Rapid evolution of *Salmonella* Enteritidis during chronic bacteraemia in an IL12/23 beta-1 receptor deficient patient

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Patient History

Year

1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013

1996: Aged 12

- A 12 year old boy presents with: **fever**, lymphadenopathy, and splenomegaly, recurrent vasculitic lesions
- **Bacteraemic for *Salmonella* spp.** (probably Enteritidis)
- Diagnosed with **IL12/23 beta 1 receptor deficiency**
- Treated with **antibiotics** and interferon gamma

1999: Aged 16

- Diagnosed with purulent pericarditis
- *Salmonella* grown from blood and pericardial fluid

2000-2005

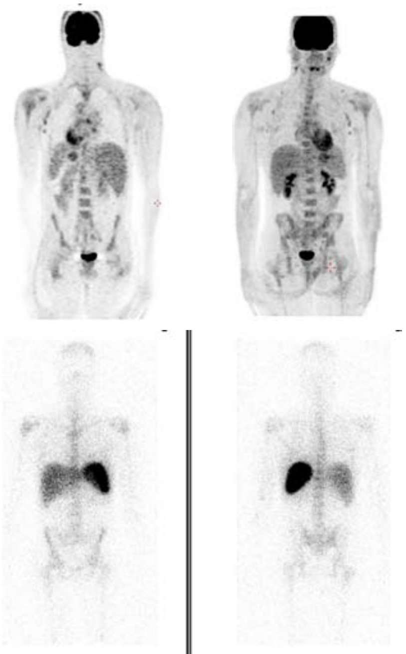
- >50 episodes of **bacteraemia**
- Salmonella Enteritidis but culture and **typing difficult**
- Vasculitic rash, arthitis and malaise
- Becoming **less clinically severe** over time

2006: Aged 22

- Care transferred to Addenbrookes hospital Cambridge
- **Elective cholecystectomy**: no *Salmonella* grown
- R cervical chain **lymph node removed**: no *Salmonella* grown
- Patient and family: **no stool carriage**
- Multiple **imaging / biopsy** studies fail to identify focus of infection

2006 - present

- Frequent bacteraemia relapses
- Salmonella difficult to grow / type
- Patient not particularly unwell
- But…Hg and albumin dropping, CRP increasing, IgG increasing – **chronic inflammation**



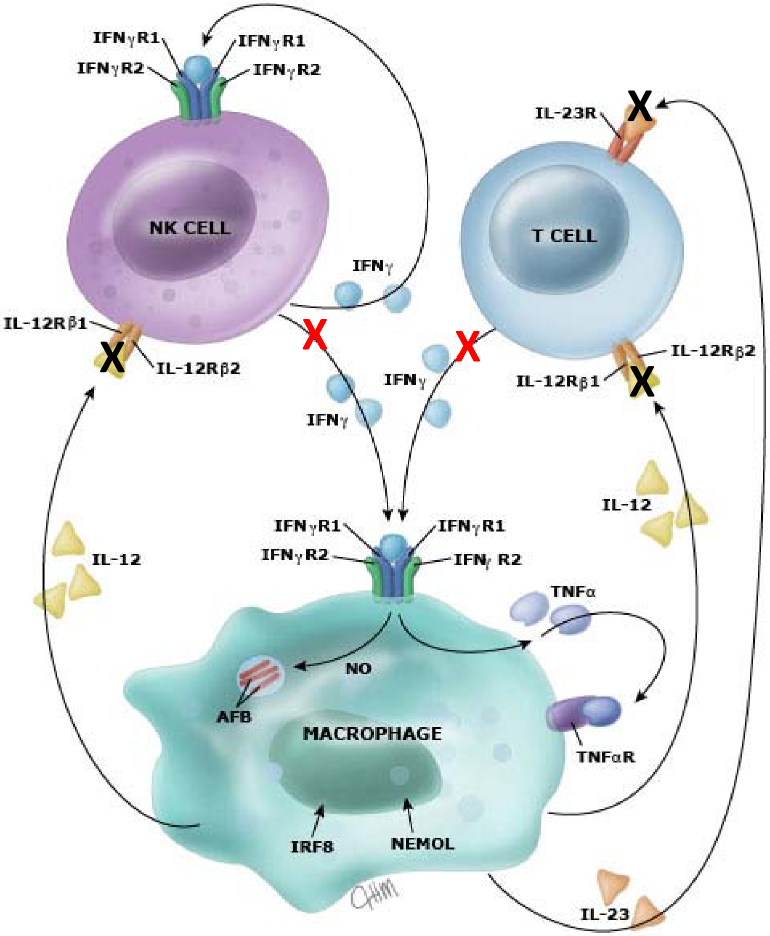
IL12/23 beta 1 receptor deficiency

IL12/23 beta 1 receptor deficiency

- autosomal recessive genetic lesion
- Impaired IFN-γ mediated immunity due to defective IL-12 / 23 signalling
- Associated with environmental mycobacteria and *Salmonella* infection

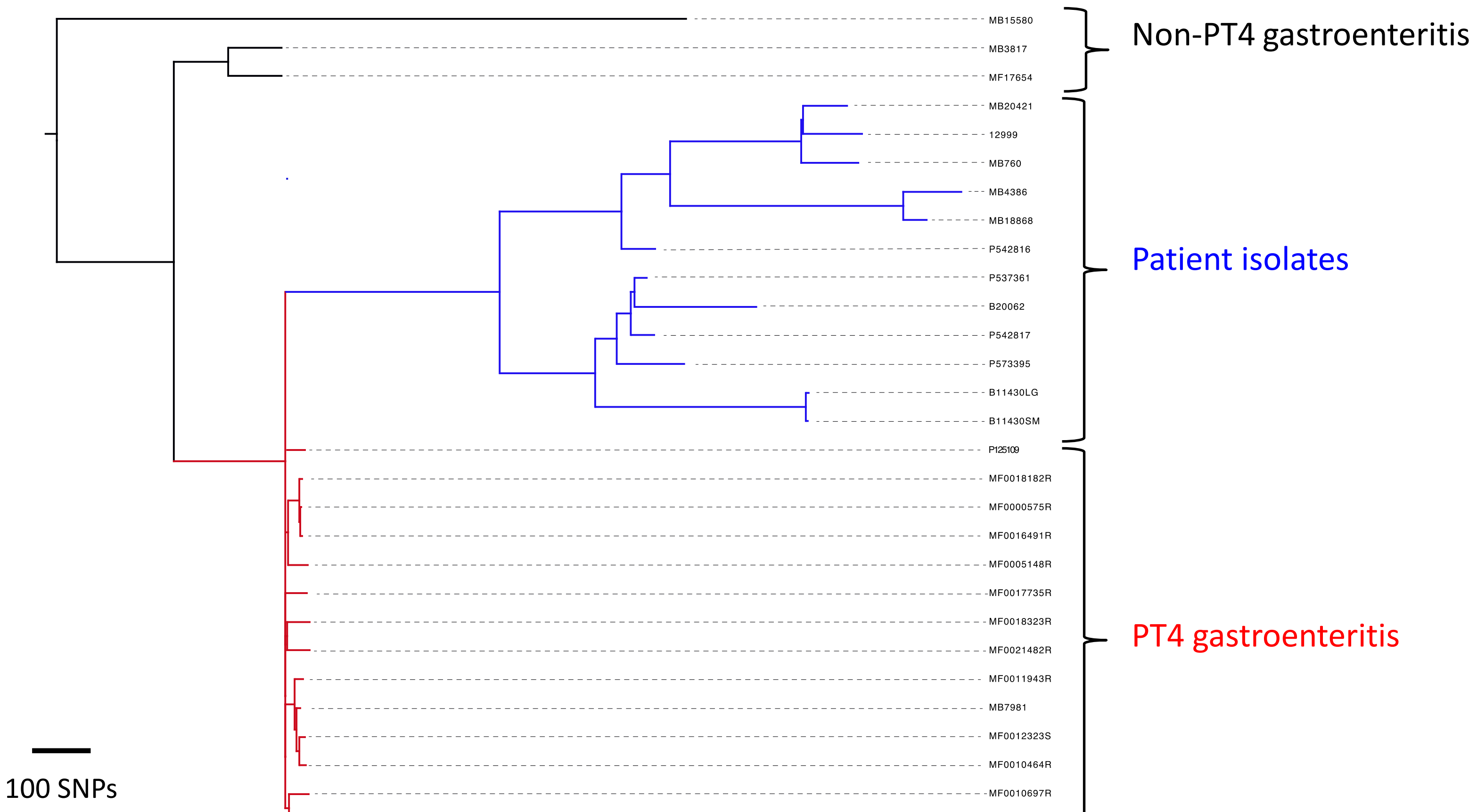
X – receptor deficiency

X – impaired cytokine response



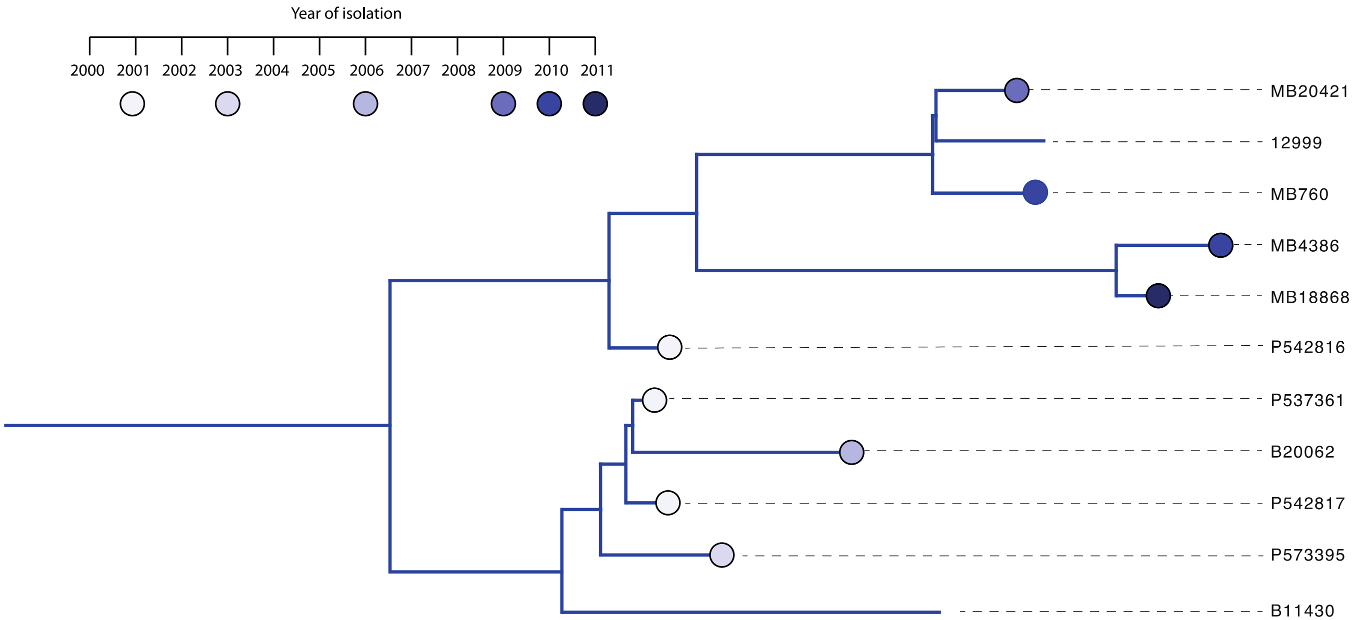
Phylogenetic analysis of S. Enteritidis gastroenteritis isolates and patient isolates

17 *S*. Enteritidis from cases of gastroenteritis (red/black)
11 isolates from the patient (blue)

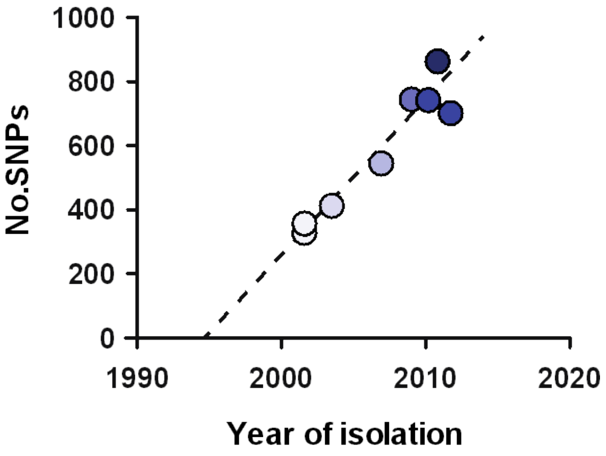


Phylogenetic analysis of S. Enteritidis gastroenteritis isolates and patient isolates

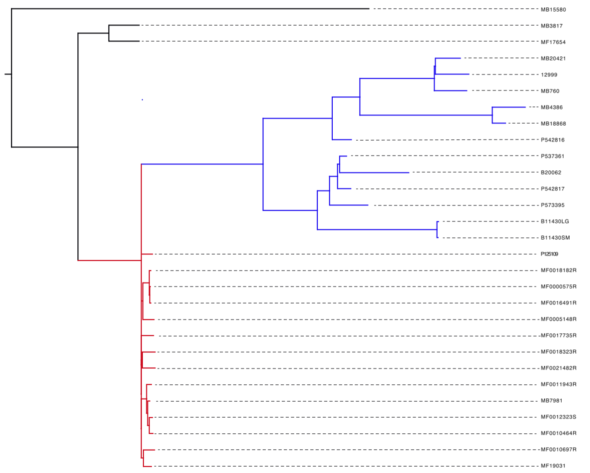
Maximum likelihood tree – 11 patient isolates



Root-to-tip SNP analysis



Bacteraemia isolate lineages exhibit distinct substitution frequencies

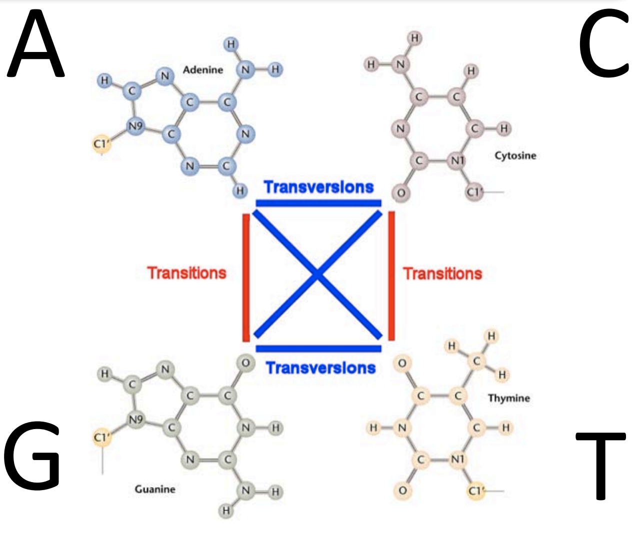


Elevated transition rate

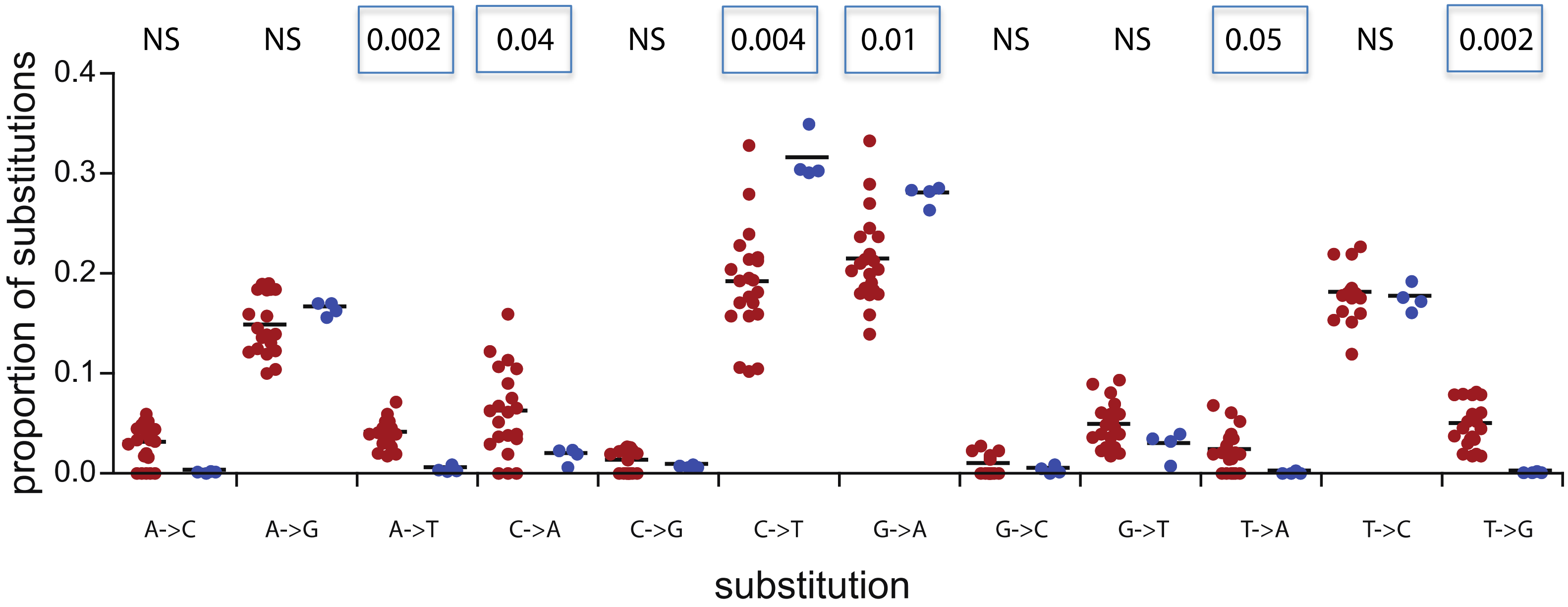
- C->T
- G->A

Decreased transversion rate

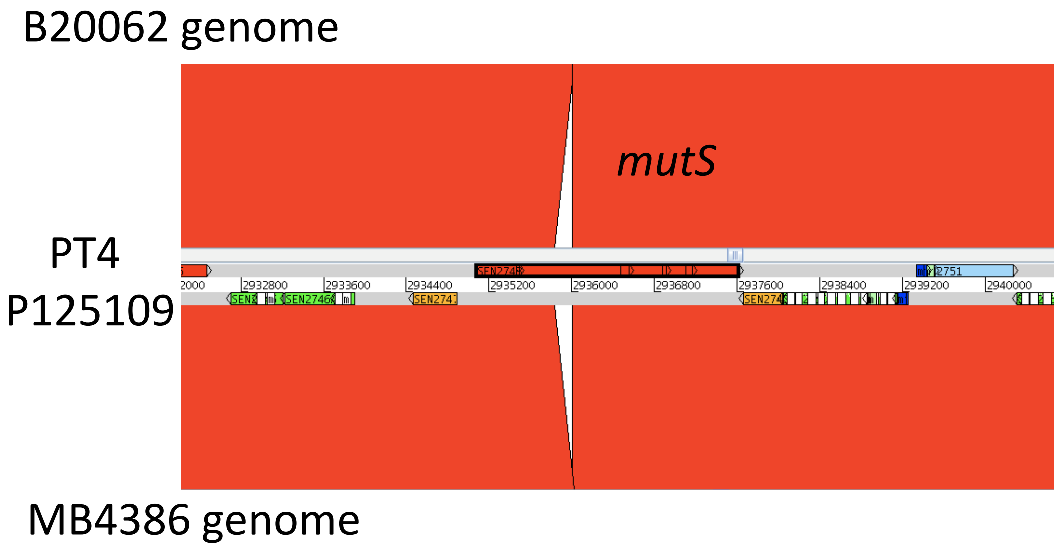
- A->T
- C->A
- T->A
- T->G

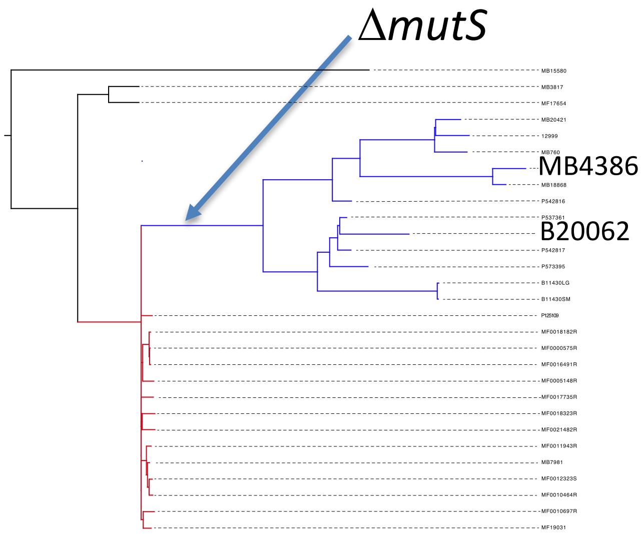


Transition *versus* Transversion mutations



All patient isolates contain an in-frame deletion in *mutS* gene

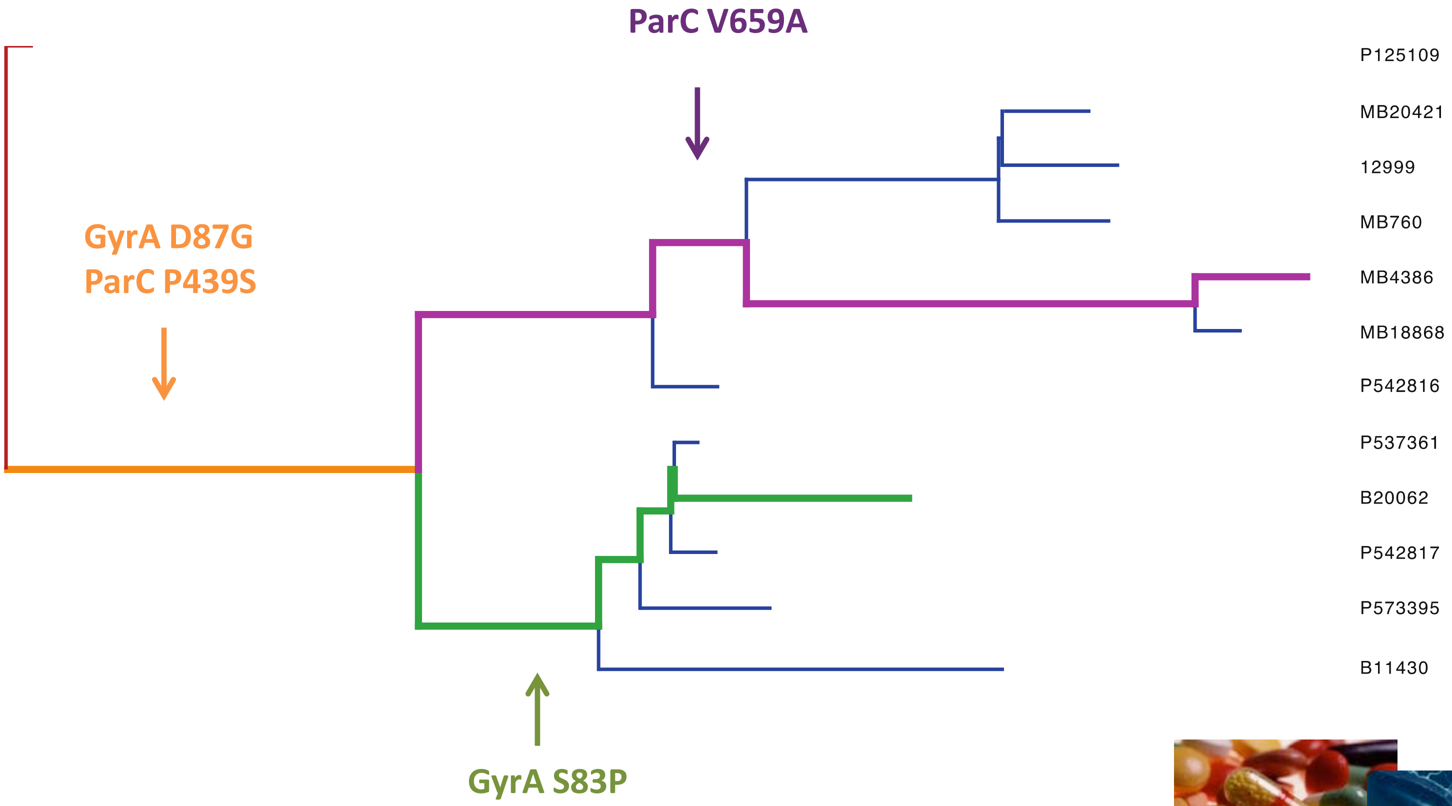




- In-frame deletion in all patient isolates
- MutS is involved in mis-match repair
- *mutS* mutations linked with
  - Elevated substitution rates up to 1000x wt
  - Altered patterns of substitution
- A hypermutator strain explains:
  - Diversity of patient isolates
  - Atypical substitution frequency
  - Accumulation of polymorphisms that may impact phenotype
  - Evasion of therapies

Polymorphisms: linked to fluroquinolone resistance

Substitutions in the topoisomerase subunit GyrA and associated protein ParC



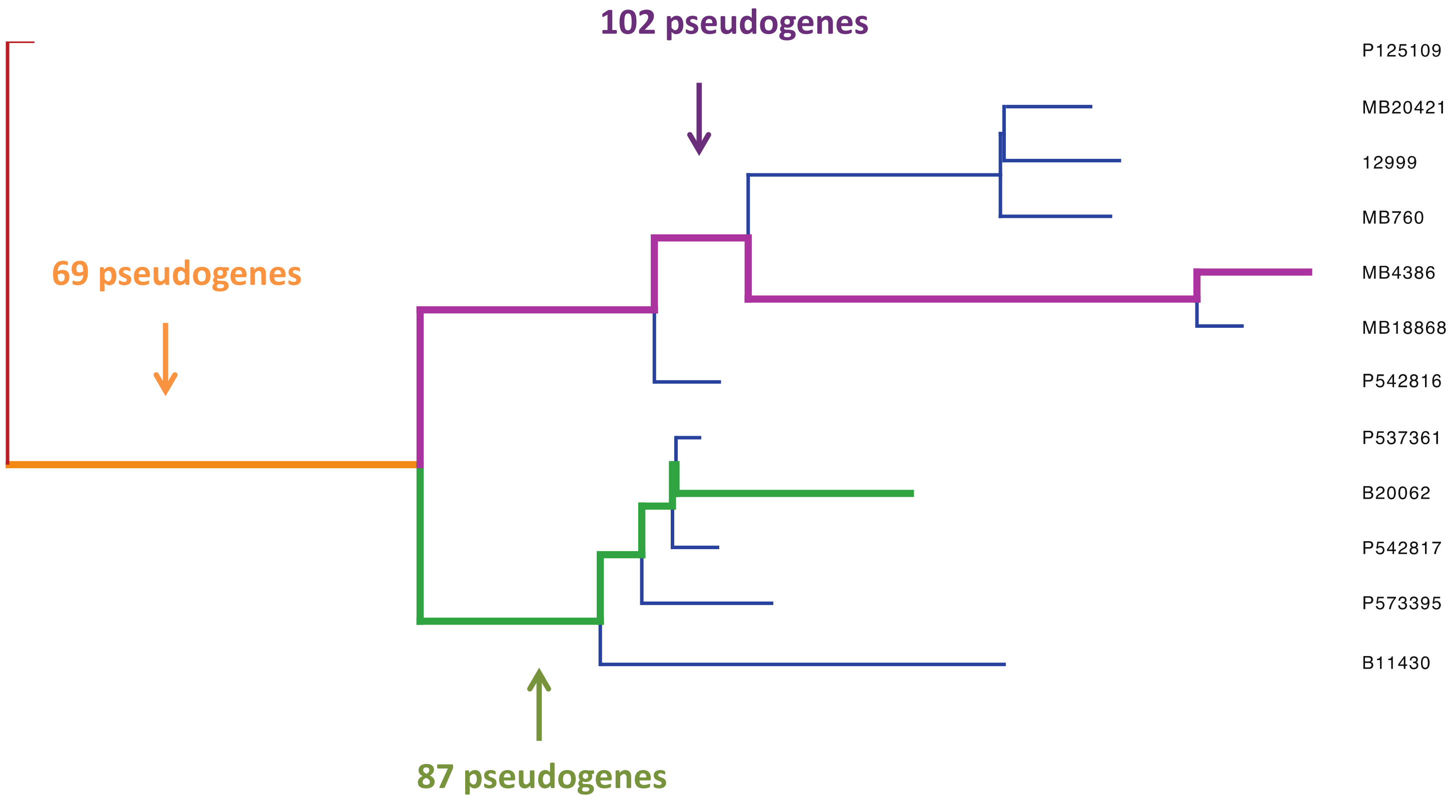
ParC V659A

GyrA D87G
ParC P439S

GyrA S83P

Considerable genome degradation - pseudogenes

Substitutions in the topoisomerase subunit GyrA and associated protein ParC



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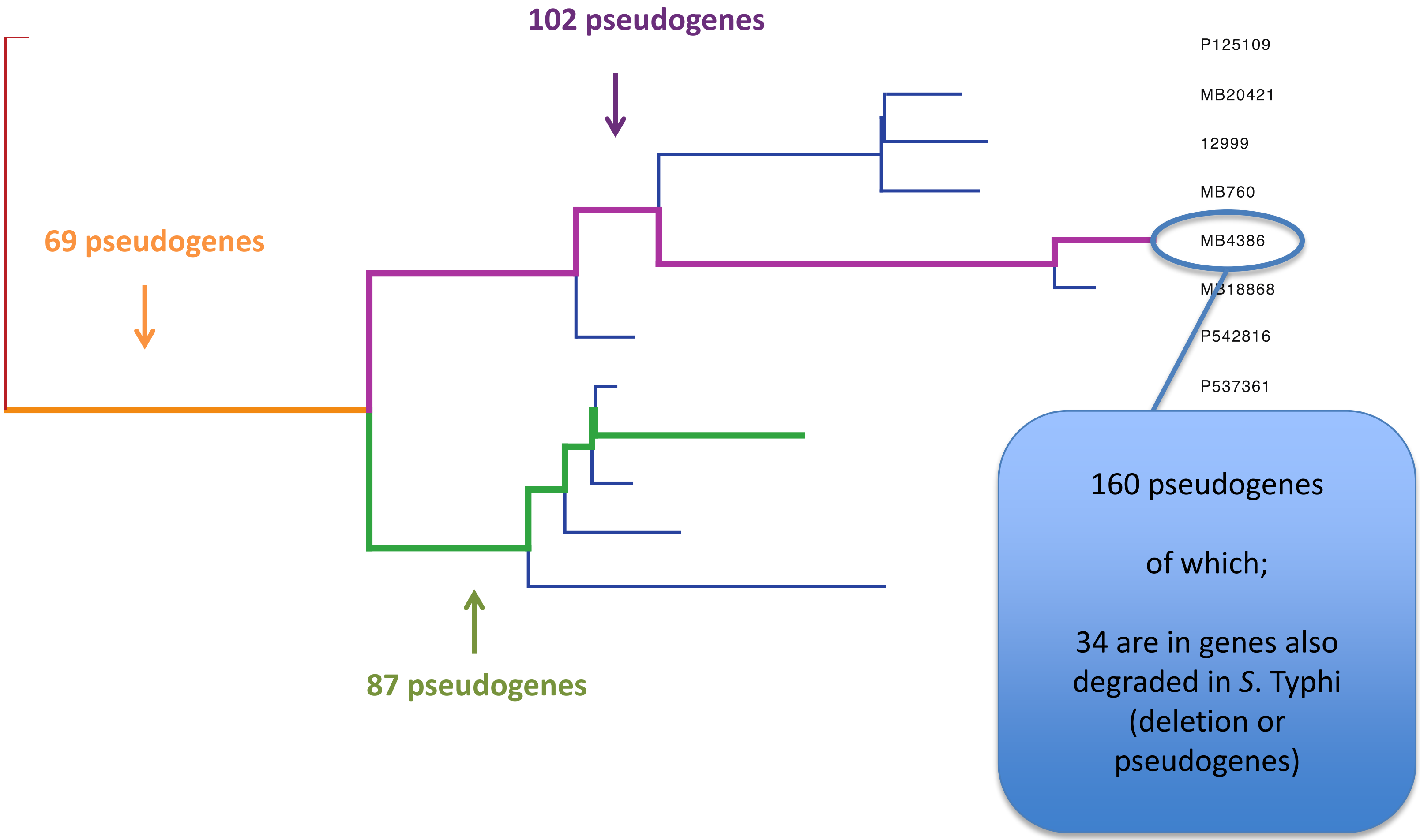
Substitutions in the topoisomerase subunit GyrA and associated protein ParC



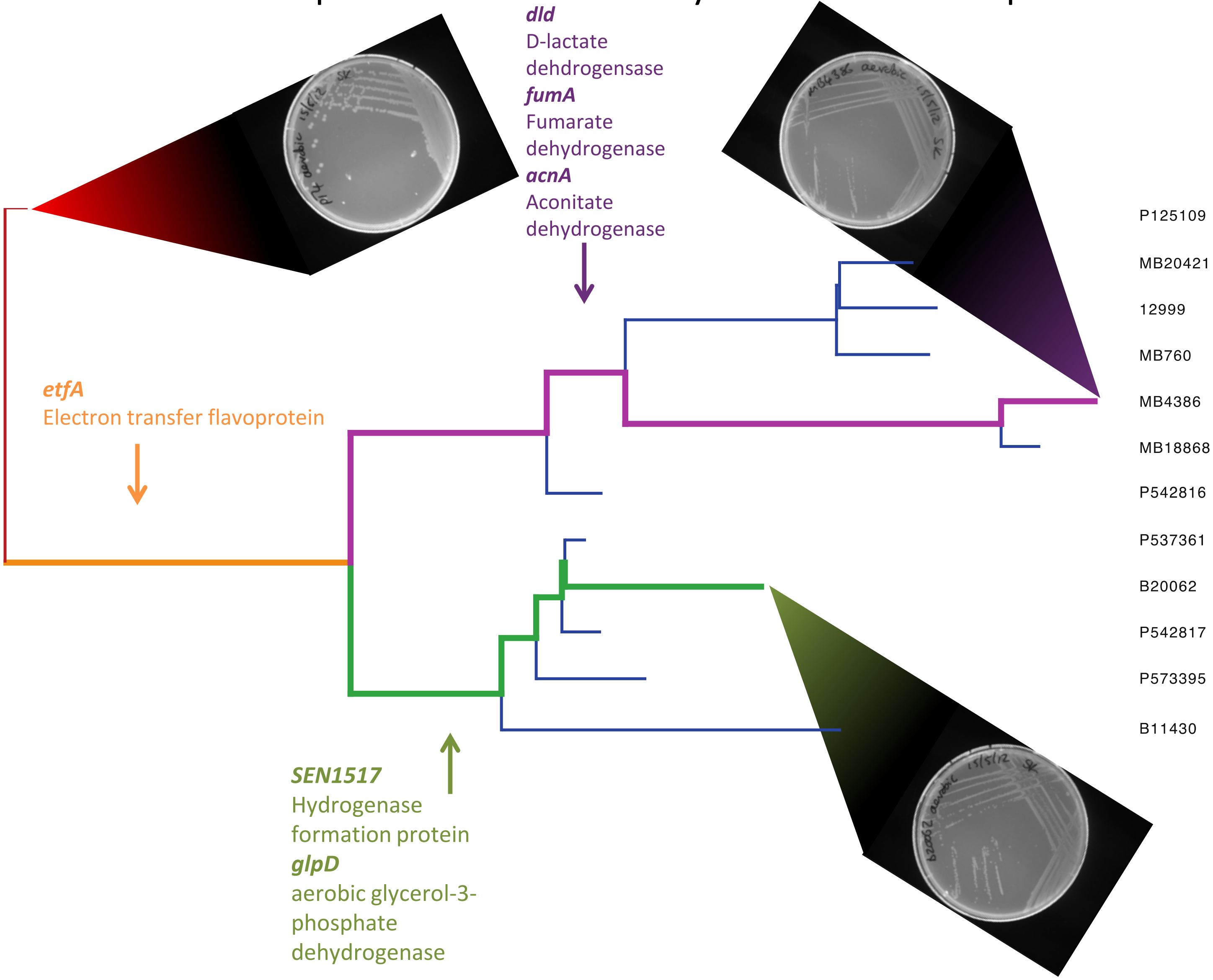
160 pseudogenes

of which;

34 are in genes also degraded in *S.* Typhi (deletion or pseudogenes)

Genome degradation - pseudogenes

Substitutions in the topoisomerase subunit GyrA and associated protein ParC

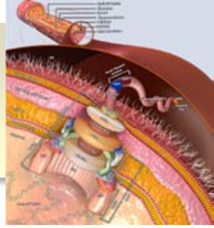


dld
D-lactate dehdrogensase
fumA
Fumarate dehydrogenase
acnA
Aconitate dehydrogenase

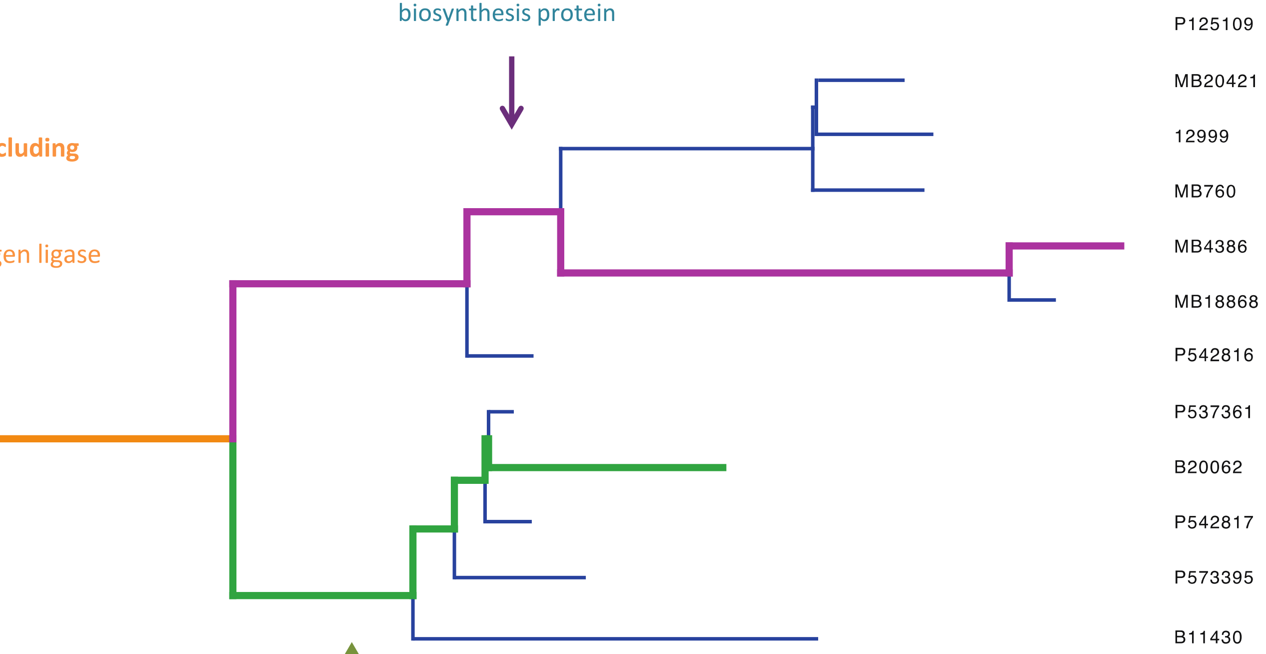
etfA
Electron transfer flavoprotein

SEN1517
Hydrogenase formation protein
glpD
aerobic glycerol-3-phosphate dehydrogenase

Genome degradation: Outer-surface proteins / macromolecules



Over-representation of degradation of outer surface proteins / macromolecules



29 including

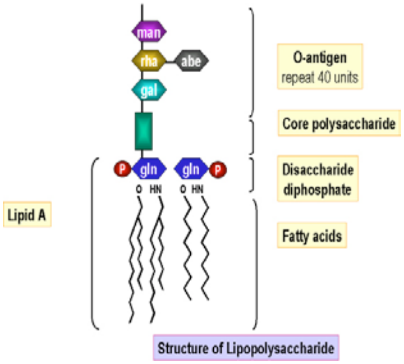
waaY
lipopolysaccharide core biosynthesis protein

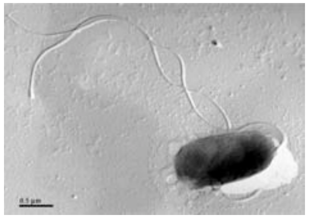
15 including

waaL
O-antigen ligase

38 including

fliC
Flagella major antigen
waaK
Lipopolysaccharide 1,2-N-acetylglucosaminetransferase

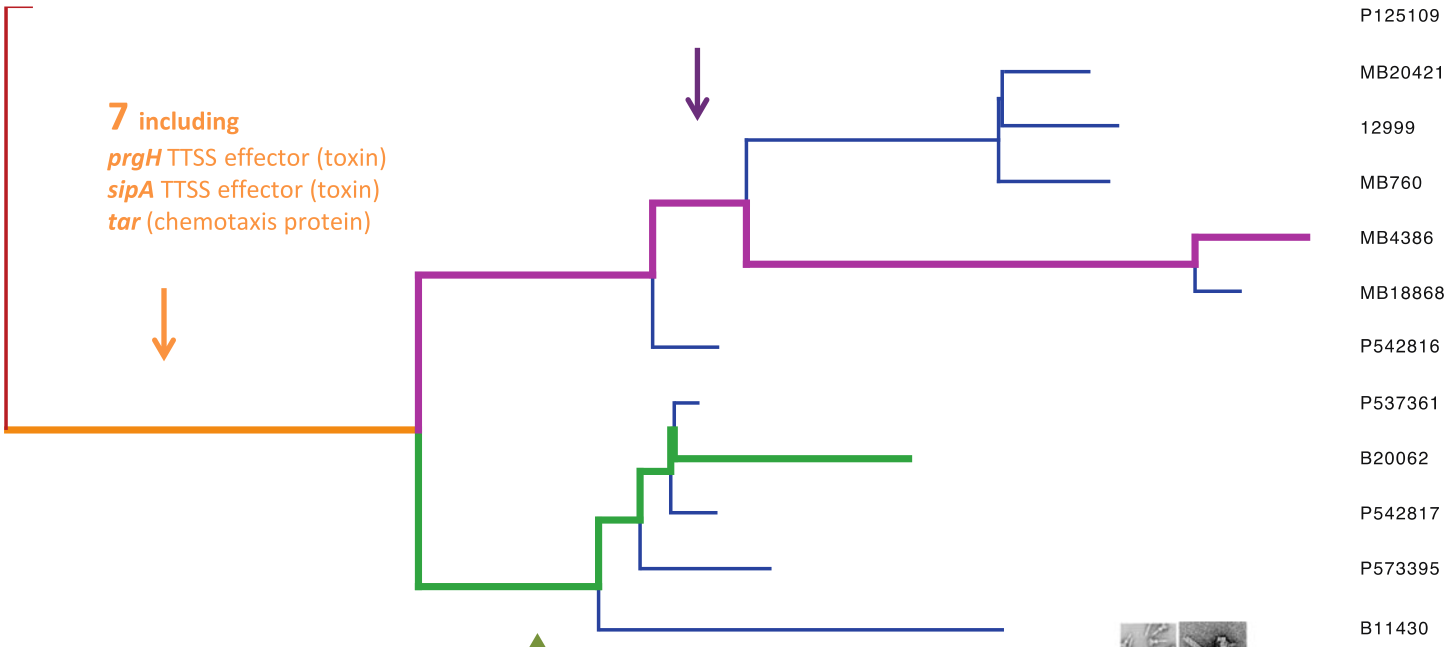




Genome degradation: virulence genes

Extensive degradation in SPI-1 encoded TTSS - Enterocyte invasion

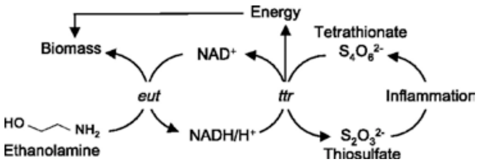
No degradation in SPI-2 encoded TTSS - Intracellular survival



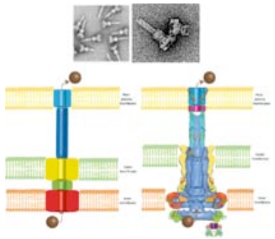
7 including
***sopA* TTSS effector (toxin)**
***cheR* chemotaxis protein**

7 including
prgH TTSS effector (toxin)
sipA TTSS effector (toxin)
tar (chemotaxis protein)

9 including
motB
Motility protein
ttrB
Tetrathionate reductase



Respiration in the inflamed intestine

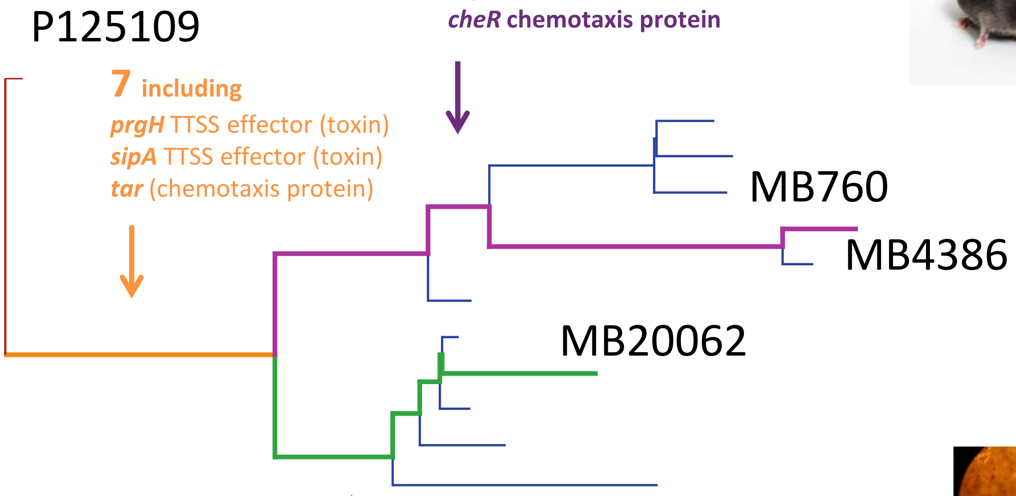


Type III secretion system (TTSS)

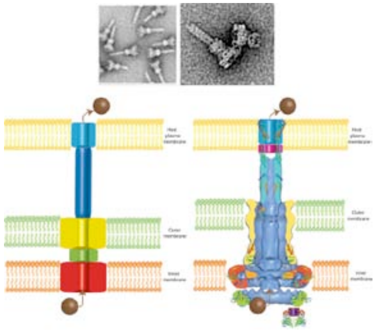
Genome degradation impacts virulence cell invasion

7 including
sopA TTSS effector (toxin)
cheR chemotaxis protein

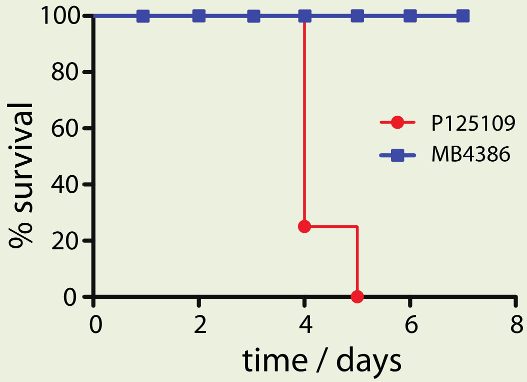
7 including
prgH TTSS effector (toxin)
sipA TTSS effector (toxin)
tar (chemotaxis protein)



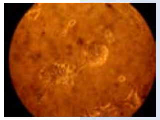
9 including
motB
Motility protein

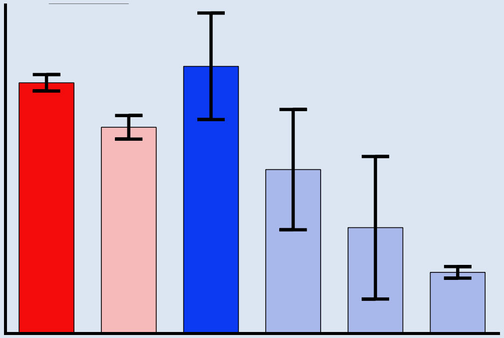


Type III secretion system (TTSS)



Virulence in C57bl/6 mice





Invasion of T84 cells

Summary

- Phylogenomics defined a rapidly evolving and clonally expanding chronic infection

- Rapid evolution / adaptation due to *mutS* mutation

- Altered phenotypes including attenuated virulence

- Insight into the host-pathogen interactions that drive this evolution

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