Novel Vaccine Targets and Assessment of Cellular Immunity

Gregory D. Wiens

National Center for Cool and Cold Water Aquaculture

My interest in the *Renibacterium* genome

1. Comparative genomics

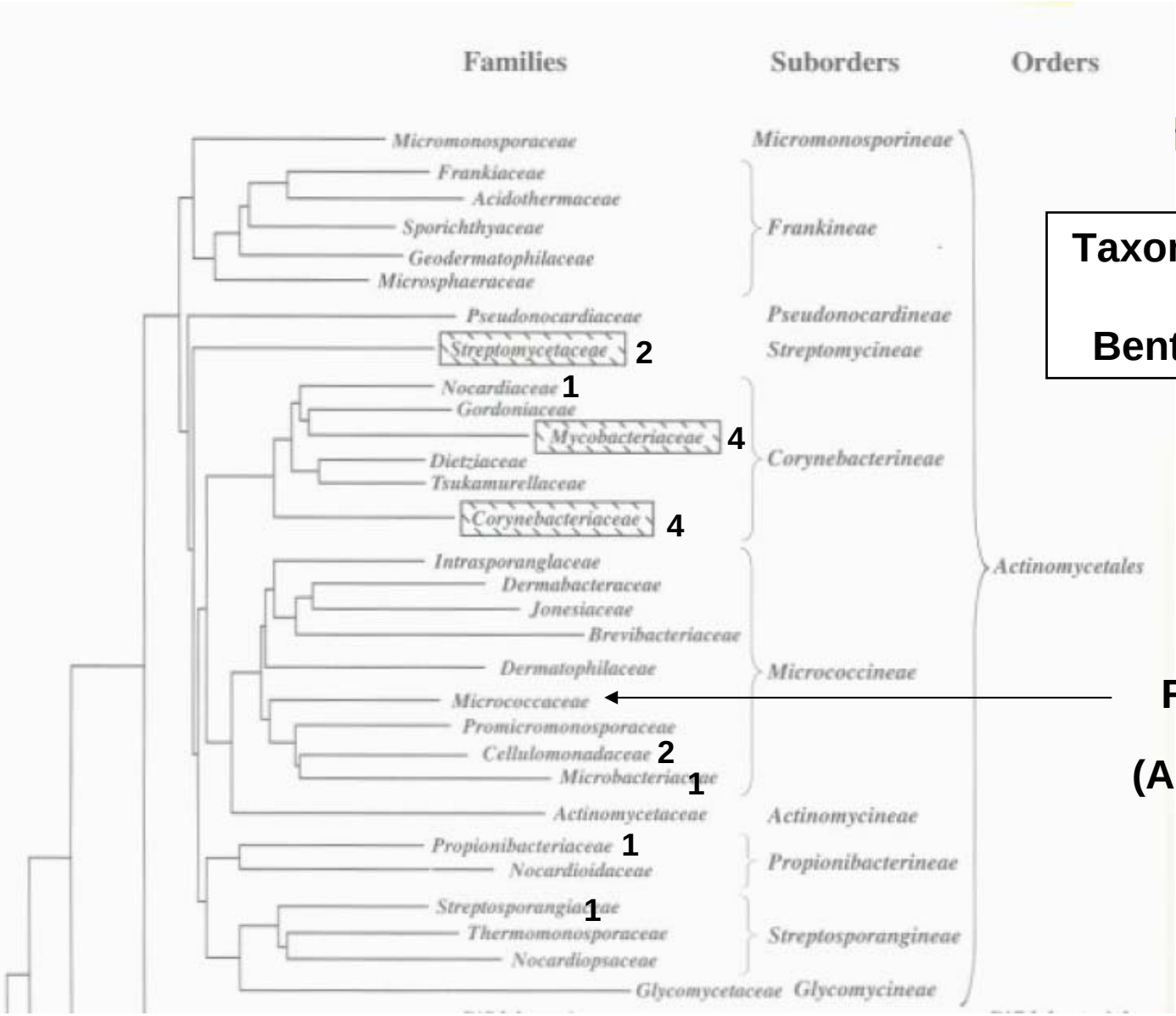
2. Identification of new vaccine candidates

3. System to study mechanisms of cellular immunity

4. Antigenic variation

   (tomorrow)

Taxonomy of High G+C Gram-Positives



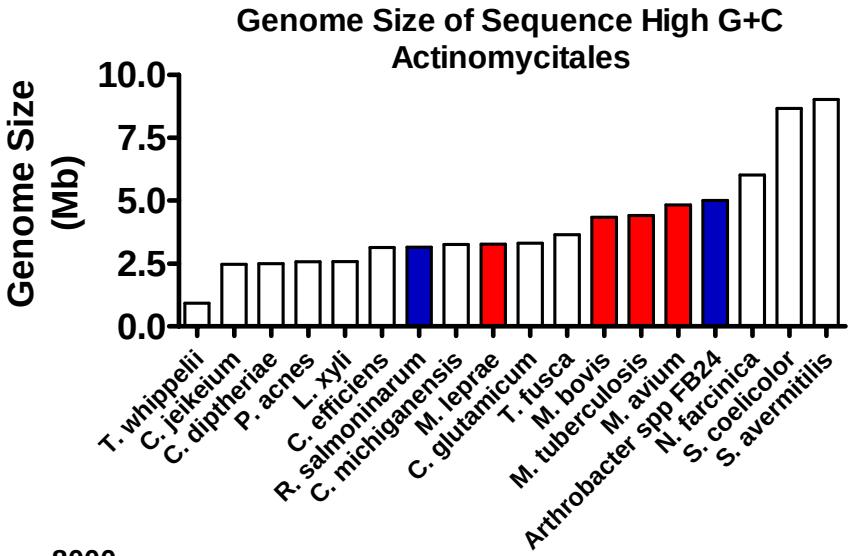
Taxonomy based on 16s rRNA Bentley et al. 2004

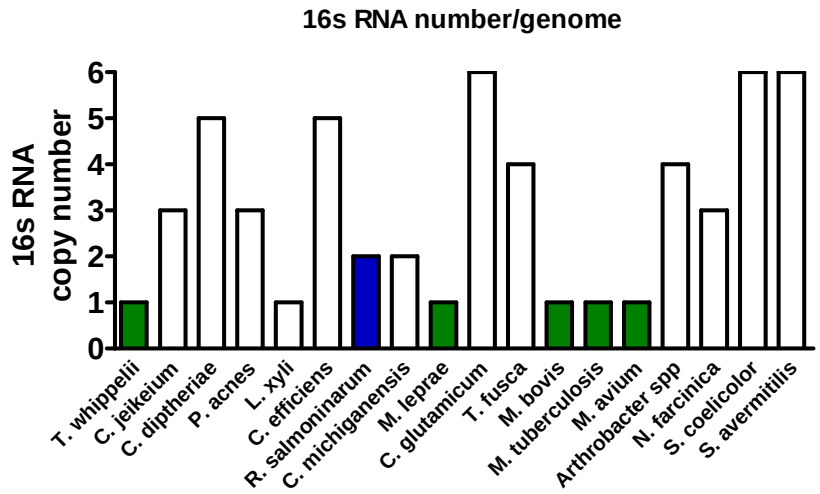
Renibacterium

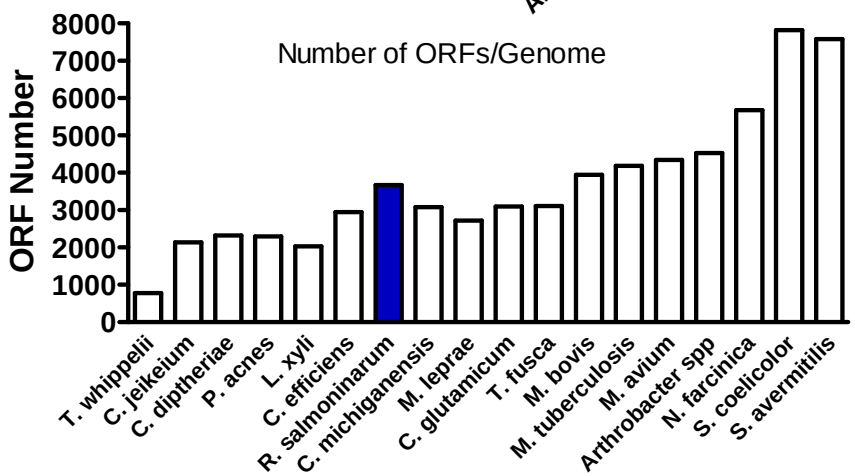
(Arthrobacter spp)

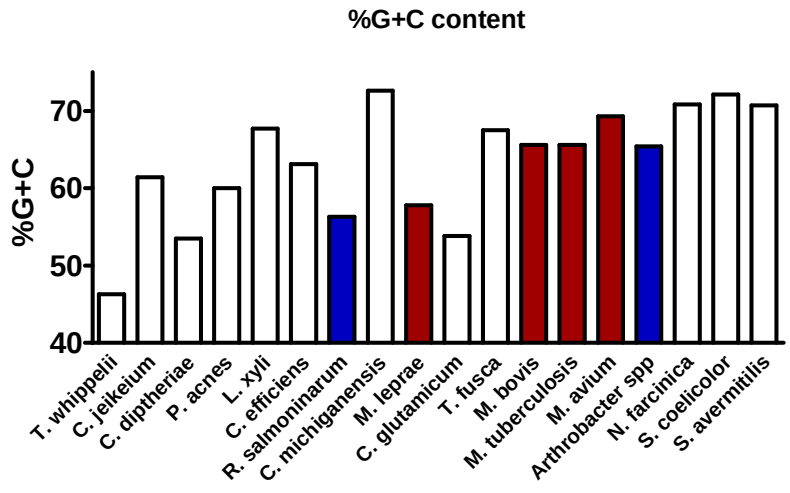
N=16

Comparative genomics



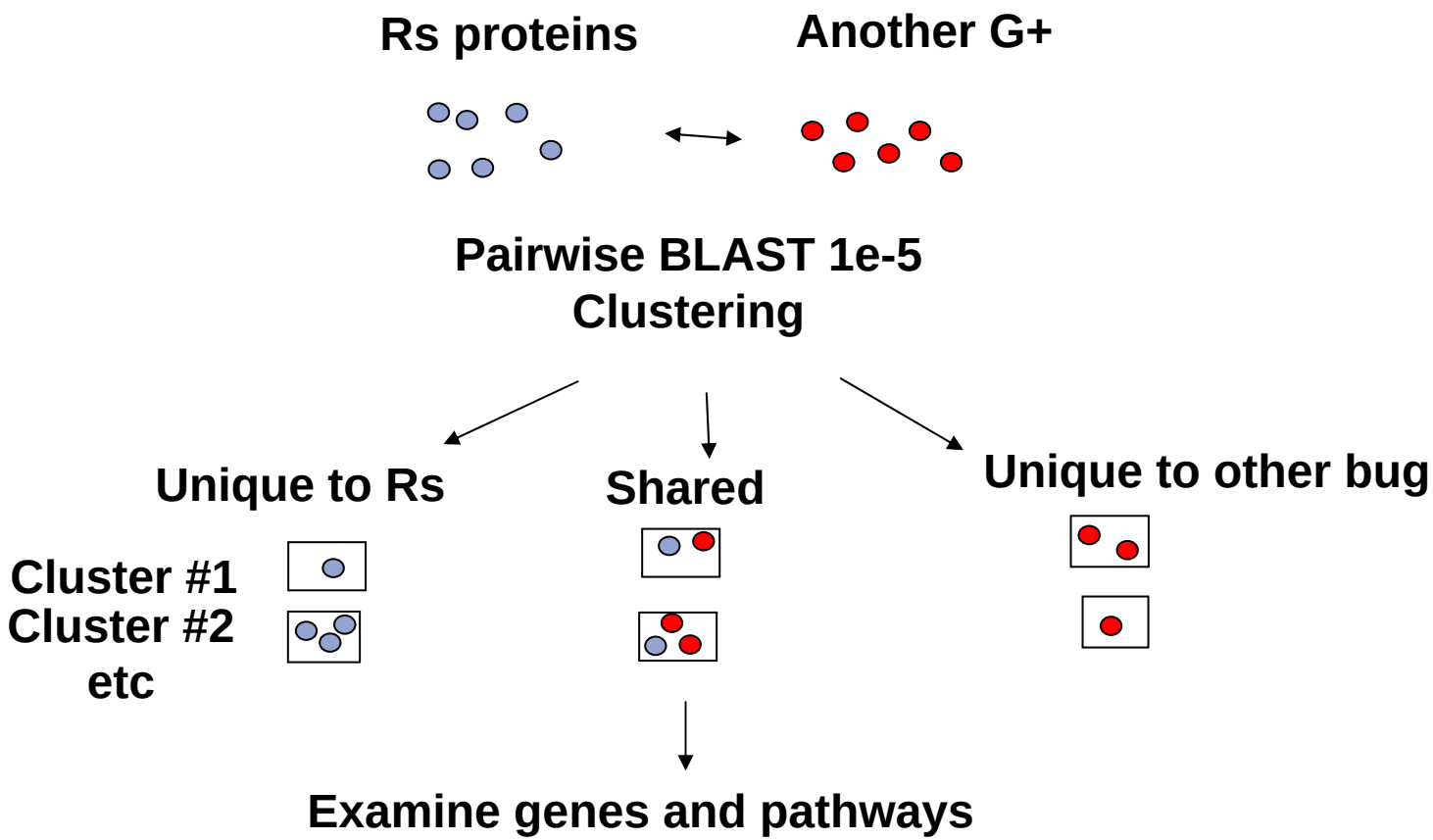




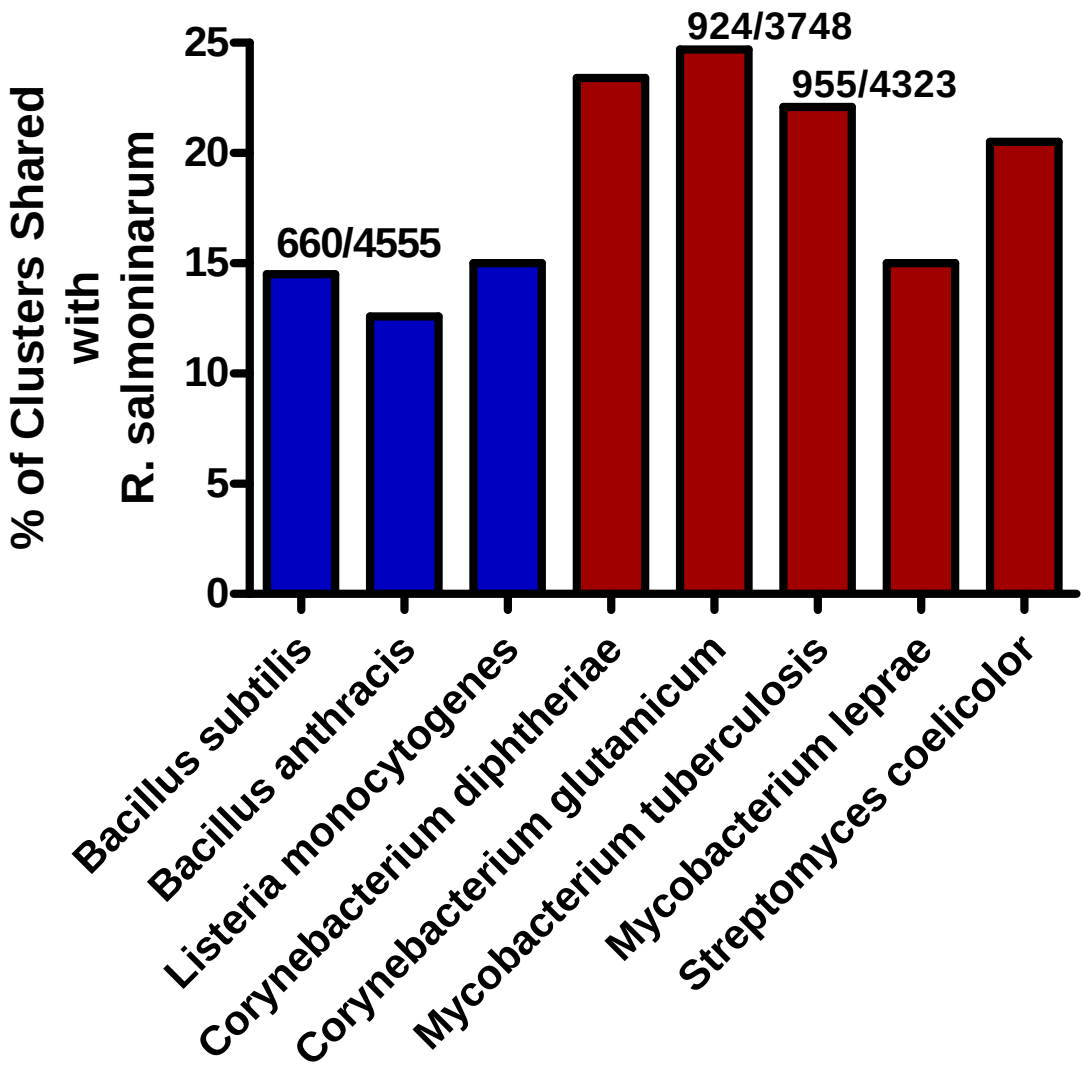


How many genes does *R. salmoninarum* share with other G+'s

- **ERGO Workbench-Pairwise protein comparison**



Numbers of protein clusters shared between *Renibacterium* and other G+'s



R.s. shares more proteins clusters with high G+C vs low G+C

More unique clusters than shared clusters

Identification of vaccine targets

- **1. Are there conserved vaccine targets in the Rs genome?**
- **2. Bioinformatic identification of surface and secreted proteins**

Vaccine targets in Corynebacteria and Mycobacteria

- *Corynebacterium diphtheria*
  - Integrated phage produces toxin
    - (other examples botulism toxin, cholera toxin)
  - No obvious integrated phage/toxins
- *Mycobacterium tuberculosis*
  - Promising subunit vaccines (Anderson and Doherty 2005)
  - 27 candidates:
    - Rv0125 serine protease – Rs 2170
    - Rv0934 phosphate uptake – Rs 654
    - Rv1884 resuscitation promoting factor proteins

Resuscitation-promoting factor proteins

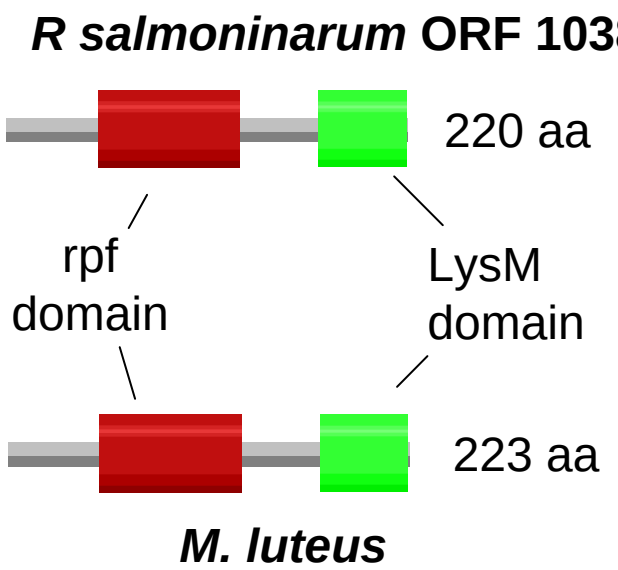
Micrococcus luteus resuscitation-promoting factor (*rpf*)

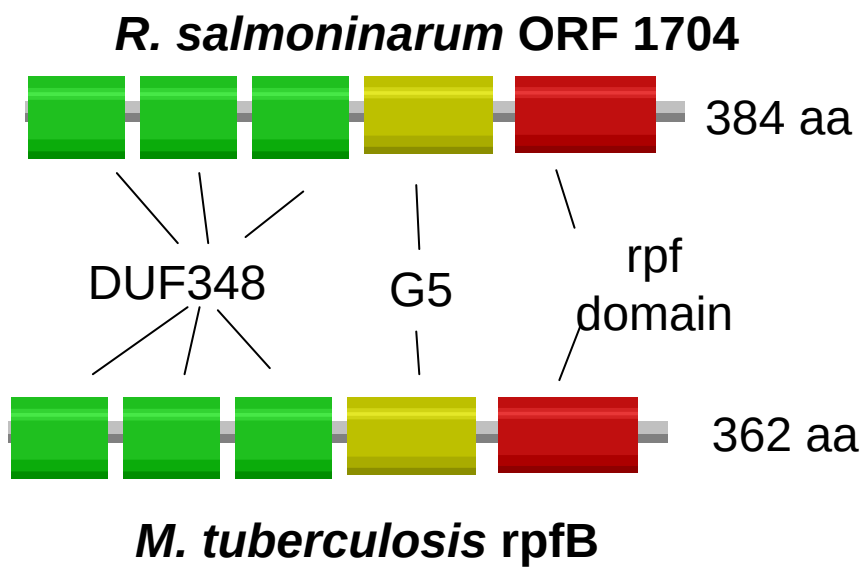
-promotes recovery of “dormant” bacteria
-stimulates growth of washed bacteria
-single gene, essential for survival

Mycobacterium tuberculosis rpfs

-5 genes
-stimulate growth
-proteins are immunogenic in mice and induce protective immunity
-hypothesized to play role in reactivation of bacteria in granulomas

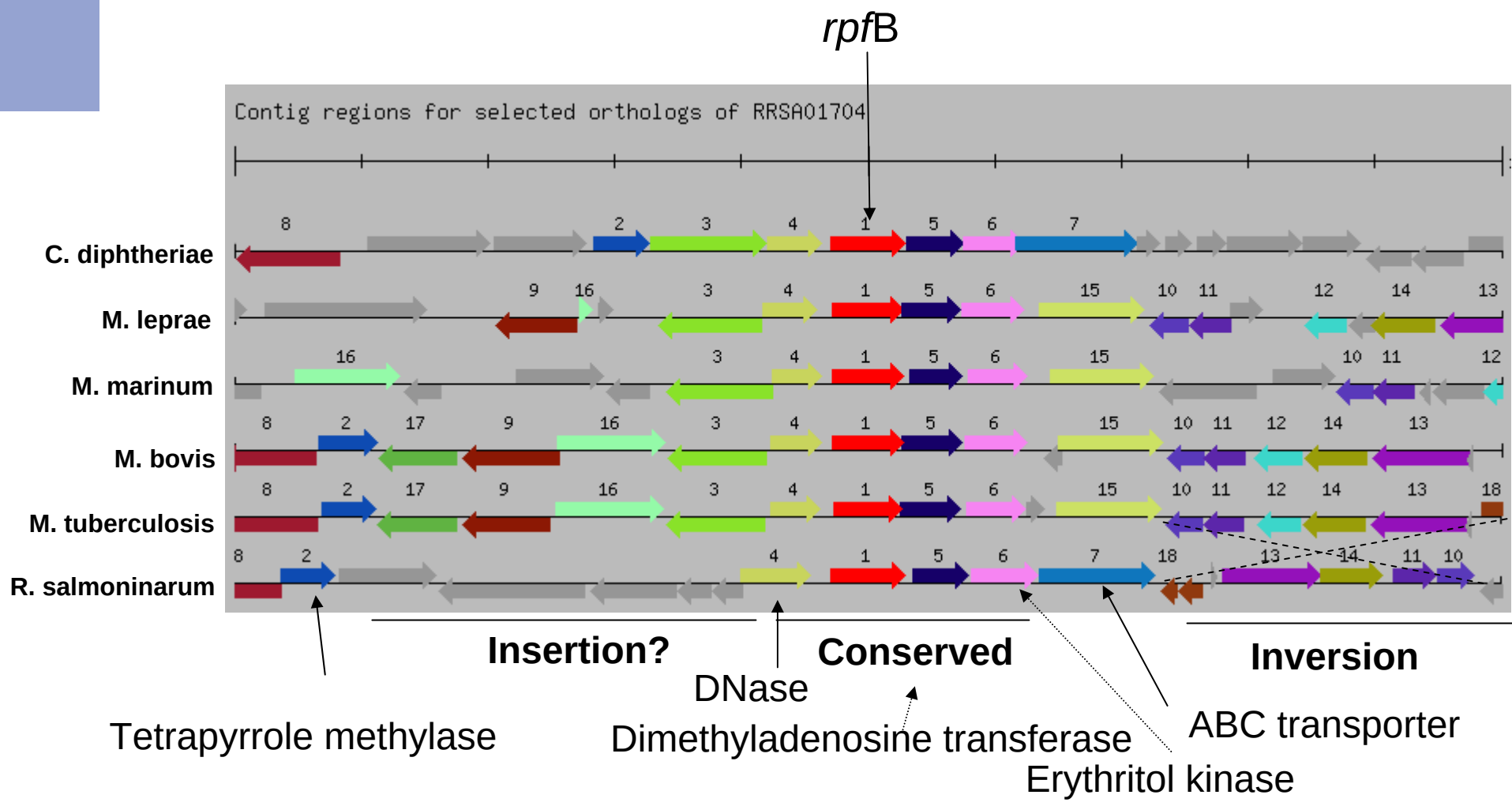
R. salmoninarum has two proteins with Rpf domains





*Renibacterium salmoninarum rpf*B is in a highly conserved cluster of genes

ERGO Pinned region analysis

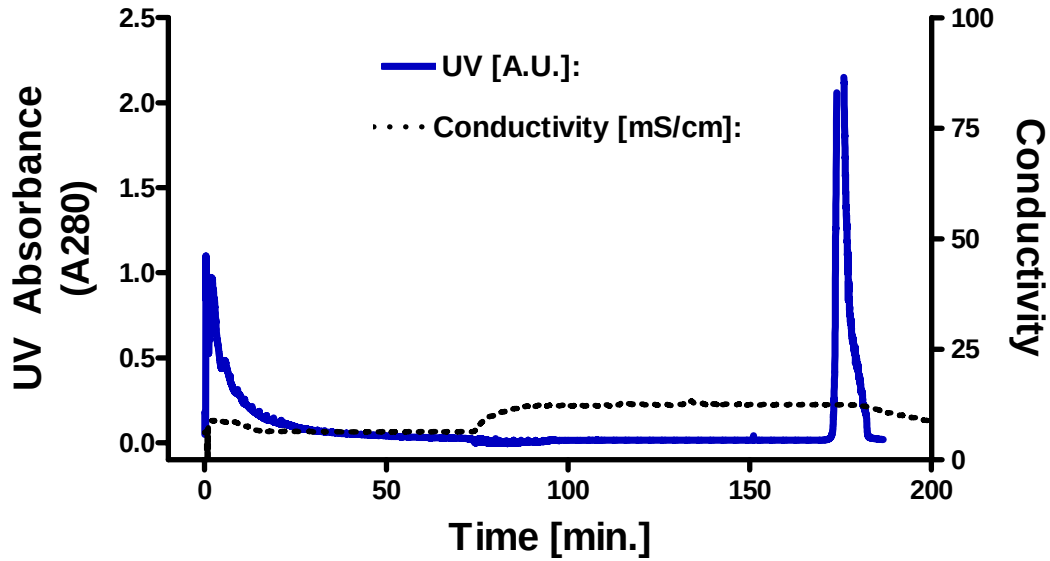


Purification of rpfA (ORF 1038) protein from *E. coli*

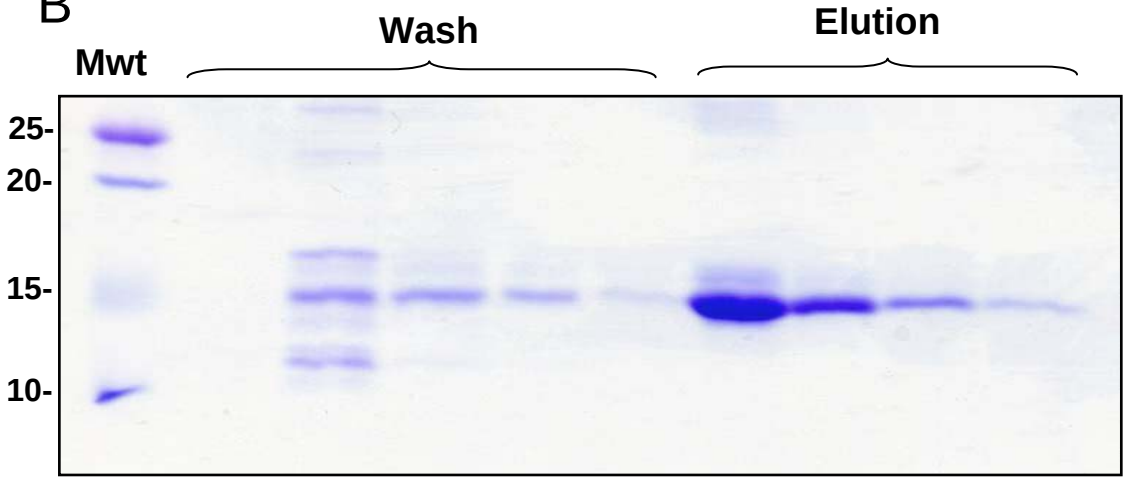
Methods

1. 1L culture
2. IPTG
3. Lysis of bacteria
4. Ni-column
5. Refolding on column
6. Elution with 0.5 M Imidizol
7. Pooling fractions
8. Dialysis in PBS
9. Yield from 1L= ~2.4 mg

A

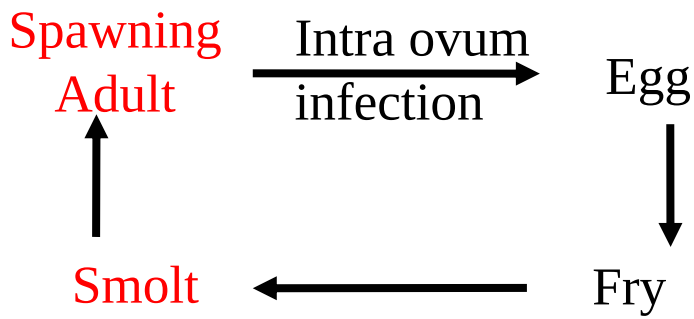


B



Future work with Rpf proteins

- No antibody response in rainbow trout at 9 wks. (Adjuvant or boost necessary?)
- Do the purified rpf proteins have growth promoting activity?
  - Nurse culture technique or spent broth supplement augments Rs isolation (Evelyn et al. 1990).
- Are the proteins produced during infection?
- Does exogenous rpf addition convert a subclinical infection into active disease?



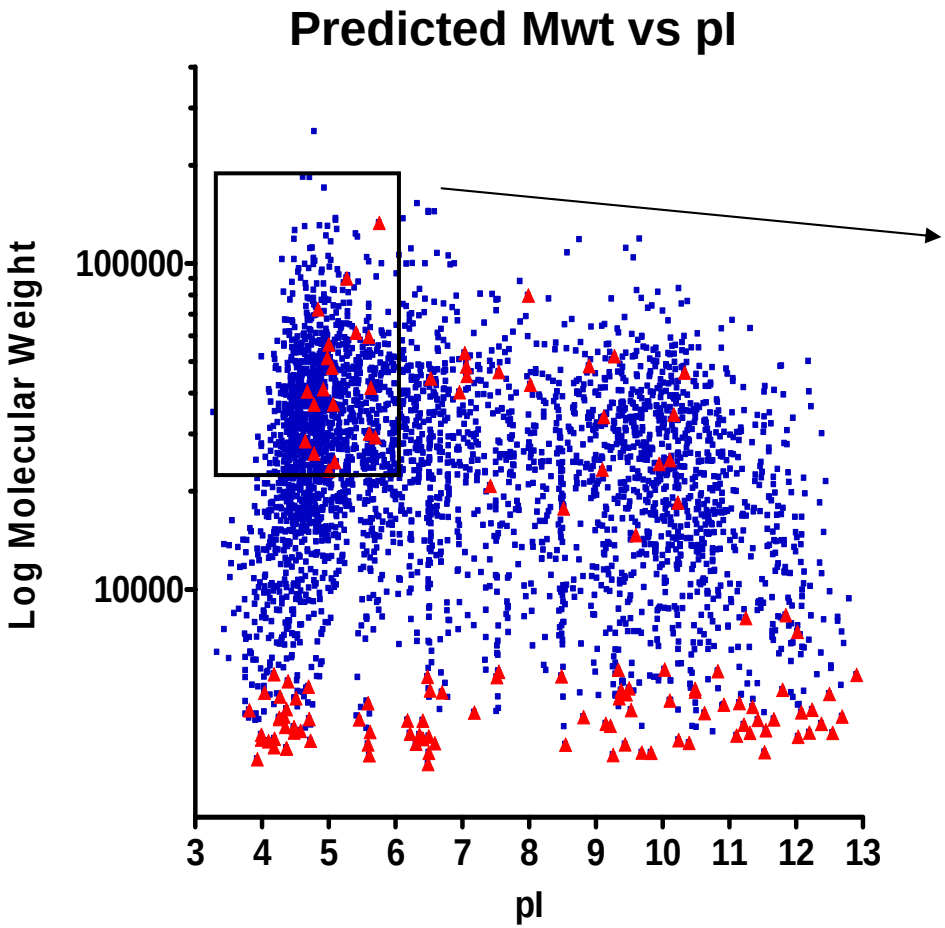
Bioinformatic identification of surface and secreted proteins

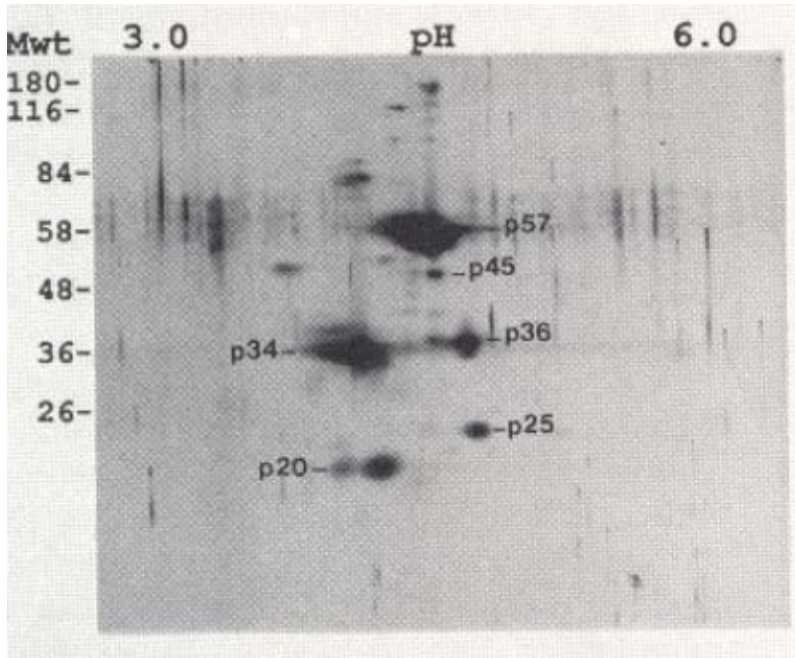
- PSORTb v2.0 – Program for prediction of bacterial protein subcellular localization.
  - Purpose to identify potential diagnostic, drug and vaccine targets.
  - Gardy et al. 2005 Bioinformatics 21:617-623
- PSORT uses a number of program modules to predict signal peptides, transmembrane helices, homology to proteins of known localization, amino acid composition and motifs. Module predictions are weighted and integrated to generate a final prediction.
- 576 Gram-positive proteins of known localization.
  - Precision of 96% for Gram +

PSORTb v 2.0 Output

Localization	% proteome (all predicted orfs)		% localized ORFs	
	R. sal. (3667 orfs)	Avg of 45 Gram + genomes	*R. sal.* (2399 orfs)	Avg of 45 Gram + genomes
Cytoplasmic	44.3% (1626 orfs)	50.7 ± 3.5%	67.8%	68.5 ± 3.9
Cell membrane	17.0% (625 orfs)	19.7 ± 2.6%	26%	26.5 ± 3.1
Cell wall	0.7% (16 orfs)	0.9 ± 0.5%	0.7%	1.2 ± 0.6
Extracellular	3.6% (132 orfs)	2.8 ± 1.5%	5.5%	3.0 ± 2.1
Unknown	34.6% (1268 orfs)	25.9%		

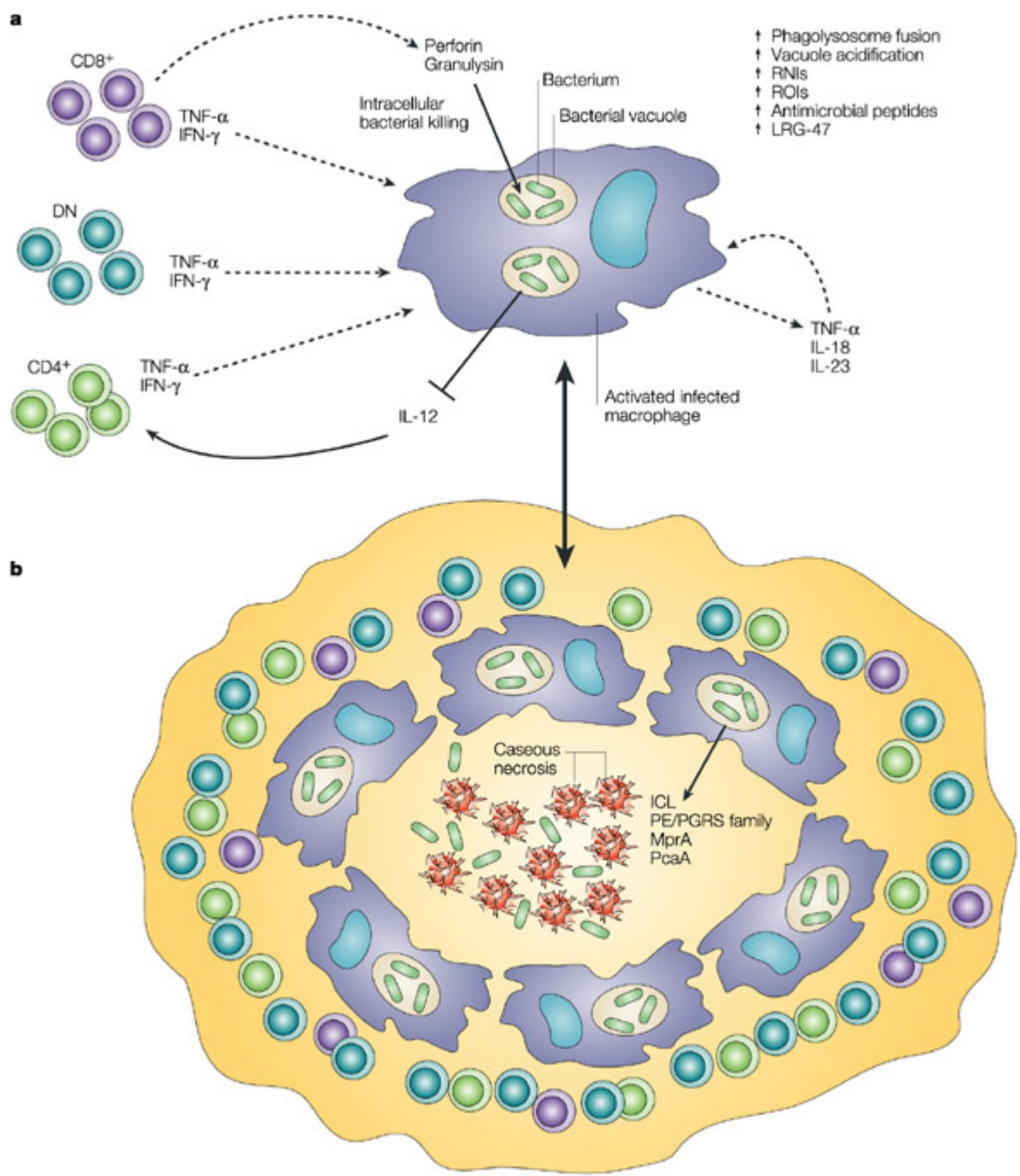
Proteome analysis of *Renibacterium salmoninarum*





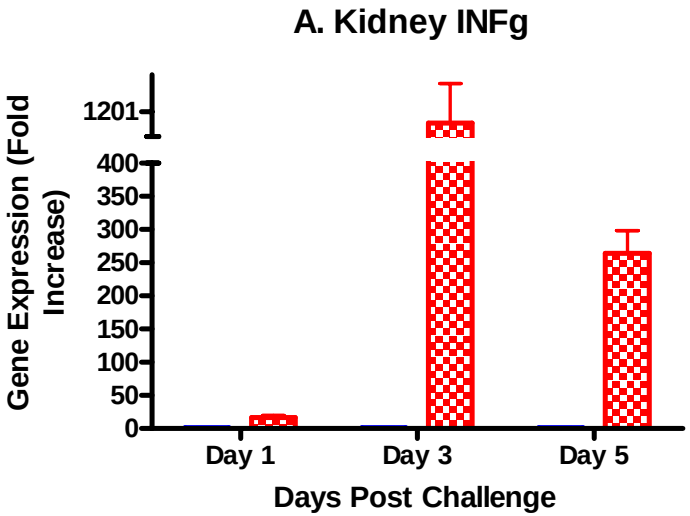
Wiens and Kaattari 1991

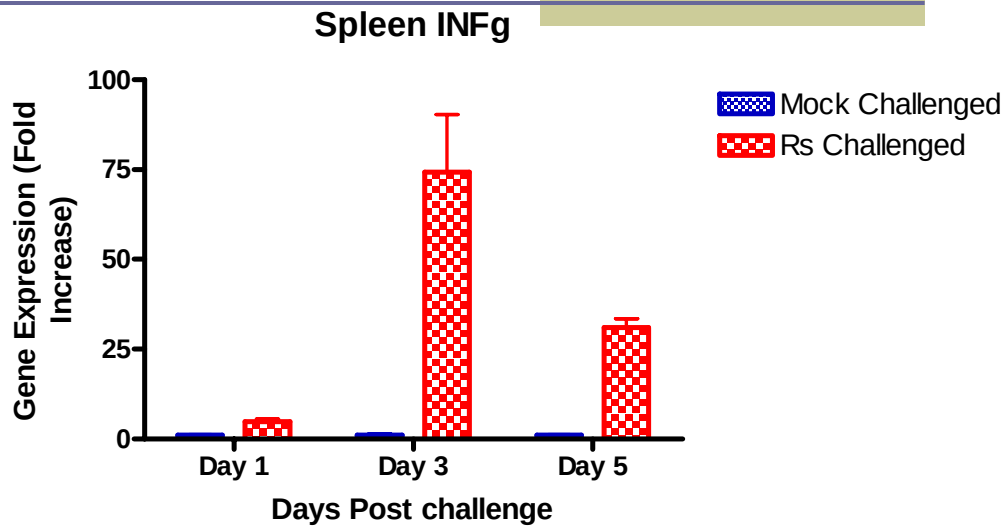
Mammalian Cellular Immunity to MtB

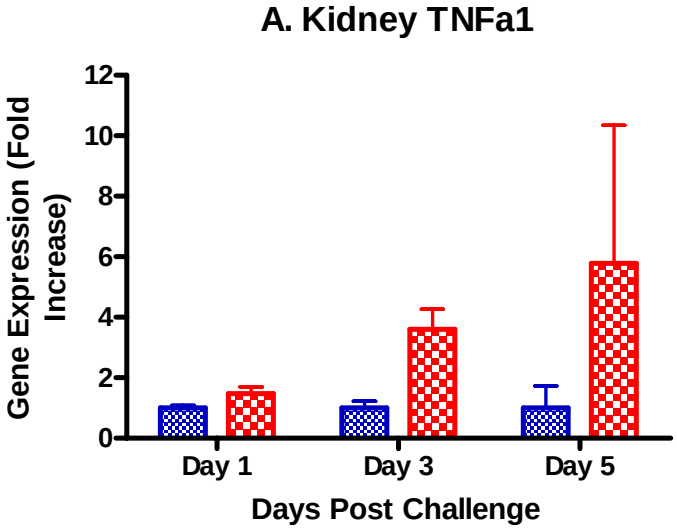


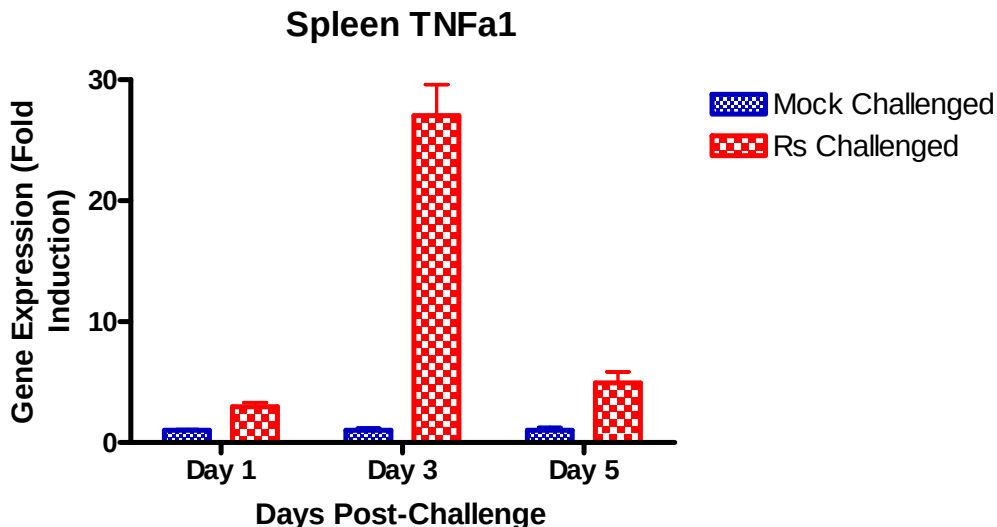
Monack Mueller and Falkow 2004

Increased TNFα and INFγ mRNA in Rainbow trout challenged with *R. salmoninarum* ATCC 33209

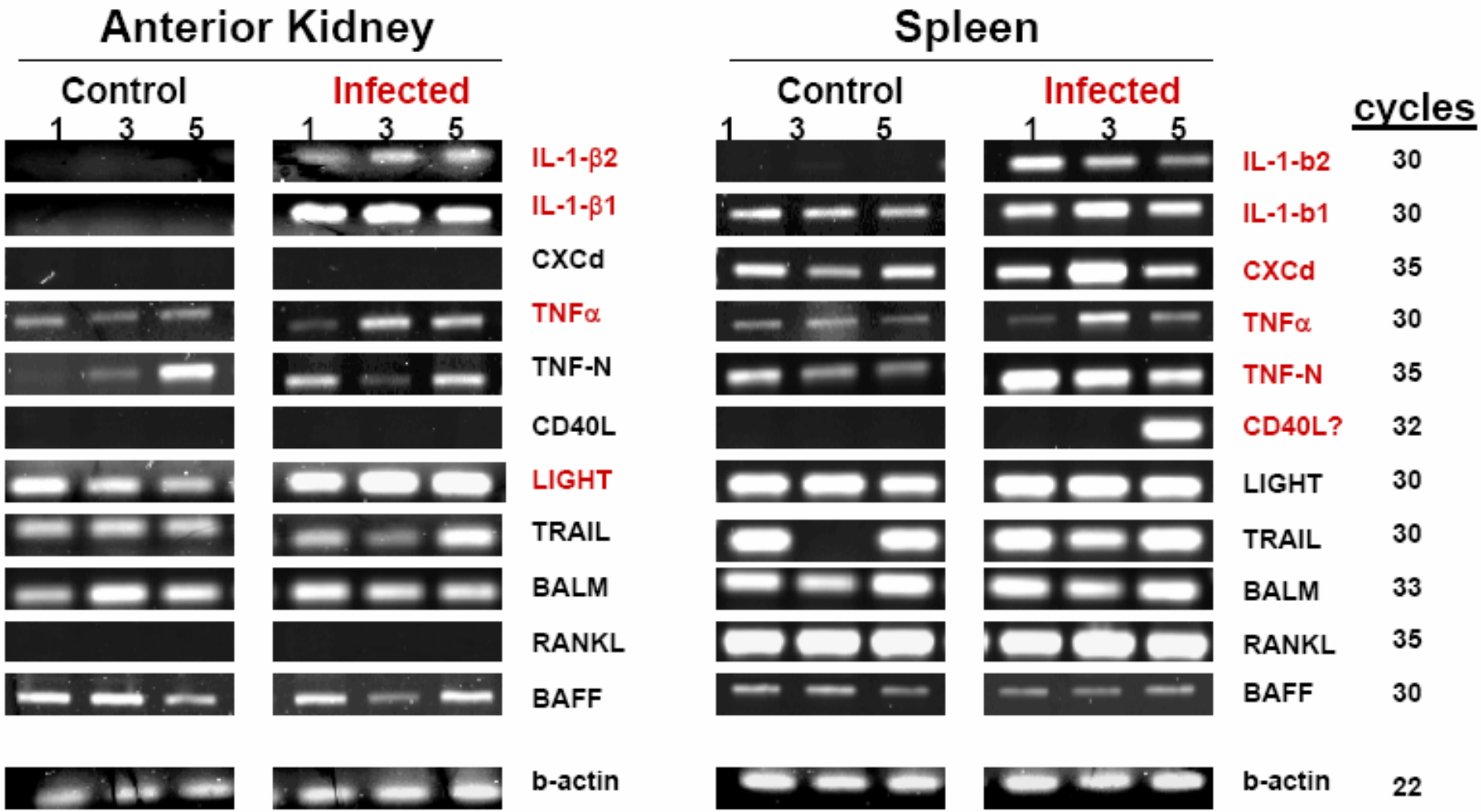








Immune gene expression in *R.salmoninarum* challenged trout



Summary

- Renibacterium genome
  - Smaller genome than Arthrobacter – gene loss?
  - Shares more genes with high G+C vs low G+C Gram +'s
- Only a few vaccine targets are conserved
  - Two rfp genes are present in the genome
  - Recombinant proteins are not immunogenic
- PSORTB predicted localization of 65.4% of the *R. salmoninarum* proteome
  - Many small ORF were predicted as extracellular.
- TNFα, INFγ, IL1-β and several novel putative cytokines are strongly induced in *R. salmoninarum* challenged trout. Assays useful for evaluating cellular immunity and vaccine candidates.

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