

# *Sodalis glossinidius* Symbiosis Islands

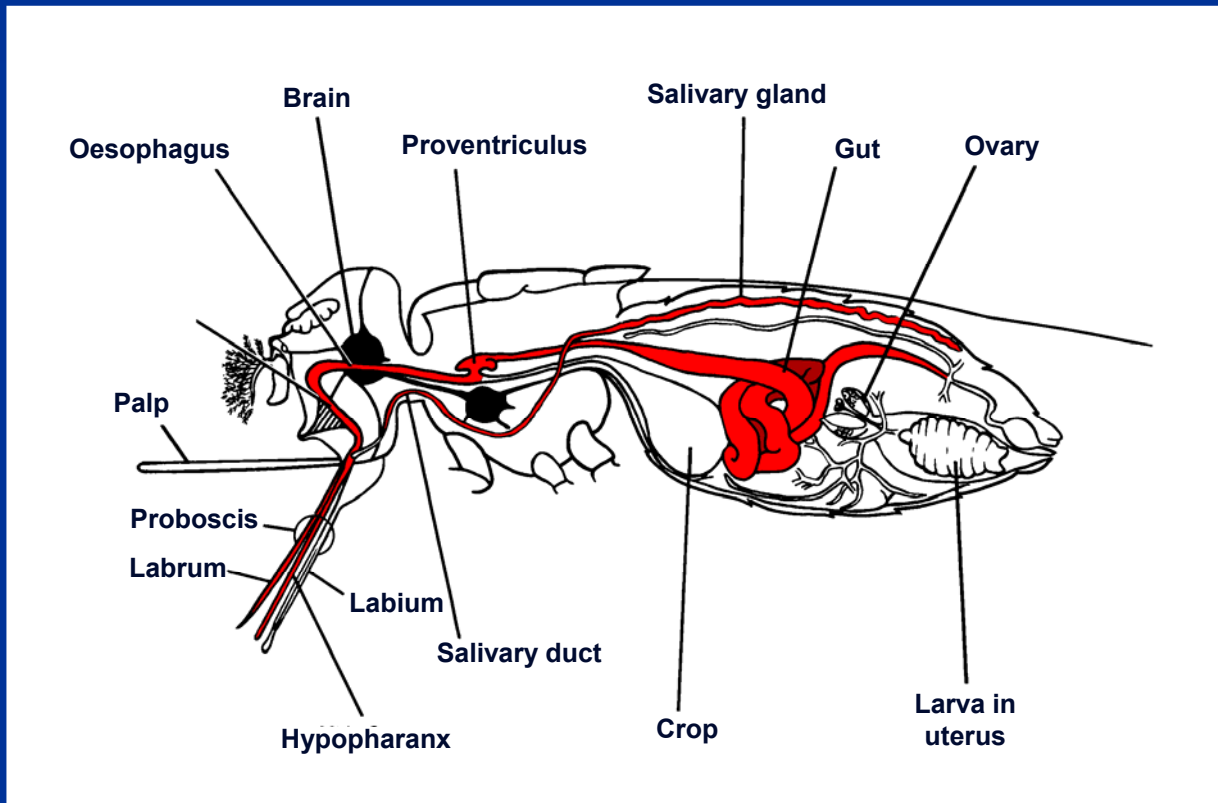
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## *Sodalis glossinidius*

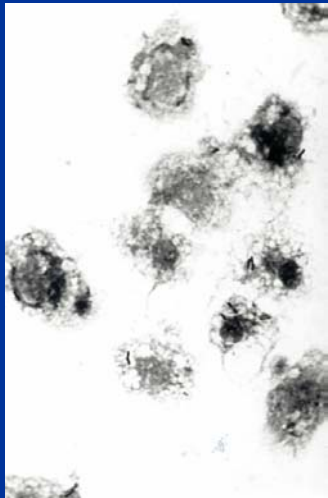
- Maternally inherited secondary endosymbiont of tsetse flies
- Found inter- and intracellularly in multiple tissues



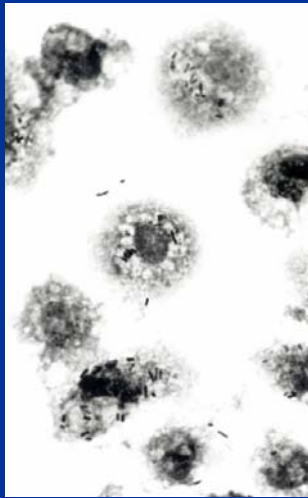
## An amenable endosymbiont

- Can be cultured in liquid media and with insect cell lines
- Invasive activity in co-culture with *Aedes albopictus* (mosquito) C6/36 cells

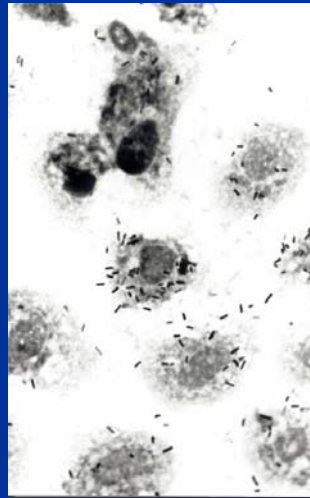
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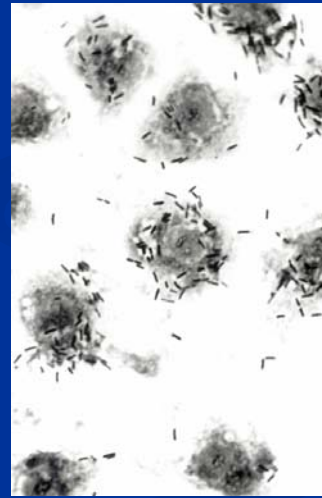
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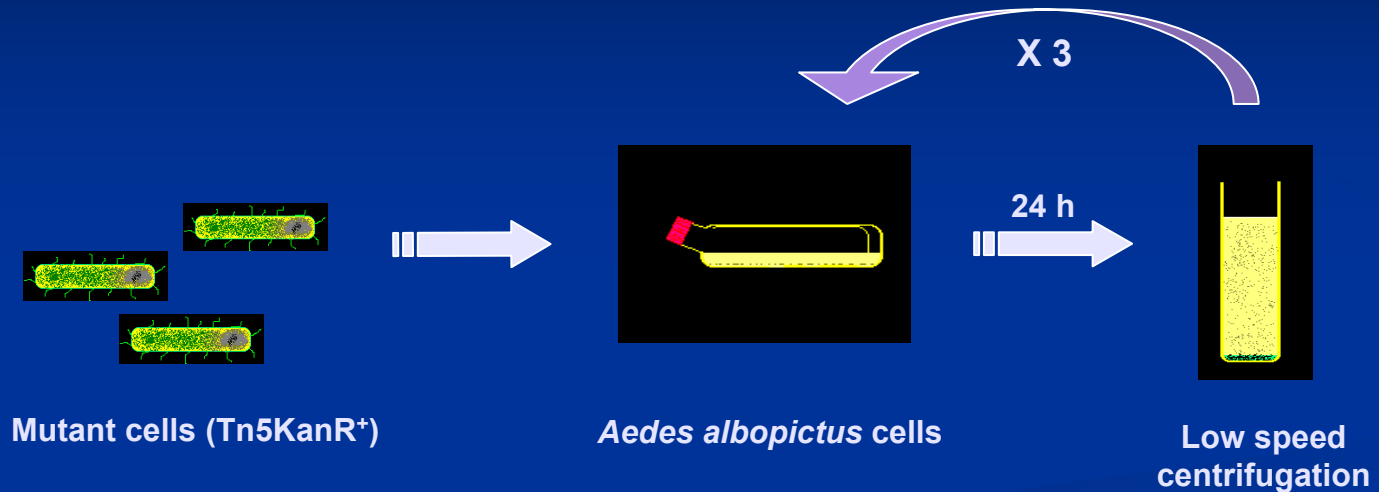
72



96 h

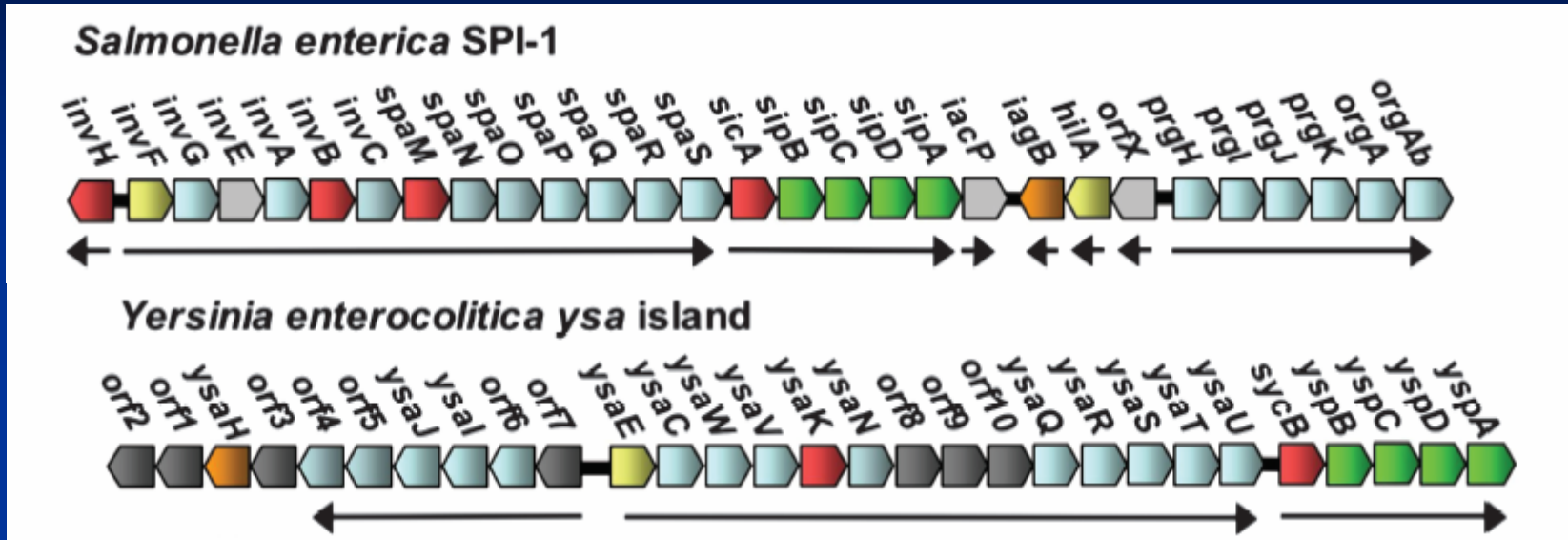


## Invasion determinant screen



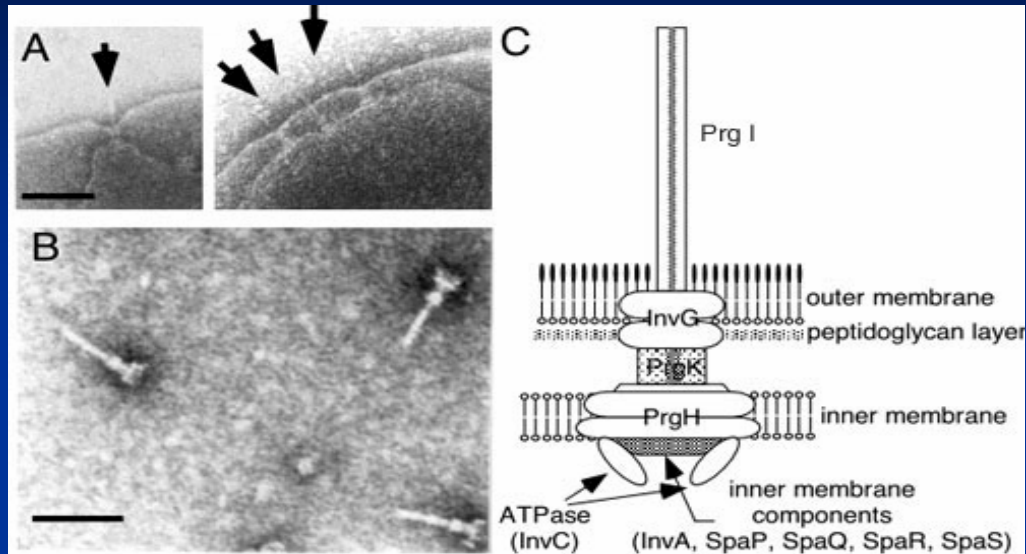
- Single clone D18 - bacteria attach to *Aedes* cells but don't invade
- 977 bp fragment - 490 bp sharing 57% amino acid identity with *Salmonella enterica* InvC
- *invC* located in *S. enterica* pathogenicity island 1 (SPI-1)
- SPI-1 encodes genetic components of a type III protein secretion system
  - Specialised secretion system utilised for interaction between bacteria and eukaryotic cells

## Type III protein secretion gene clusters



- Most TTSS gene clusters are similar in size (~30 kb) and organization - either plasmid encoded or chromosomal
- Initially identified in animal and plant pathogens
- Also environmental bacteria – e.g. *Desulfovibrio*, *Myxococcus* and *Verrucomicrobium*
- Multiple distinct TTSS's - *Salmonella*, *Yersinia* and *Chromobacterium* spp. each have 2, *Burkholderia pseudomallei* has 3 TTSS's

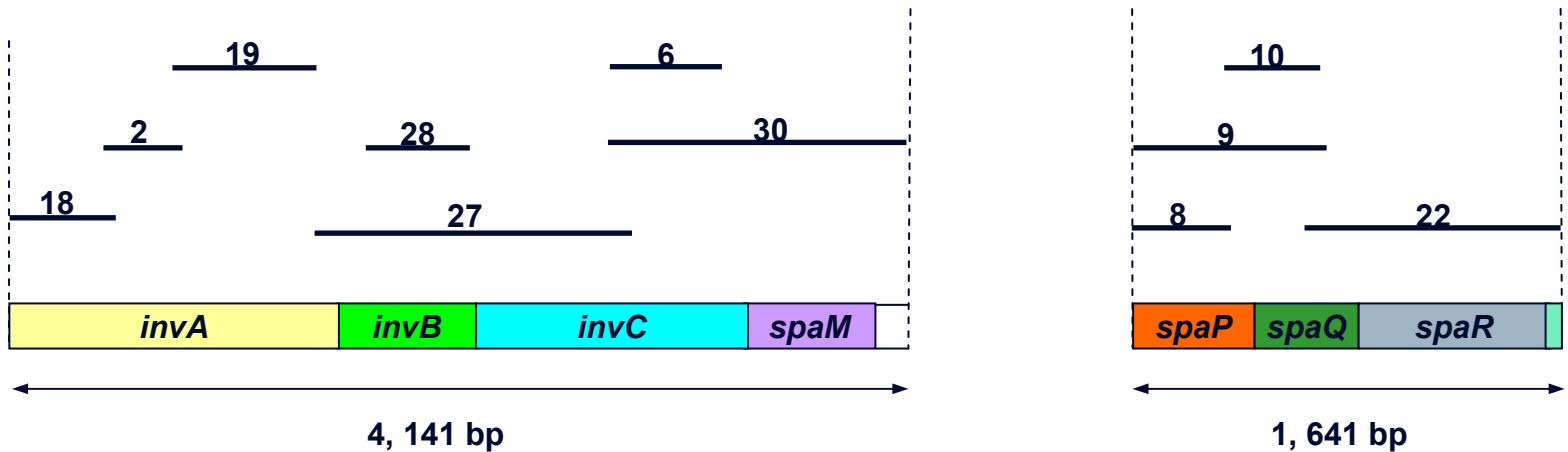
# Type III protein secretion apparatus



- 'Molecular syringe' expressed upon contact with host cells
  - translocate proteins across bacterial inner and outer membranes to cytosol of host cells
- Effector proteins subvert host cell processes - cytoskeletal rearrangements, intracellular signalling, apoptotic pathways
- TTSS enable invasion of eukaryotic cells
- Some TTSS enable bacterial replication/persistence post-invasion in host-enclosed vacuoles

# Investigation of a *Sodalis* type III protein secretion system

- Aim: Identify type III secretion genes in *Sodalis glossinidius* by PCR
- Designed degenerate oligonucleotide primers based on consensus of aligned TTSS homologs (*Salmonella*, *Shigella*, *Yersinia*, *Pseudomonas*)

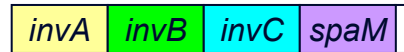


*Salmonella enterica* SPI-1

12,399 bp

## An intact type III protein secretion gene cluster?

### *Sodalis glossinidius*



54 32 57 28

56 71 46

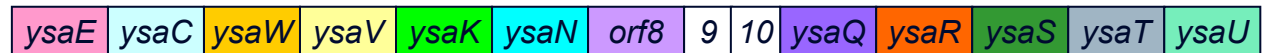
### *Salmonella enterica* SPI-1



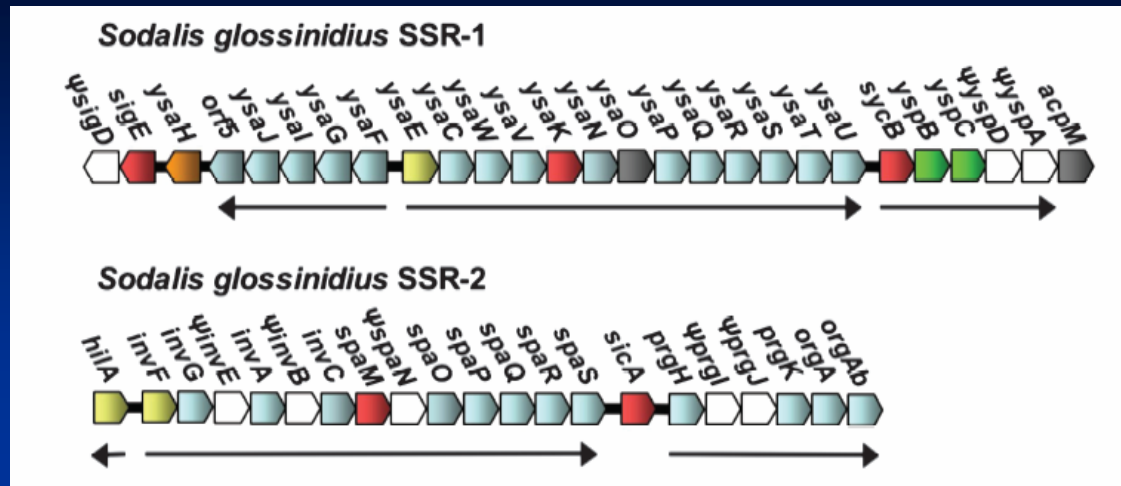
51 27 53 20

57 64 38

### *Yersinia enterocolitica* ysa



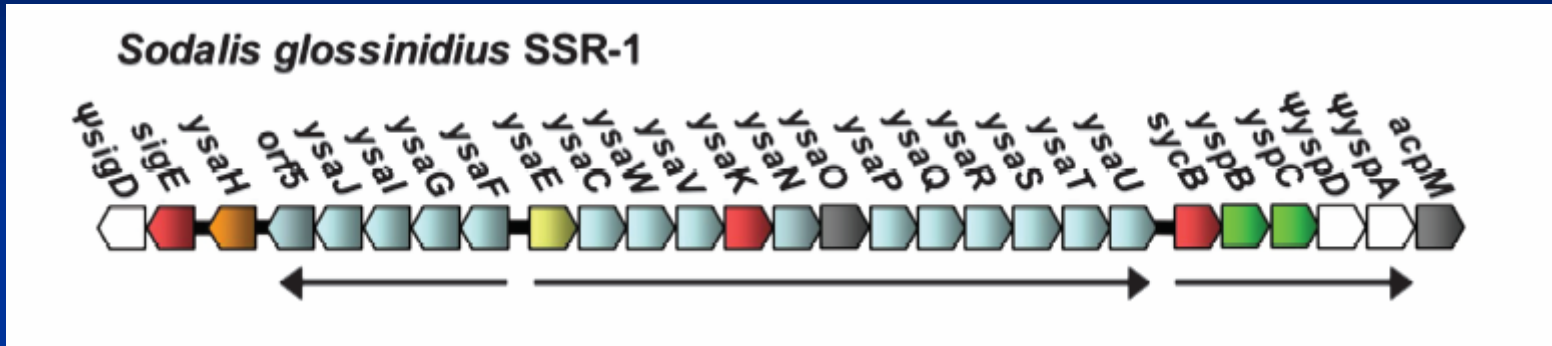
Dale C., Young S.A., Haydon D.T. and Welburn S.C. (2001) The insect endosymbiont *Sodalis glossinidius* utilizes a type III secretion system for cell invasion. *Proc. Natl. Acad. Sci. U.S.A.* **98**. 1883-8.



Dale C, Jones T & Pontes M (2005) Degenerative evolution and functional diversification of type-III secretion systems in the insect endosymbiont *Sodalis glossinidius*. *Molecular Biology and Evolution* **22**:758-766

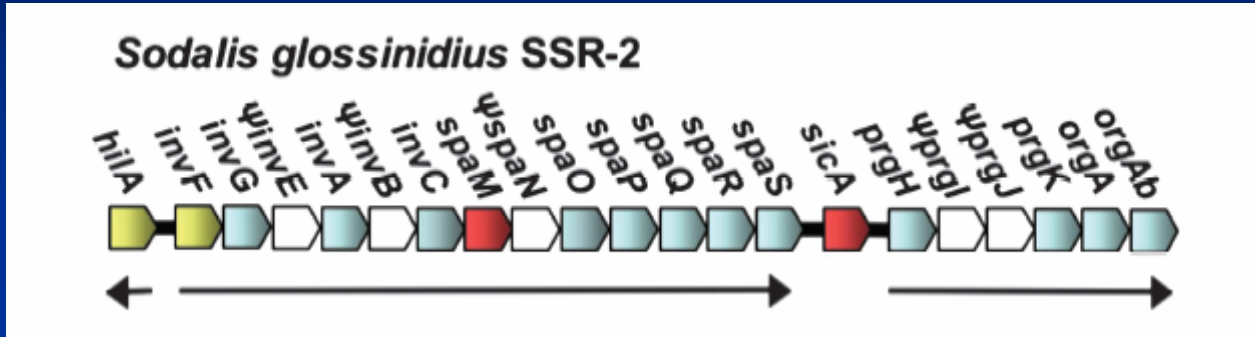
- PCR screened BAC library of *S. glossinidius* genomic DNA
- Complete shotgun sequencing of positive BAC clones
- Revealed **two** distinct type III secretion system gene clusters

## Sodalis symbiosis region 1



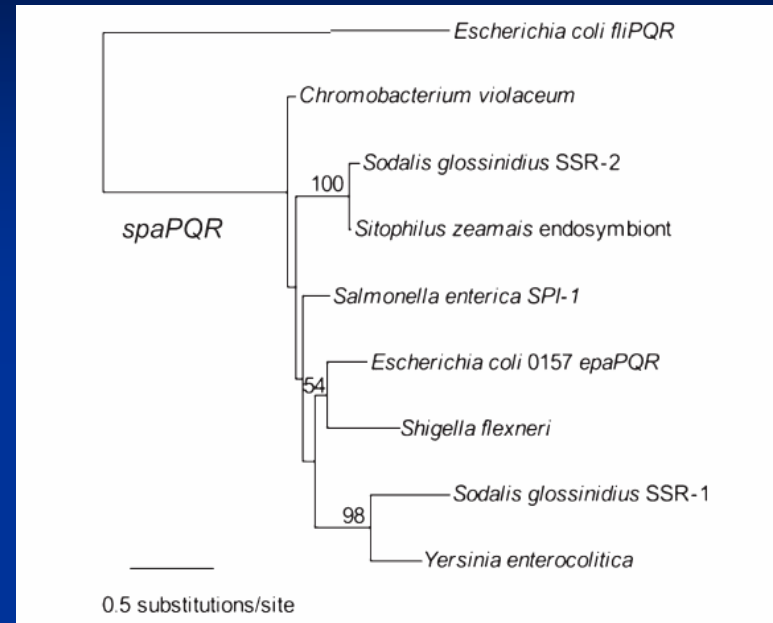
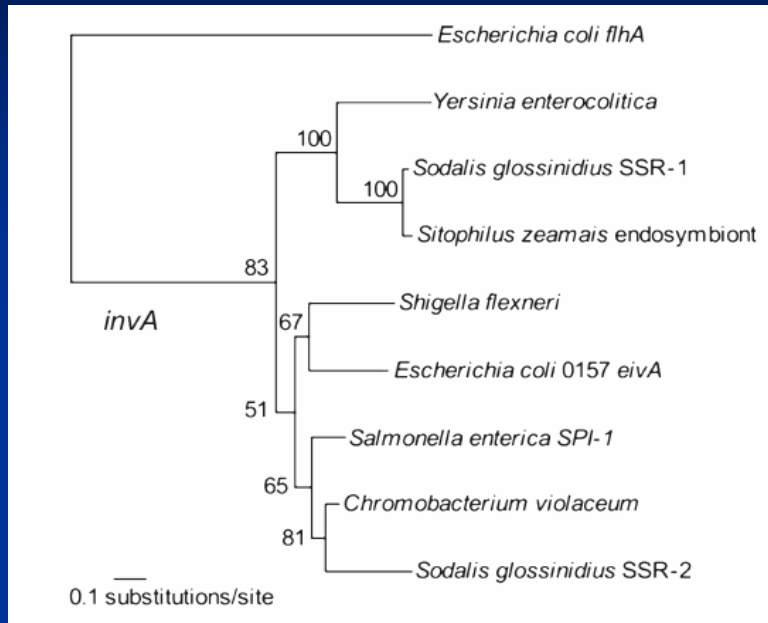
- 28.5-kbp - 23 intact ORFs, 3 pseudogenes
- 48.9 mol% G+C significantly lower than chromosome (54.9 mol% G+C)
- Resembles TTSS-encoding *ysa* pathogenicity island of *Yersinia enterocolitica*
- SSR-1 has all protein components needed to produce a functional secretion apparatus
- Effector proteins homologous to the *Salmonella* Sip proteins that affect host cytoskeletal modifications associated with bacterial invasion

## Sodalis symbiosis Region 2



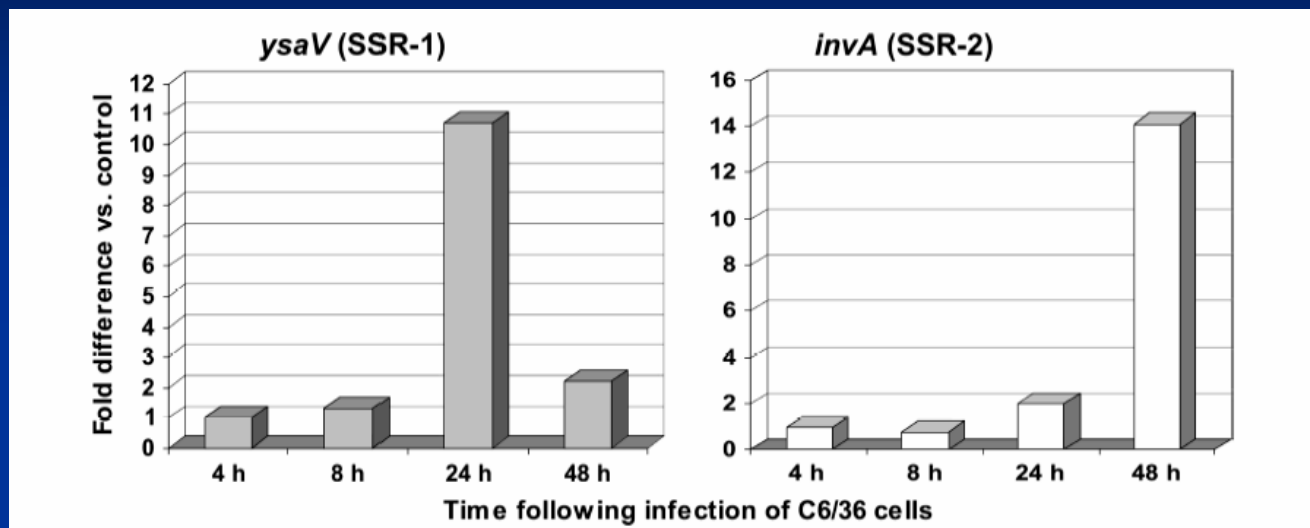
- 28-kbp - 16 intact ORFs, 5 pseudogenes
- 56.3 mol% G+C similar to chromosome (54.9 mol% G+C)
- Gene organization resembles reduced version of *Salmonella enterica* SPI-1 TTSS island.
- 'SSR-2 has genes necessary to produce intracellular and membrane-bound components of the TTSS syringe but lacks functional homologs for needle structure and secreted effectors'

# Ancestry of symbiosis regions



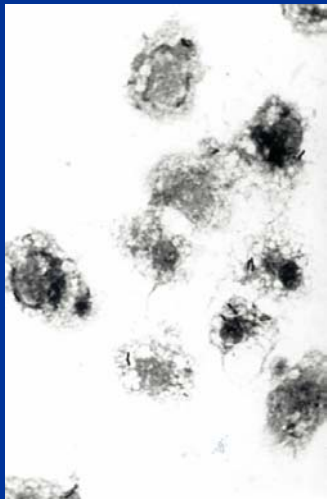
- Bootstrap analyses provided no support for trees derived from wide range of Gram -ve bacteria
  - Focused on smaller well-supported clade
- Maximum-likelihood trees presented remain unclear
- Close phylogenetic relationship between genetic components of SSR-1 and SSR-2 suggests possible functional complementation between two secretion systems

## Differential expression of SSR-1 and SSR-2

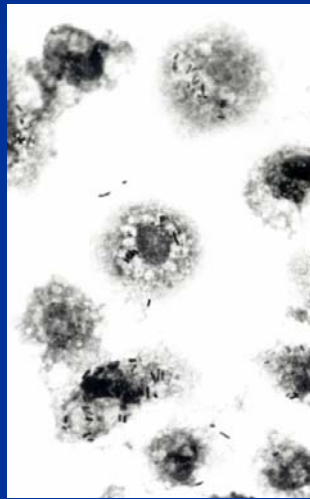


- Used quantitative RT-PCR to analyse the expression profiles of *ysaV* and *invA*
- Expression level relative to control gene *rpIB*
- Maximal expression of *ysaV* (SSR-1) at 24 h coincides with invasion of *Aedes* cells by *S. glossinidius*
- Maximal expression of *invA* (SSR-2) at 48 h coincides with intracellular stage

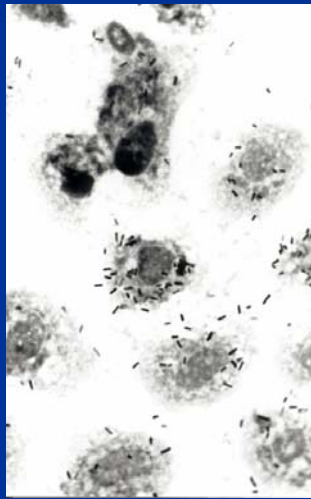
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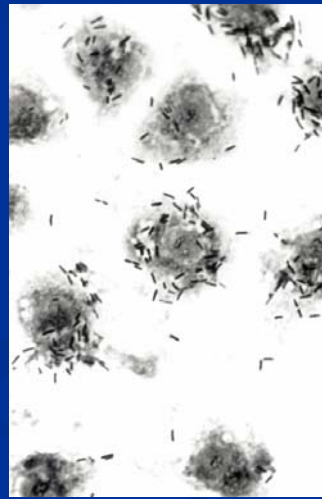
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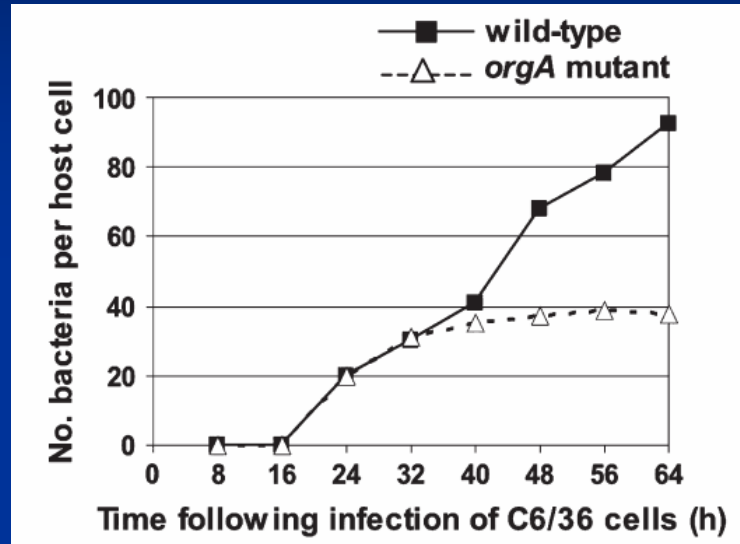
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96 h



## *S. glossinidius* lacking *orgA* demonstrate impaired replication in host cells



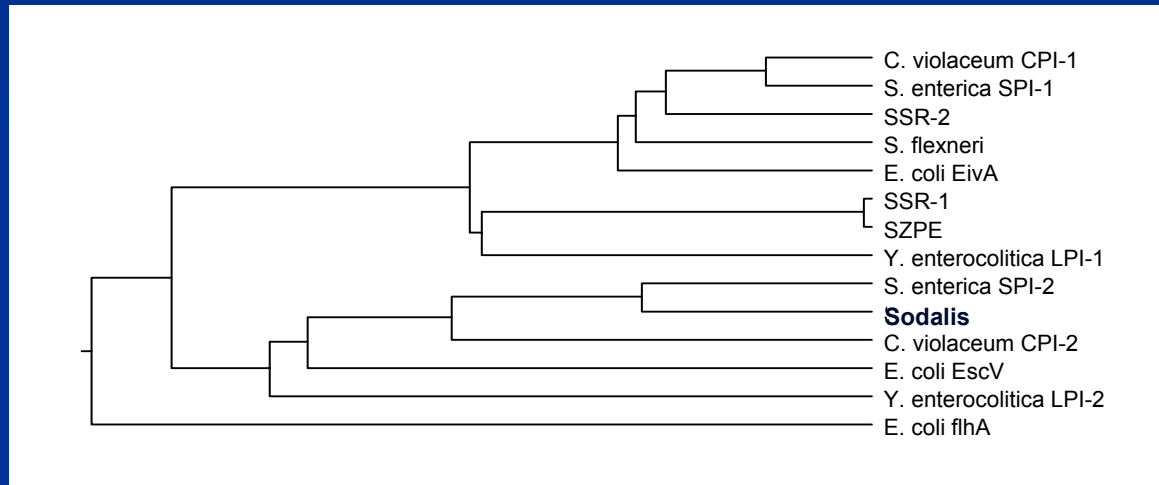
- *orgA* (SSR-2) mutant remains invasive but loses ability to replicate in host cells
- SSR-2 has a role enhancing proliferation of *S. glossinidius* inside insect cells

# Conclusions

1. *S. glossinidius* symbiosis regions have different roles:
  - SSR-1 in invasion
  - SSR-2 in intracellular persistence
  - only determined from *Aedes* cell culture
2. Distinct G+C composition, gene organisation suggests independent ancestry
  - though possibility of complementation
3. SSR-1 like *Y. enterocolitica* *ysa* TTSS, SSR-2 like *S. enterica* SPI-1
  - both members of the *inv/mxi/spa* group
4. SSR-1 and SSR-2 both present in presymbiotic ancestor of *S. glossinidius* and SZPE
  - specialisation of SSR-2 presymbiosis?
5. SSR-2 possible descendent of SPI-1 but function modulated to resemble SPI-2
  - lack of needle and effectors dissimilar to SPI-2

## A third type III protein secretion system in *S. glossinidius*?

- 465 bp PCR product – 82% identity over 155 aa to *S. enterica* SPI-2 SsaV



- S. glossinidius* has a **third** type III secretion system that is a true SPI-2 like TTSS (utilised for persistence within host cells)

OR

- ssaV* sequence is a pseudogene possibly part of larger non-functional TTSS

# Summary

*Sodalis glossinidius* first symbiont to have two functional type III secretion systems

– likely SZPE will prove similar

As a vertically transmitted symbiont *Sodalis* must ensure its survival

1. By being passed to the tsetse larvae
2. By being present in the correct cell tissues during larval development

It is highly likely that *Sodalis glossinidius* uses these type III secretion systems to maintain its symbiosis within the tsetse fly

Applications in tsetse/trypanosomiasis control

## Manipulation of type III secretion systems

- Biological Control - Secretion of insecticidal proteins into tsetse midgut
- Cellular therapy - Reconstitution of functional activity through secretion of enzyme into deficient cells
- Antigen Delivery - Efficient stimulation of host immune response using type III secreted effectors as carrier proteins
- Bacterial invasion - Use of reporter gene technology to examine invasion and *in vivo* virulence gene expression
- Inhibitors of invasion - Screen bacteria with inhibitory compounds using invasion-linked reporter gene

# Acknowledgements

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