Abstract: A strain of Francisella species wherein a gene which encodes for part of the glutamate metabolic pathway has been inactivated, and which is able to produce a protective immune response in an animal, for use as live prophylactic or therapeutic vaccine against infection by said Francisella species. Particularly effective strains include those where the cpB gene is deleted. Other embodiments of the invention describe strains which comprise a further genetic mutation wherein a gene which encodes for another component of the cell is also inactivated. Pharmaceutical compositions comprising said strains, together with methods which utilise such strains are also described and claimed.
Published: For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
Live Vaccine Strain

This invention relates to live strains of *Francisella* species, their use as prophylactic or therapeutic vaccines, to compositions comprising these strains, and their use in the prevention or treatment of bacterial infection.

*Francisella tularensis* is an extremely pathogenic Gram-negative bacterium and is the etiological agent of the zoonotic disease Tularemia. There are four recognised subspecies of *F. tularensis*, including subspecies *tularensis, holarctica*, and *novicida*, which exhibit a high degree of genetic conservation. The most virulent subspecies is *Francisella tularensis* subspecies *tularensis*, which has an infectious dose in humans of as little as 10 cells via the airbourne route.

At present there is no available vaccine against *F. tularensis* infection although it has been demonstrated previously that an undefined attenuated strain of *Francisella tularensis*, which has been designated *Francisella tularensis* live vaccine strain (LVS), is capable of providing protection against the most virulent subspecies, *tularensis*. However, the LVS vaccine is not registered and has only been used to vaccinate at-risk individuals under special license. This license has now been withdrawn. The LVS strain is likely to remain unlicensed because the genetic changes that are responsible for the attenuating phenotype are not understood at the molecular level. Therefore, there exists a possibility that the vaccine strain could revert back to the fully virulent form. Further, it has been shown that whilst LVS provides effective protection in the mouse model of infection, protection is not complete. The protection afforded by LVS against an aerosol challenge of the most virulent *tularensis* subspecies is sub-optimal. Clearly, a vaccine which is genetically stable and which provides complete protection is highly desirable.

The fact that naturally occurring, attenuated strains of *F. tularensis* can induce protective immunity does suggest that an attenuated strain with properly defined genetic mutations in the organism's virulence factors is a feasible approach in vaccine development.

Unfortunately, however, relatively little is known about the virulence mechanisms of *F. tularensis* and, as such, virulence factors have proven to be very difficult to predict; the recent completion of the genome sequence of the virulent strain *F. tularensis*
subspecies *tularensis* SchuS4 has so far failed to reveal the presence of classical virulence factors such as toxins or type-III secretion systems, which are predominant in so many other pathogenic bacteria.

Some efforts to identify new vaccine strains have focused on naturally occurring strains (other than LVS) or on spontaneously attenuated strains of *F. tularensis*, such as the FSC043 mutant of SchuS4 reported by Twine *et al.* (*Infection and Immunity* Vol 73, 2005, pp8345-8352). Examination of these attenuated mutants has confirmed that mutations can lead to attenuated strains which afford some level of protection against tularemia. However, the molecular basis of this attenuation and protection is unknown and, in any case, the protection afforded is not better than that provided by LVS. The problem remains, therefore, to find defined mutations which give complete protection against the most virulent forms of *Francisella*.

A live vaccine strain of *Francisella* derived from the subspecies *novicida*, which contains a single genetic mutation, is described in co-pending International Application number PCT/GB2004/001264, the contents of which are hereby incorporated by reference. This application shows that a genetic lesion in the purine enzyme pathway provides attenuated strains which are also protective in the mouse model of infection.

Whilst this clearly represents a significant advance in the development of a vaccine for tularemia, it is widely recognised that an additional genetic mutation would be required to enable such a strain to obtain licensed status. The problem remains, therefore, to determine further mutations which result in attenuated strains but which also provide complete protection against all strains of *Francisella tularensis*.

New live vaccines, containing well defined mutations and which are fully protective against tularemia are therefore required.

The applicants have found that by modifying strains of *Francisella* in a particular way, attenuated strains which are protective can be produced. These live strains can be used as the basis for new vaccines against tularemia.

The present invention therefore provides a strain of *Francisella* species wherein a gene which encodes for part of the glutamate metabolic pathway has been inactivated, and which is able to produce a protective immune response in an animal, for use as a live prophylactic or therapeutic vaccine against infection by said *Francisella* species.
As used herein, the term "metabolic pathway" means the sum total of the chemical processes occurring in a cell, in which the processes occur in steps, through which compounds are gradually built up or broken down. Each step of the metabolic pathway is catalysed by an enzyme, whose structure is encoded by a gene.

As used herein the term "glutamate metabolic pathway" means the chemical processes whereby glutamate is synthesised or broken down, including those steps wherein an enzyme catalyses the formation of glutamate or catalyses the use of glutamate to form another material.

Thus, genes that encode for part of the metabolic pathway in Francisella species include those genes which encode for the formation of glutamate and those which encode for enzymes which are utilised in the glutamate metabolic pathway.

Inactivation of such a gene is likely to interrupt the normal metabolic pathway such that, for example, glutamate is not produced by the cell or that the amount of glutamate produced is significantly altered when compared with the virulent Francisella species, such as Francisella tularensis subspecies tularensis SchuS4, in which the same gene having been inactivated.

The presence, or absence, of glutamate (or aminoglutaric acid) may be readily determined using 13C, 15N or 1H Nuclear Magnetic Resonance Spectroscopy (NMR), as described by the papers by Robertson, D.E. et al (Applied and Environmental Microbiology 1990, vol. 56 pp1 504-1508 and Biochimica et Biophysica Acta 1989, vol. 992, pp320-326) and the quantities of glutamate produced by strains of the present invention may be readily determined using techniques such as Liquid Chromatography - Mass Spectrometry (LC-MS), which are routine in the art.

As used herein "glutamate" refers to the amino acid glutamate or glutamic acid, which may exist in the form of β-glutamate (beta-glutamate) or γ-glutamate (gamma-glutamate).

The inventors have found that inactivating a gene which encodes for part of the glutamate metabolic pathway provides a strain which is both attenuated and protective against exposure to virulent Francisella species. Without wishing to be bound by theory, it is thought that part of the glutamate metabolic pathway may include, or have
an effect on the formation of extracellular capsule. Thus, genes which encode for the
synthesis of capsule, or which are analogous to these genes when compared with
established capsule encoding genes from other organisms, may fall within the definition
of genes that encode for part of the metabolic pathway, as described above.

As used herein, the term "capsule" means an extracellular component, commonly a
layer, of polysaccharide and/or protein which protects a bacterial cell and which, in
association with pathogenic bacteria, serves as a barrier against phagocytosis by the
white blood cells of an animal host, in which the pathogenic bacteria are present.

As used herein, the term "capsule-encoding gene" means a gene which encodes for a
protein, or other molecule, which is involved in the synthesis of the capsule component
of a bacterial cell or a gene which is homologous to a gene which has been assigned
as having some involvement in bacterial capsule biosynthesis, including involvement in
the synthesis of capsule or any component thereof and involvement in the assembly
and/or transport of said components to form a capsular structure.

As used herein, the terms "homologous" and "homology" means, at the protein level,
the similarity of two amino acid sequences are such that the two sequences share
greater than 30% identity. Identity in this instance can be judged for example using the
BLAST program (vs. 2.2.12) found at http://www.ncbi.nlm.nih.gov/BLAST/ or the
algorithm of Lipman-Pearson with, for example, Ktuple:2, gap penalty:4, Gap Length
Penalty:12, standard PAM scoring matrix or other suitable parameters as readily
determined by a person skilled in the art (Lipman, DJ. and Pearson, W.R., Rapid and

Genes which encode for the glutamate metabolic pathway (including those which
encode for a capsule component) of the Francisella bacterium may be determined by
analysis of the relevant genome sequence and/or by comparison with other bacteria
which have well-defined capsule encoding genes.

Gene inactivation can be carried out using any of the conventional methods known in
the art. Typically, the strain is transformed with a vector which has the effect of down-
regulating or otherwise inactivating the gene. This can be done by mutating control
elements such as promoters and the like which control gene expression, by mutating
the coding region of gene so that any product expressed is inactive, or by deleting the
gene entirely. Alternatively, the gene can be inactivated at the RNA or protein level, by transforming the cell so that it expresses a sense or anti-sense construct which binds to DNA or RNA encoding the gene to prevent transcription thereof.

Preferably however, the gene is inactivated by complete or partial deletion mutation or by insertional mutation.

Specifically, the applicants have found that it is preferable to inactivate one or more of the genes which has been annotated in the *Francisella tularensis* genome as being a capsule-encoding gene, for example *capB* or *capC* (hereinafter "cap"genes). In particular the applicants have found that it is preferable to inactivate a *cap* gene in *Francisella* species such as *F. tularensis* subspecies *tularensis* or subspecies *holarctica*. In a preferred embodiment, the *capB* gene (FTT0805) and/or the capC gene (FTT0806) of *F. tularensis* subspecies *tularensis* is inactivated to provide one strain according to the present invention. In other embodiments, the corresponding genes of other *Francisella* species are deleted to provide other strains according to the present invention. The corresponding *cap* genes from other *Francisella* species will usually have at least 80%, preferably at least 85% homology and more preferably at least 90% homology to, and similar function to, the *cap* genes of *F. tularensis* subspecies *tularensis*. In a more preferred embodiment, the *capB* gene (FTT0805) of *F. tularensis* subspecies *tularensis* strain SchuS4 is inactivated to provide a preferred strain.

In particular, the applicants have found that a strain of *Francisella tularensis* which has a *cap* gene inactivated is attenuated and is protective against challenge from virulent *Francisella* species. As illustrated hereinafter, a strain of *F. tularensis* subspecies *tularensis* which has an inactivated *cap* gene is protective in mice. In particular the applicants have shown that deletion of the *capB* gene from *F. tularensis* subspecies *tularensis* SchuS4 strain provides an attenuated strain which is protective against aerosol challenge with the virulent SchuS4 strain.

The strain of the invention suitably has a further defined mutation or lesion so as to reduce the risk of the bacterium reverting to a virulent form. In this case, the mutation is in a gene which is selected so that the strain is suitably attenuated, but can still retain the ability to stimulate a sufficient immune response to provide long term protection.

Suitable additional mutations can be identified using conventional methods, and examination and analysis of the current live vaccine strain (LVS) or other attenuated
strains may assist in the identification. Examples of these mutations include, but are not limited to, mutations to the FTT0918 and FTT0919 genes. Alternatively the further defined mutation may advantageously involve a gene which encodes for another component of the cell, which is not part of the glutamate metabolic pathway. Such further mutations include, but are not limited to, mutations which inactivate pilin genes, for example mutations to pilA and/or pilE and/or pilC genes or other mutations such as those described in co-pending British patent application number GB051 1722.1 (the contents of which are hereby incorporated by reference), mutations which inactivate genes which encode enzymes in the purine pathway, for example mutations to purA and/or purF genes and other purine pathway genes such as those described in co-pending International Application number PCT/GB2004/001264 (the contents of which are hereby incorporated by reference).

Particular preferred examples of further defined mutations are inactivation of the purF and/or inactivation of the pilA gene and/or inactivation of the FTT0918 gene.

It will be understood by the skilled person that such further defined mutations can be achieved by using any conventional method as hereinbefore described but that in preferred embodiments of the inventions the gene which encodes for other components of the cell is inactivated by complete or partial deletion mutation or by insertional mutation.

Since the strains of the present invention have been found to be protective against infection by Francisella species in the mouse model of infection, the strains also provide useful vaccines against the diseases caused by Francisella infections and, in particular, tularemia. It is therefore preferred that the strains are formulated into pharmaceutical compositions, in which they are combined with a pharmaceutically acceptable carrier. Such pharmaceutical compositions form a second aspect of the invention.

Suitable carriers may be solid or liquid carriers as is understood in the art. They may suitably be formulated for administration to mucosal surfaces (for example for oral use, of for administration by inhalation or insufflation) or for parenteral administration.

In particular they are formulated as sterile aqueous or oily solutions for intravenous, subcutaneous, intramuscular or intramuscular dosing.
Alternatively they are formulated for administration to mucosal surfaces and in particular for intranasal application. Such formulations may include microencapsulation of the strain in the composition, or microencapsulation of the entire composition. Such microencapsulation techniques are commonly known in the art.

Compositions are suitably prepared in unit dosage forms, as conventional in the art. They are administered at dosages which are determined using clinical practice, and depend upon factors such as the nature of the patient, the severity of the condition, and the precise vaccine strain being employed. Typically dosage units will comprise $10^5$ - $10^8$ cfu. Dosages may be boosted as appropriate or necessary.

Compositions may also contain further immunogenic reagents which are effective against *F. tularensis* infection or other diseases. They may further contain other agents such as adjuvants and the like, which enhance the host's immune response to the vaccine.

In a further aspect the present invention relates to the use of a strain of *Francisella* species wherein a gene which encodes for part of the glutamate metabolic pathway has been inactivated, and which is able to produce a protective immune response in an animal, in the preparation of a live prophylactic or therapeutic vaccine against infection by *Francisella* species. In particular, such strains find use in the preparation, or manufacture, of a vaccine for the treatment of Tularaemia.

In yet a further aspect, the invention provides a method of preventing or treating infection caused by *Francisella* species, which method comprises administering to an animal, including a human being, an effective amount of a strain or of a pharmaceutical composition, each as hereinbefore described.

In particular, the method is useful in the treatment of infection caused by *Francisella tularensis* subspecies *tularensis*.

Novel strains which are suitable for vaccine use form a further aspect of the invention. In particular, the invention provides a strain of *Francisella* species wherein a gene which encodes for part of the glutamate metabolic pathway has been inactivated. In particular the *strains* which are suitable for use as vaccines are as hereinbefore
described. In a preferred embodiment the inactivated gene is capB or capC. It is further preferred that the strain is a strain of *Francisella tularensis* subspecies *tularensis*, for example the SchuS4 strain.

The invention will now be particularly described by way of non-limiting Example, with reference to the accompanying diagrammatic drawings in which:

Figure 1 shows the strategy for the construction of a suicide plasmid for the deletion of the *capB* gene from the *F. tularensis* subspecies *tularensis* strain SchuS4.

Figure 2 shows a southern blot of genomic DNA from wild type and *capB* mutant strains of *F. tularensis* subspecies *tularensis* strain SchuS4.

Figure 3 shows the effects of osmotic stress on wild type *F. tularensis* subsp. *tularensis* strain SchuS4 and the Δ*capB* mutant strain of the invention, when grown in a range of salt conditions.

Figure 4 shows survival data of BALB/c mice infected subcutaneously with wild type and *capB* mutant strains of *F. tularensis* subspecies *tularensis* strain SchuS4.

Figure 5 shows survival data of BALB/c mice, administered with a *capB* mutant strain of *F. tularensis* subspecies *tularensis* strain SchuS4, and subsequently challenged with 70 mean lethal doses (MLD) of the virulent strain, *F. tularensis* subspecies *tularensis* strain SchuS4.

Figure 6 shows survival data of Female BALB/c mice (6-8 weeks old), injected subcutaneously with 100 µl of PBS containing 10⁴ CFU *F. tularensis* subsp. *tularensis* strain SchuS4 Δ*capB*, *F. tularensis* live vaccine strain (LVS) or PBS alone and eight weeks later challenged with 10⁴ CFU administered by the subcutaneous route.

Figure 7 shows a graph depicting the colonization and clearance of bacteria from the spleens of mice administered with either wild type *F. tularensis* subsp. *tularensis* strain SchuS4 or the Δ*capB* mutant strain of the present invention.

Figure 8 shows a graph which quantifies the IL-2 and IFN-γ recall response of spleen cells harvested from mice *immunized* subcutaneously with 100 µl of PBS containing
10^4 CFU *F. tularensis* subsp. *tularensis* strain SchuS4 Δ capB, *F. tularensis* live vaccine strain (LVS) or PBS alone and then subsequently stimulated with wild type *F. tularensis* subsp. *tularensis* strain SchuS4.

**Example 1:** Construction of a plasmid containing a mutated allele of *F. tularensis* caoB

The *capB* gene of *F. tularensis* subspecies *tularensis* strain SchuS4 encodes a protein of 405 amino acids (aa) that has 36% identity to the 397 aa CAPB protein from *Bacillus anthracis* strain 'Ames Ancestor'. Regions of DNA flanking the *capB* gene of *F.tularensis* were PCR amplified from *F. tularensis* subspecies *tularensis* strain SchuS4 using the primer pairs P1/P2 and P3/P4 as shown in Table 1. The chloramphenicol resistance cassette (Cam-r) was PCR amplified from the plasmid pKK202 with the primer pair CamF/CamR.

**Table 1:**

<table>
<thead>
<tr>
<th>Primers used for the construction of PSMP42 - Sequences in bold indicate the sequence complementary to Francisella DNA and the underlined sites in the 5' extensions are restriction sites.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
</tr>
<tr>
<td>P2</td>
</tr>
<tr>
<td>P3</td>
</tr>
<tr>
<td>P4</td>
</tr>
<tr>
<td>CamF</td>
</tr>
<tr>
<td>CamR</td>
</tr>
</tbody>
</table>

The left and right flanks and the chloramphenicol resistance cassette were assembled as shown in Fig. 1, and cloned into the suicide plasmid pSMP22 to give the construct pSMP42. This gave a plasmid-borne mutant allele that could be used for recombinational exchange with the wild type chromosomal allele.

**Example 2:** Generation of a *F. tularensis* strain deleted for *capB*
The suicide plasmid pSMP42 was electroporated into the £ co// mobilising strain S-17 λpir. The plasmid was then introduced from the mobilising strain to F. tularensis subspecies tularensis strain SchuS4 by conjugal transfer. Transconjugants were selected on chloramphenicol and merodiploids arising from chromosomal integration of the suicide plasmid were resolved by plating on Thayer Martin agar containing sucrose at 5 %. Allelic replacement mutants were confirmed by Southern blot analysis as shown in Figure 2. Genomic DNA of wild type and capB deletion mutants (ΔcapB) of F.tularensis subspecies tularensis strain SchuS4 was digested with MliI and NcoI, separated by agarose gel electrophoresis and transferred to a nylon membrane. The MliI insert of pSMP42 was labelled with DIG-1 1-dUTP during PCR amplification with the primer pair P1/P4 and used as a probe to hybridise to the membrane. DNA fragments to which the probe hybridised were detected in a chemiluminescent assay (CSPD substrate, 30 min exposure, X-ray film). The ΔcapB mutant contained two hybridising fragments of 12.5Kb and 3.2Kb as shown in Figure 2.

Example 3: Comparison of phenotype - effect of osmotic stress

F. tularensis subsp. tularensis strain SchuS4 ΔcapB and F. tularensis subsp. tularensis strain SchuS4 (wild type) were suspended in a range of salt (naci) concentrations (up to 5M) and then plated onto BCGA media using 20 µl droplets. Bacterial growth was assessed after 72 hours and the results are portrayed in Figure 3. It was apparent that, as compared with the wild type, SchuS4 ΔcapB grew less efficiently at the highest salt (5 M) concentration. It appeared that ΔcapB was also more sensitive at the low salt concentrations. This indicates that the ΔcapB mutant has a different phenotype to wild type F.tularensis and exhibits different growth characteristics in osmotic environments.

Example 4: Determination of virulence of a caoB mutant in the mouse model of Tularemia

Several investigators have determined that the MLD of F. tularensis subspecies tularensis is -10 colony forming units (CFU) in the BALB/c mouse, irrespective of route of administration. In order to determine whether capB is required for virulence, groups of six female BALB/c mice, aged 6-8 weeks, were infected subcutaneously with wild-type SchuS4 and ΔcapB SchuS4 strains of F. tularensis subspecies tularensis. As previously reported mice infected with 10^2 (115) CFU of SchuS4 succumbed to
infection by day 5 post-infection. In contrast, 100% of the mice infected with $1.6 \times 10^5$ and 80% (4 out of 5) of mice infected with $1.6 \times 10^6$ and $1.6 \times 10^4$ CFU of the ΔcapB isogenic strain survived as shown in Figure 4. This level of survival following deletion of a single gene demonstrates that capB is an important gene in the virulence of *Francisella tularensis* subspecies *tularensis*.

**Example 5: Protection Afforded by a capB mutant of Francisella tularensis**

The chromosomal copy of the gene *capB* was deleted by allelic exchange as described in example 1 and 2. Genetic analysis was performed to ensure that the gene was removed (as per example 2) and a clonal population of the resultant strain was prepared. Upon subcutaneous administration to female Balb/c mice (6-8 weeks), $1.6 \times 10^5$ bacteria of the AcapB mutant strain failed to cause death in all five mice. This is in contrast to the parental wild-type strain of which only 115 bacteria were required to kill all 5 mice of a control group in 5 days. 46 days after immunisation with the capB negative strain, survivors of the infection detailed in Example 4 were challenged with 70 mean lethal doses (MLD) of a virulent strain of *F. tularensis* (SchuS4 strain). Non-immunised (naive controls) mice succumbed to infection and died within 5 days whereas mice immunised with the capB mutant were protected and did not die as shown in Figure 5.

**Example 6: Survival against virulent challenge**

Female BALB/c mice (6-8 weeks old) were injected subcutaneously with 100 µl of PBS containing $10^3$ CFU *F. tularensis* subsp. *tularensis* strain SchuS4 ΔcapB (prepared as described above), *F. tularensis* live vaccine strain (LVS) or PBS alone. Eight weeks later mice were challenged with $10^4$ CFU wild type *F. tularensis* subsp. *tularensis* strain SchuS4 administered by the subcutaneous route. Survival data, shown in Figure 6, clearly indicates that superior protection is afforded by the capB deletion mutant than the live vaccine strain, and that 100% survival was observed.
Example 7: Colonisation and clearance in vivo of \textit{Tularensis} strains

Female BALB/c mice (6-8 weeks old) were injected subcutaneously with 100 µl of PBS containing $10^4$ CFU wild type \textit{Tularensis} subsp. \textit{Tularensis} strain SchuS4 or F. \textit{Tularensis} subsp. \textit{Tularensis} strain SchuS4 Δ\textit{capB}. Groups of 4 mice were killed 3, 7, 14 and 31 days following inoculation of the bacteria. Spleens were removed and the number of bacteria per spleen determined by serial dilution in PBS followed by microbiological culture on BCGA agar plates for 96 hours at 37OC. None of the mice injected with wild type SchuS4 survived longer than five days post inoculation, which precluded determination of splenic bacterial burdens in these mice on days 7, 14 and 31 post inoculation. However, at day 3 post inoculation there was a highly significant (P<0.001) difference in the numbers of bacteria in the spleens of mice injected with wild type and the Δ\textit{capB} mutant; mice injected with F \textit{Tularensis} subsp. \textit{Tularensis} strain SchuS4 Δ\textit{capB} had substantially lower numbers of bacteria in their spleens.

Animals injected with F. \textit{Tularensis} subsp. \textit{Tularensis} strain SchuS4 Δ\textit{capB} steadily cleared the organism and no viable bacteria were detected in the spleens of these animals at 31 days post inoculation; indicating that they had effectively cleared the mutant. These results shown in Figure 7 indicate that the \textit{capB} mutant strain is less likely to cause latent infection after administration.

Example 8: IL-2 and IFN-γ recall response of mice immunized with the \textit{cabo}B mutant strain of example

Female BALB/c mice (6-8 weeks old) were injected subcutaneously with 100 µl of PBS containing $10^4$ CFU F. \textit{Tularensis} subsp. \textit{Tularensis} strain SchuS4 Δ\textit{capB}, F. \textit{Tularensis} live vaccine strain (LVS) or PBS alone. 40 days later groups of immunised 4 mice were killed and their spleens removed. Single cell suspensions of spleen cells were prepared in culture media (RPMI-1640) (Sigma, UK) supplemented with 10% heat inactivated foetal bovine serum (FBS) (Sigma, UK); 1% penicillin / streptomycin / glutamine (Sigma, UK) and 50µM 2-mercaptoethanol (Sigma, UK). Cells were stimulated overnight in triplicate with either heat killed F. \textit{Tularensis} subsp. \textit{Tularensis} strain SchuS4 (5 µg ml⁻¹ protein) and supplemented RPMI 1640 or supplemented RPMI 1640 alone. IL-2 and IFN-γ secretion from the cells was determined using cytokine bead array technology (BD Biosciences, Oxford UK). As compared with mice injected with PBS, spleen cells from animals immunized with SchuS4 Δ\textit{capB} or LVS secreted
significant quantities of IL-2 and IFN-γ when re-stimulated in vitro with inactivated *F, tularensis* strain SchuS4. However, when spleen cells from animals immunized with SchuS4 Δ*capB* or LVS were cocultured with media alone, no significant IL-2 or IFN-γ secretion was detected. These results are shown in Figure 8.
Claims

1. A strain of *Francisella* species wherein a gene which encodes for part of the glutamate metabolic pathway has been inactivated, and which is able to produce a protective immune response in an animal, for use as live prophylactic or therapeutic vaccine against infection by said *Francisella* species.

2. A strain according to claim 1 wherein the gene encodes for the synthesis of capsule

3. A strain according to claim 1 or 2 wherein the gene is selected from the group of genes consisting of FTT0808, *capB*, and *capC*.

4. A strain according to claim 3 wherein the gene is *capB*.

5. A strain according to claim 3 wherein the gene is *capC*.

6. A strain according to any of claims 1 to 5 wherein said gene is inactivated by complete or partial deletion mutation or by insertional mutation.

7. A strain according to any of claims 1 to 6 which is a strain of *Francisella tularensis*.

8. A strain according to claim 7 which is a strain of *Francisella tularensis* subspecies *tularensis*.

9. A strain according to claim 7 which is a strain of *Francisella tularensis* subspecies *holarctica*.

10. A strain according to either or claims 7 or 8 which is *Francisella tularensis* subspecies *tularensis* SchuS4

11. A strain according to any preceding claim which comprises a further genetic mutation wherein a gene which encodes for another component of the cell is also inactivated.
12. A strain according to claim 11 wherein the gene which encodes for another component of the cell is selected from the group consisting of FTT0918, FTT0919 and FTT1564.

13. A strain according to claim 11 wherein the gene which encodes for another component of the cell is a gene which encodes for a pilin subunit or is a gene which encodes for an enzyme in the purine pathway.

14. A strain according to claim 13 wherein the gene which encodes for another component of the cell is a gene selected from the group consisting of pilA, pilE, pilC, purA and purF.

15. A strain according to any of claims 11 to 14 wherein the gene which encodes for another component of the cell is inactivated by complete or partial deletion mutation or by insertional mutation.

16. A pharmaceutical composition comprising a live strain of a Francisella species wherein a gene which encodes for part of the glutamate metabolic pathway has been inactivated, and which is able to produce a protective immune response in an animal, in combination with a pharmaceutically acceptable carrier.

17. A pharmaceutical composition according to claim 16 wherein the gene is selected from the group of genes consisting of FTT0808, capB, and capC.

18. A pharmaceutical composition according to claim 17 wherein the gene is capB.

19. A pharmaceutical composition according to claim 17 wherein the gene is capC.

20. A pharmaceutical composition according to any of claims 16 to 19 wherein said gene is inactivated by complete or partial deletion mutation or by insertional mutation.

21. A pharmaceutical composition according to any of claims 16 to 20 wherein said live strain is a strain of Francisella tularensis.
22. A pharmaceutical composition according to claim 21 wherein said strain is a strain of *Francisella tularensis* subspecies *tularensis*.

23. A pharmaceutical composition according to claim 21 wherein said strain is a strain of *Francisella tularensis* subspecies *holarctica*.

24. A pharmaceutical composition according to either of claims 21 or 22 wherein said strain is *Francisella tularensis* subspecies *tularensis* SchuS4.

25. The use of a strain of *Francisella* species wherein a gene which encodes for part of the glutamate metabolic pathway has been inactivated, and which is able to produce a protective immune response in an animal, in the preparation of a live prophylactic or therapeutic vaccine against infection by *Francisella* species.

26. The use according to claim 25 wherein the gene is selected from the group of genes consisting of FTT0808, *capB*, and *capC*.

27. The use according to claim 26 wherein the gene is *capB*.

28. The use according to claim 26 wherein the gene is *capC*.

29. The use according to any of claims 25 to 28 wherein said gene is inactivated by complete or partial deletion mutation or by insertional mutation.

30. The use according to any of claims 25 to 29 wherein said live strain is a strain of *Francisella tularensis*.

31. The use according to claim 30 wherein said strain is a strain of *Francisella tularensis* subspecies *tularensis*.

32. The use according to claim 30 wherein said strain is a strain of *Francisella tularensis* subspecies *holarctica*.

33. The use according to either of claims 30 or 31 wherein said strain is *Francisella tularensis* subspecies *tularensis* SchuS4.
34. The use of a strain according to any of claims 1 to 15 in the preparation of a vaccine for the treatment of tularemia.

35. A method of preventing or treating infection caused by *Francisella* species, which method comprises administering to an animal an effective amount of a strain according to any of claims 1 to 15.

36. A method of preventing or treating infection caused by *Francisella* species, which method comprises administering to an animal an effective amount of a pharmaceutical composition according to any of claims 16 to 24.

37. A strain of *Francisella* species wherein a gene which encodes for part of the glutamate metabolic pathway has been inactivated.

38. A strain according to claim 37 wherein the gene is *capB*.

39. A strain according to claim 37 or 38 wherein the *Francisella* species is a strain of *Francisella tularensis* subspecies *tularensis*. 
Figure 2

1. F. tularensis SchuS4 wild type genomic DNA
2. F. tularensis SchuS4 ΔcapB genomic DNA
3. F. tularensis SchuS4 ΔcapB genomic DNA

Genomic arrangement of capB locus in wildtype F. tularensis SchuS4
Genomic arrangement of capB locus in F. tularensis SchuS4 ΔcapB

Probes - Dig labelled MluI fragment from pSMP42

Kb
9.4
6.6
4.4
2.3
1
2
3
Figure 5

All mice challenged with 70cfu SchuS4

Percent survival

Days

- SchuS4ΔcapB 1.6x10^6 cfu
- SchuS4ΔcapB 1.6x10^5 cfu
- SchuS4ΔcapB 1.6x10^4 cfu
- naive controls
Figure 7:
Figure 8:

[Graph showing cytokine levels (IL-2 and IFN-gamma) for different treatments: CAP B, LVS, PBS.]

- Media
- Killed FT

Cytokine Levels:
- IL-2
- IFN-gamma