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## Non-antibiotic selectable markers for *Francisella tularensis* strains LVS and SchuS4

S. A. Smith<sup>1</sup>, F. G. White<sup>1</sup>, A. Lair<sup>1</sup>, K. Moore<sup>1</sup>, S. Soni<sup>2</sup>, J. S. Gunn<sup>2</sup>

<sup>1</sup>Battelle Memorial Institute, Columbus, United States, <sup>2</sup>The Ohio State University, Columbus, United States

**Aims:** The aim of this project was to identify genes that could serve as non-antibiotic selectable markers for genetic manipulation of *F. tularensis* LVS and SchuS4.

### Methods:

- 1) A review of the annotated genomes revealed that both organisms lack critical genes in the biosynthetic pathways for each of 12 amino acids found to be absolutely required for growth in Chamberlain's defined medium (CDM). From the isoleucine-leucine-valine pathway, *ilvD*, encoding dihydroxy acid dehydratase, is present in SchuS4 but not in LVS, while *ilvC*, encoding ketol acid reductoisomerase, is present in LVS but not SchuS4. We tested with reformulated CDM whether the metabolic intermediates, 2-isovalerate and 3-methyl-2-oxopentanoate could rescue the dependency on valine and isoleucine, respectively, which would be highly suggestive that *ilvD* and *ilvC* could serve as non-antibiotic selectable markers.
- 2) In a second line of experimentation we tested whether the Calvin Cycle enzyme phosphoribulokinase (PRK) would interfere with the pentose phosphate pathway, leading to a casamino acid (CAA)-dependent phenotype in CDM. The hypothesis was that the PRK phenotype could be rescued by a second key enzyme from the Calvin Cycle, ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). PRK from the cyanobacterium *Synechococcus* PCC7942 was codon optimized and expressed from the *Francisella groE* promoter, and it was also co-expressed with codon-optimized RubisCO from the bacterium *Rhodospirillum rubrum*.

### Results:

- 1) When added to reformulated CDM, 2-isovalerate relieved the requirement for valine, and 3-methyl-2-oxopentanoate rescued the dependency upon isoleucine, for both LVS and SchuS4.
- 2) PRK transformants could not be obtained on rich medium, even when co-transformed with RubisCO. As such, the phenotype on CDM could not be tested.

### Conclusions:

- 1) The outcomes with 2-isovalerate and 3-methyl-2-oxopentanoate suggest that *ilvD* and *ilvC* will serve as non-antibiotic selectable markers in *Francisella*. The genes have been amplified and cloned for further testing.
- 2) The use of RubisCO as a selectable marker could not be tested, due to the toxicity of PRK even in rich medium.

This outcome suggests that tighter control of PRK expression will be required to test RubisCO as a selectable marker. As such, we are now examining use of the *Francisella ttp* promoter as an inducible promoter for better control of PRK expression.