Towards stable isotope breath test detection of inhalational tularemia

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Abstract
A mass outbreak of inhalational tularemia caused by bioweapon (BW) aerosol exposure to *Francisella tularensis* remains a potentially devastating threat. Here we describe early steps in the development of a novel breath test diagnostic for pneumonic tularemia BW events. *F. tularensis* expresses a unique enzyme and known virulence factor citrulline ureidase, (CTU) an enzyme important in disrupting the NO mediated innate immunity, and in counteracting against intracellular acidification in phagosomes. CTU catalyzes the hydrolysis of citrulline to ornithine, ammonia and CO_2, and we hypothesized we could use selectively ^{13}C-labeled citrulline to produce labelled carbon dioxide by CTU activity in a similar manner to urease breath testing. The significant biosafety challenges in studying a Tier 1 select agent both in vitro and in vivo (mouse model) were overcome and may prove informative for breath test studies of other high containment organisms. Detection of CTU activity was possible in vitro and in breath from the mouse model.

Results

F. tularensis subsp.	Clinical Manifestation	Representative strain	CTU Expression
tularensis	Fulminant, potentially fatal	SCHU S4	Yes
holarctica	Self-resolving	LVS	No
		R96-0246	No

Table 1. Clinical Manifestion and CTU expression in *F. tularensis* subsp.

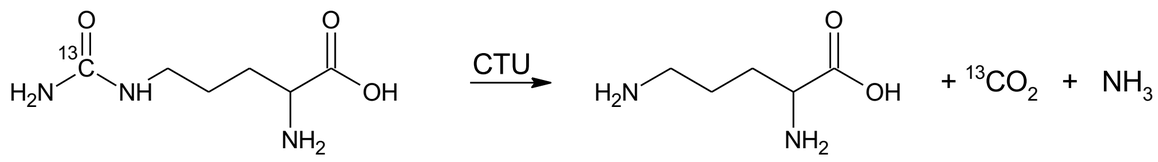

Fig. 1. CTU action on ^{13}C-citrulline produces $^{13}CO_2$

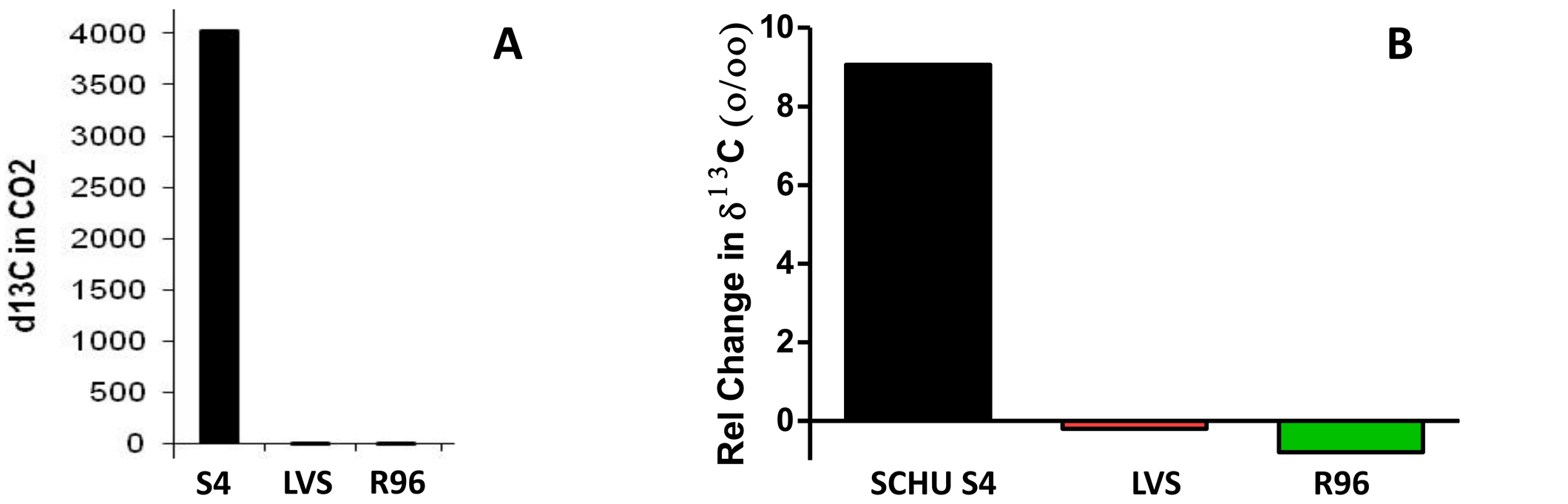

Fig 2. Only virulent *F. tularensis* SCHU S4 enriched ^{13}C. (A) SCHU S4 (S4), LVS, and R96-0246 (R96) were lysed by sonication, and the bacterial lysates were incubated with ureido-^{13}C-citrulline. The amount of ^{13}C in the head space was measured by mass spectrometry of absorbed sodasorb. (B) ^{13}C-citrulline was added to actively growing cultures in a sealed tube, and CO_2 was captured for 24 h using Sodasorb. The amount of ^{13}C was measured by mass spectrometry, and presented as change in $\delta^{13}C$ relative to media only

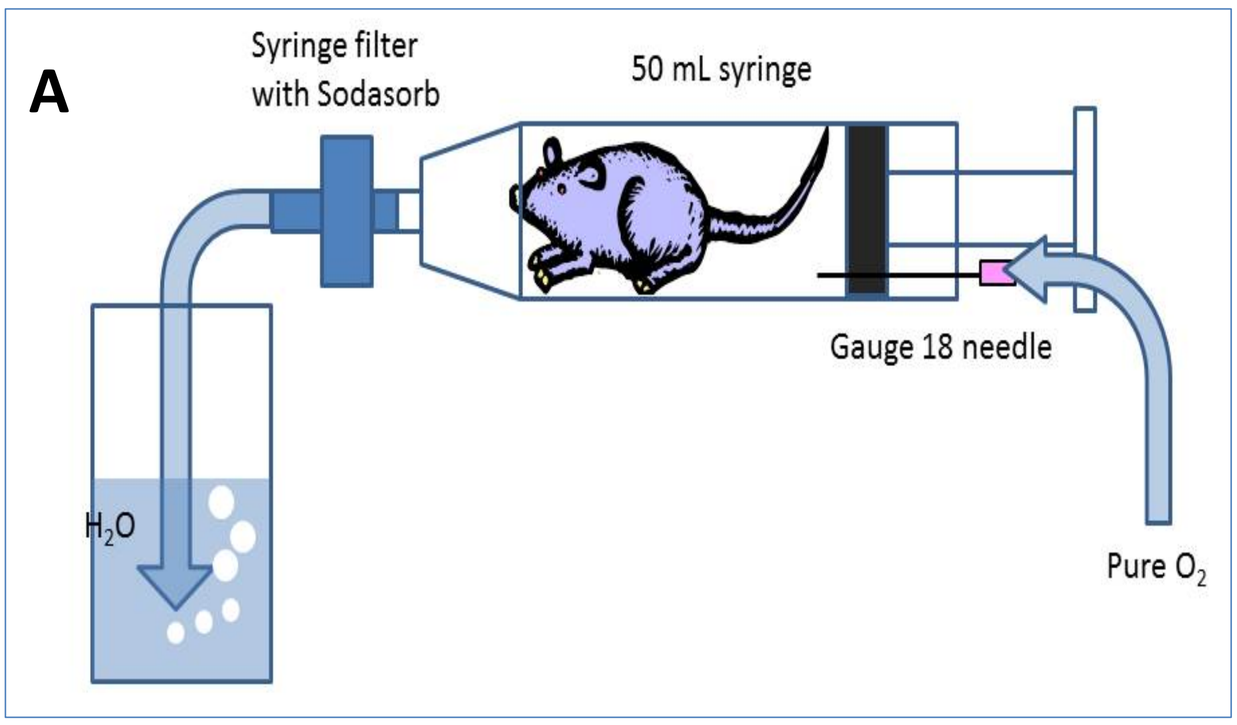

Fig. 3. Breath samples from mice infected with SCHU S4 contain increased level of $^{13}CO_2$. (A) Apparatus used to capture expired breath from infected mice using sodasorb CO_2 capture. (B) Three C57BL/6 mice were infected intranasally with 200 CFU SCHU S4. Two days after challenge, a baseline (B) breath sample was collected before the infected mice were injected with 1 mg ^{13}C-citrulline, and two samples (1 and 2) were collected sequentially after injection. Each breath sample was collected over a period of 30 mins mean +/- sd and measured by IRMS using Elemental Analyzer desorption of CO_2.

Conclusions Absorption of carbon dioxide on sodasorb enables us to autoclave samples out of a select agent ABSL3 laboratory safely, with high temperature desorption using an elemental analyzer upstream of a GC-IRMS. The enzyme activity was measured in infected mice by breath test.

Limitations of this study. Animal numbers were low, we didn't use inhaled delivery of labelled citrulline, we didn't use control infections with LVS or R96 strains that don't express CTU, and we only studied 1 post-infection time point.

Further details. US Patent 9,074,237, Method for diagnosing Francisella tularensis infections, Timmins *et al.* including synthetic methods.