by the enzyme ID was effective against both auxotrophic and prototrophic microbes. These observations support the notion that, for bacteria, amino acid biosynthesis is more energetically costly than their capture from the environment. The necessity to biosynthesize amino acid in a depleted environment may thus reduce the capacity of the bacterium to replicate.

CONCLUDING REMARKS

Genetic screens have clearly established the critical importance of the aromatic amino acids biosynthetic pathway for *F. tularensis* virulence. The fact that inactivation of almost every gene in this pathway lead to reduced virulence suggests that the available pool of aromatic amino acids is limiting in the infected host. Similarly, the severe intracellular growth defect of mutants unable to synthesize purines *de novo*, also suggest that macrophages contain limiting concentrations of purines.

Altogether, experimental data and predictive models favor the notion that *F. tularensis* preferentially utilizes specific amino acids for energy and fatty acids as gluconeogenic substrates rather than carbohydrate sources during cytosolic multiplication. Intracellular transcriptomic studies revealed that significant changes occurred in the expression of genes encoding enzymes involved in carbohydrate metabolism as well as in genes encoding putative amino acid and carbohydrate transporters. Hence, the importance of nutrition in *F. tularensis* virulence can be seen as a fine balance between its ability to capture nutrients from the host (transport) and the regulation of its metabolism in response to the amounts of nutrients available (metabolism). Mutations affecting either one or the other (or both) of these two functions lead to an impaired fitness and are likely to cause a reduced virulence.

Intracellular pathogens often co-regulate their metabolic needs and the production of dedicated virulence factors by using pleiotropic (mainly transcriptional) regulators. The stringent response is a stress response that occurs in bacteria in reaction to amino acid or carbon starvation. The stringent response is signaled by the alarmone ppGpp. Notably, the expression of many virulence regulators is mediated by ppGpp, thereby coupling pathogenesis to metabolism (Dalebroux et al., 2010). *F. tularensis* also uses ppGpp to control the activity of its major regulator of virulence, MglA (Charity et al., 2009). In particular, ppGpp

was shown recently to promote physical interactions between the MglA–SspA complex and the putative DNA binding factor PigR (also designated FevR) to control the PigR-dependent activation of the *Francisella* pathogenicity island (Charity et al., 2009). These data suggest a link between nutrient availability and virulence. They are in agreement with the transciptomic analysis of a *mglA* knock-out mutant of *F. tularensis* subsp. *novicida* (Brotcke et al., 2006). Indeed, among the 102 MglA-regulated genes identified, 20 were predicted to play a role in metabolism, particularly in amino acid metabolism.

A systematic mutational analysis of *F. tularensis* metabolic pathways, coupled to thorough biochemical and biophysical characterization of its metabolic capacities will be required to fully understand the complex interplays between metabolism and virulence.

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