Paratransgenesis as a tool to block trypanosome transmission by tsetse.

Jan Van Den Abbeele

THIRD FAO/IAEA INTERNATIONAL CONFERENCE ON AREA-WIDE MANAGEMENT OF INSECT PESTS:
INTEGRATING THE STERILE INSECT AND RELATED NUCLEAR AND OTHER TECHNIQUES
VIENNA, 22 - 26 MAY 2017

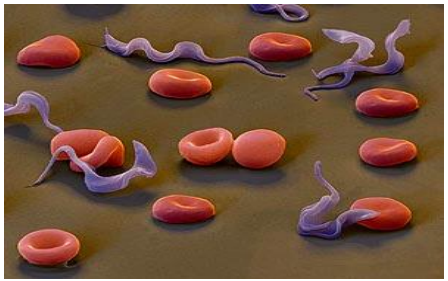
Outline of the presentation

- Background on tsetse-transmitted trypanosomiases and its control
- Potential role of paratransgenic refractory tsetse flies within SIT programs
- Paratransgenesis in tsetse fly: proof-of-concept; current bottlenecks

Tsetse-transmitted African trypanosomiasis

- Parasitic disease(s) caused by *Trypanosoma* sp. (protozoa, kinetoplastidae) in different host species (man, bovine, goat,…).
- Distribution: sub-Saharan Africa

Tsetse fly (*Glossina* sp.)



Trypanosoma sp.

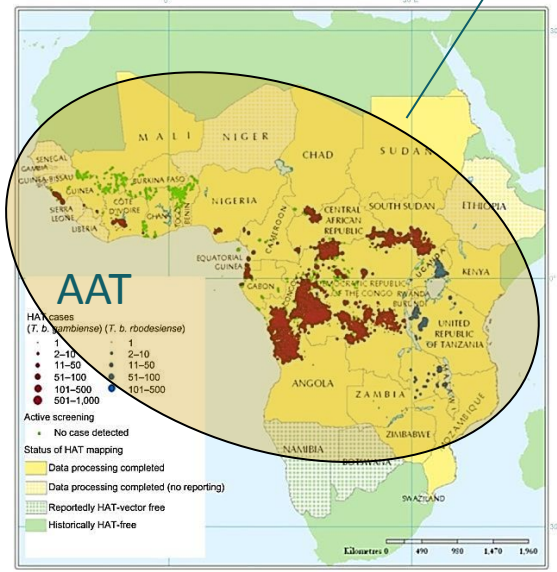
HAT; sleeping sickness
T.brucei gambiense; T.brucei rhodesiense

AAT; nagana
T.congolense; T.vivax; T.brucei brucei

Tsetse-transmitted African trypanosomiasis

- HAT (in 2015)
  - DRC: 2.351 new cases (>85%);
  - CAR: 146;
  - 100 cases in surrounding countries
  - → remote, rural areas
  - > 60 million people are still at risk

WHO: → elimination by 2025?
e.g. TRYP-ELIM program in DRC (ITM, PNLTHA, LSTM – B&M funding)



- AAT

Remains one of the biggest infectious disease constraints to productive livestock rearing in sub-Saharan Africa:

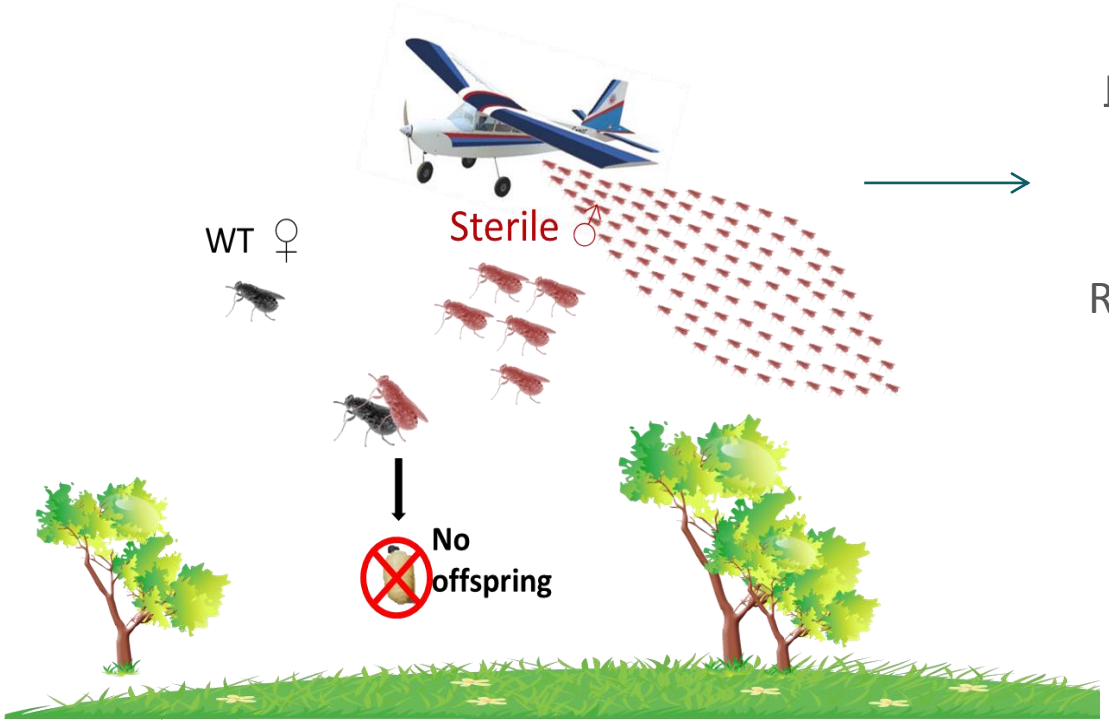
- cattle breeding (increase of mortality and morbidity)
- reduction of meat / milk production: lower income, reduction of nutritional proteins
- losses in animal traction power: reduction of the yields and the surface area that can be cultivated; restricted land usage

Tsetse-transmitted African trypanosomiasis: control

- HAT:
  - Active case detection
  - Accurate and rapid diagnosis; stage determination
  - Treatment: limited amount of drugs available; drug resistance?
  - Tsetse fly control: tiny targets

- AAT:
  - Diagnosis by the local vet/farmer,…
  - **Treatment**: two main drugs: isometamidum chloride, diminazene aceturate; homidium; important issues: quality of the drugs on the local market; drug resistance.
  - Trypanotolerant cattle
  - **Tsetse fly control**:
    - use of insecticides: impregnated screens/targets; selective application on animals; sequential aerosol technique – SAT;…
    - **Sterile Insect Technique** (SIT)

Tsetse-transmitted African trypanosomiasis: SIT



Regular, massive release of potential vectors during a long-time period

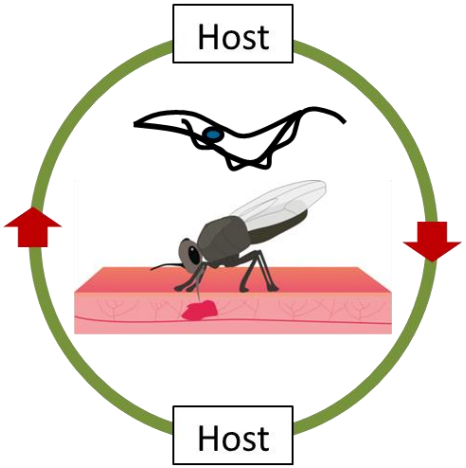
↓

Release of 100% trypanosome refractory flies?

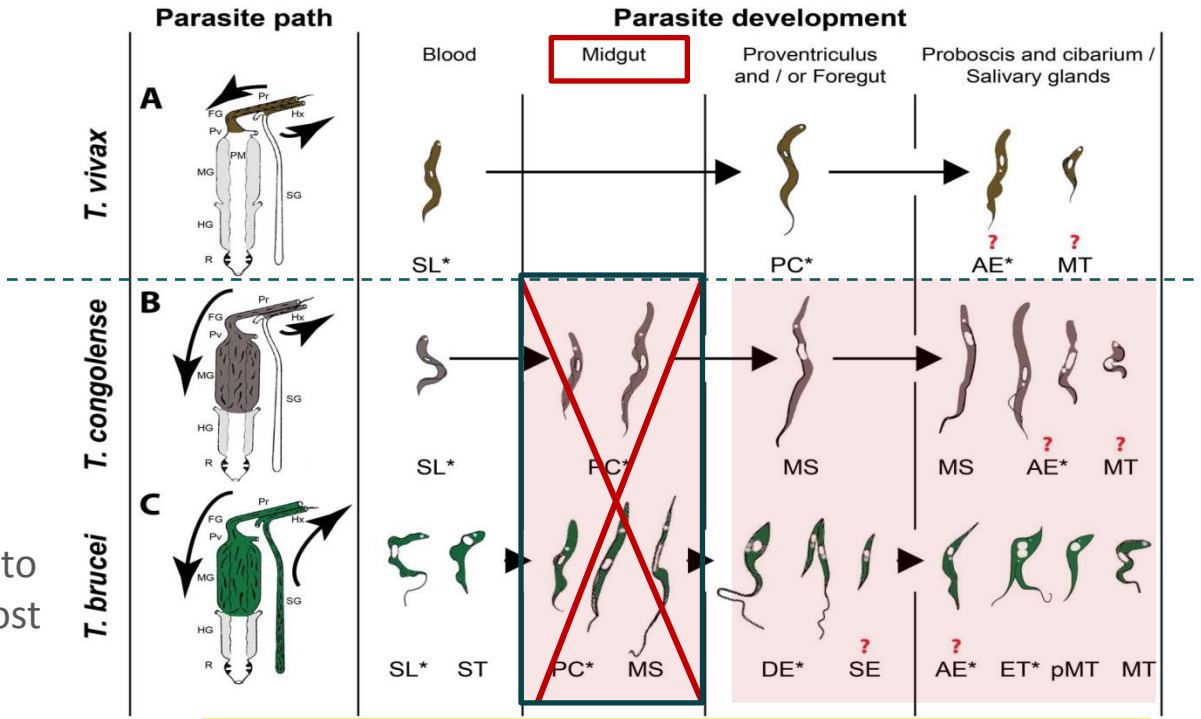
↓

Paratransgenesis to block trypanosome transmission by the tsetse fly

Trypanosome transmission by the tsetse fly

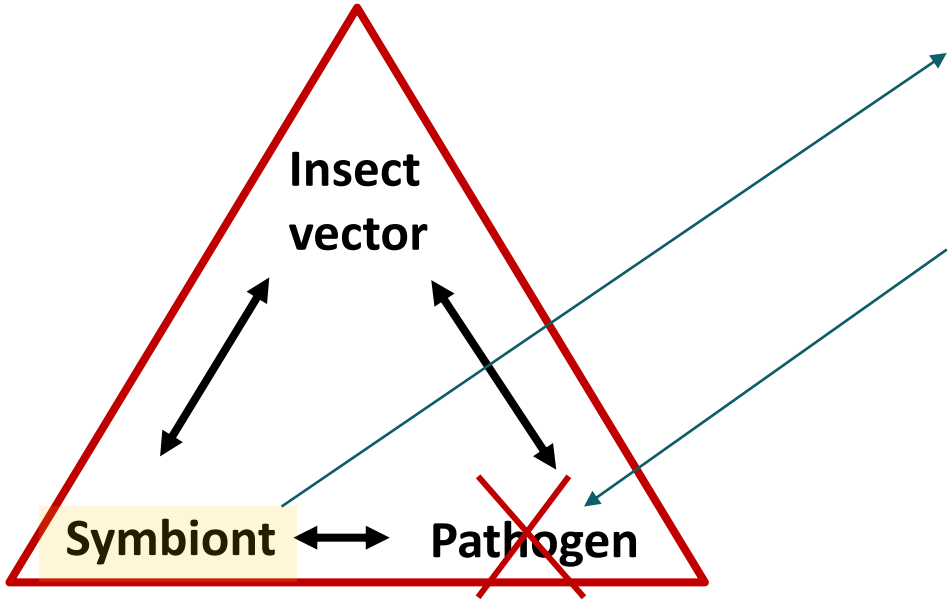


Biological transmission: obligatory developmental cycle in tsetse; simple to complex cfr. *Trypanosoma* species; most of tsetse flies are refractory for trypanosomes

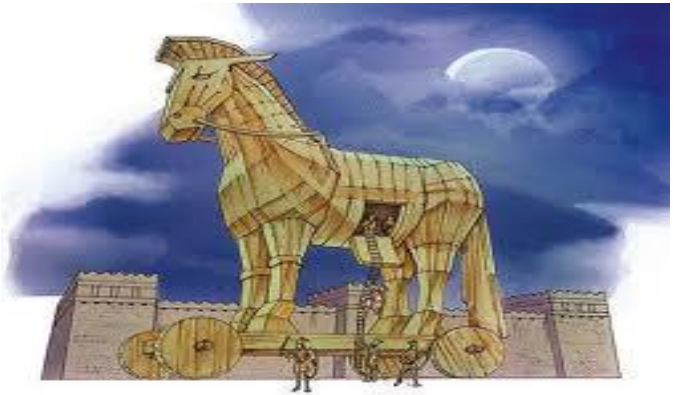


→ Can we achieve this through **paratransgenesis** ?

Paratransgenesis



Trojan horse concept: **Genetically modified symbiotic microorganism** as an *in situ* delivery system for effector molecules that target the insect-pathogen interplay (paratransgenesis)

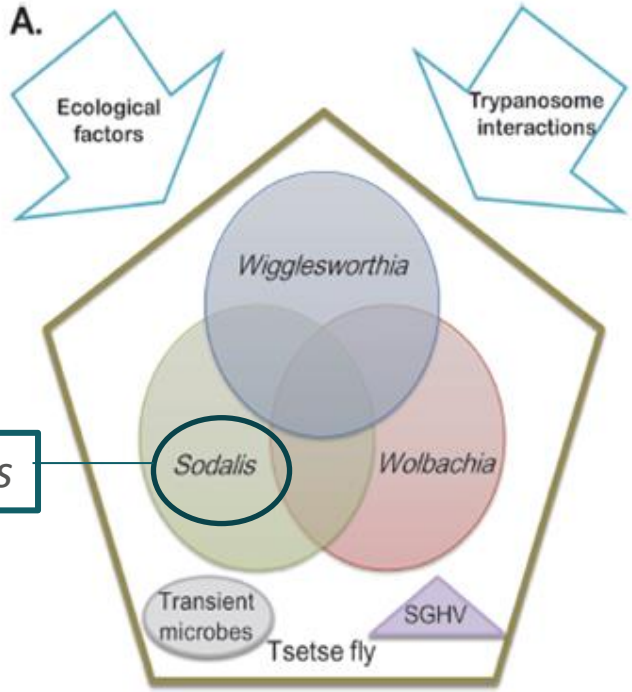


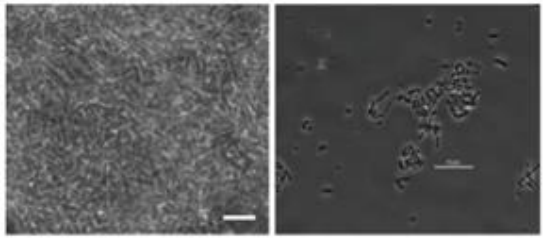
Paratransgenesis

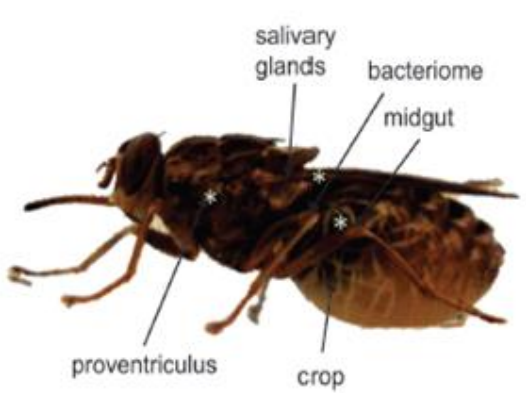
- In order to perform paratransgenesis, there are several requirements:
  - The commensal microorganism (e.g. bacteria) can be grown *in vitro* easily.
  - It can be genetically modified, such as through transformation with a plasmid containing the expression construct/desired gene or by genomic integration.
  - The engineered microorganism is stable and safe.
  - There is a tight and unique association between vector and symbiont
  - Field delivery is possible and easily handled.

Tsetse candidate microorganisms: microbiome

Sodalis glossinidius





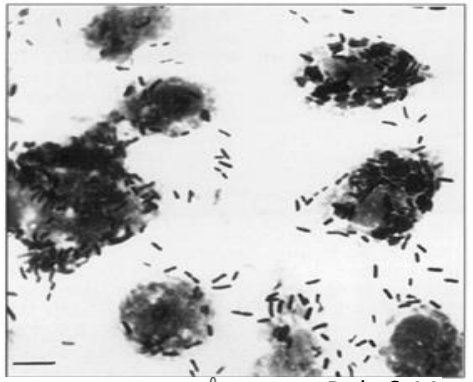


Snyder & Rio, 2013

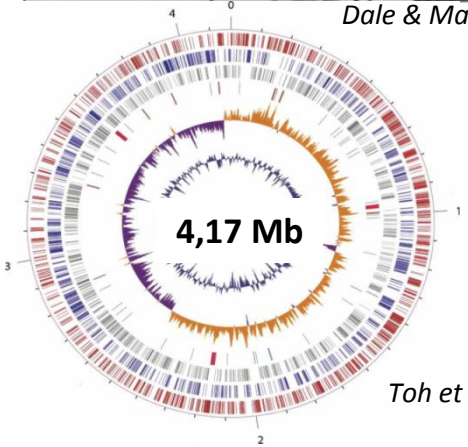
Facultative commensal: *Sodalis glossinidius*

- Fam. Enterobacteriaceae;
- Role in tsetse not clear; present in all lab colonies; prevalence in natural populations is highly variable: 0-70%;
- Vertical transmission to subsequent generations: mother → offspring through the intrauterine nourishment of the larva;
- Present in the midgut, hemolymph, salivary glands, milk glands; intra- and extracellular;
- Genome: 4.17Mb; 2,432 protein coding sequences; reduced coding capacity of 51%; 972 pseudogenes;
- Availability of an *in vitro* culture system and methodologies for genetical modification;

→ suitable candidate 'vehicle' for paratransgenesis

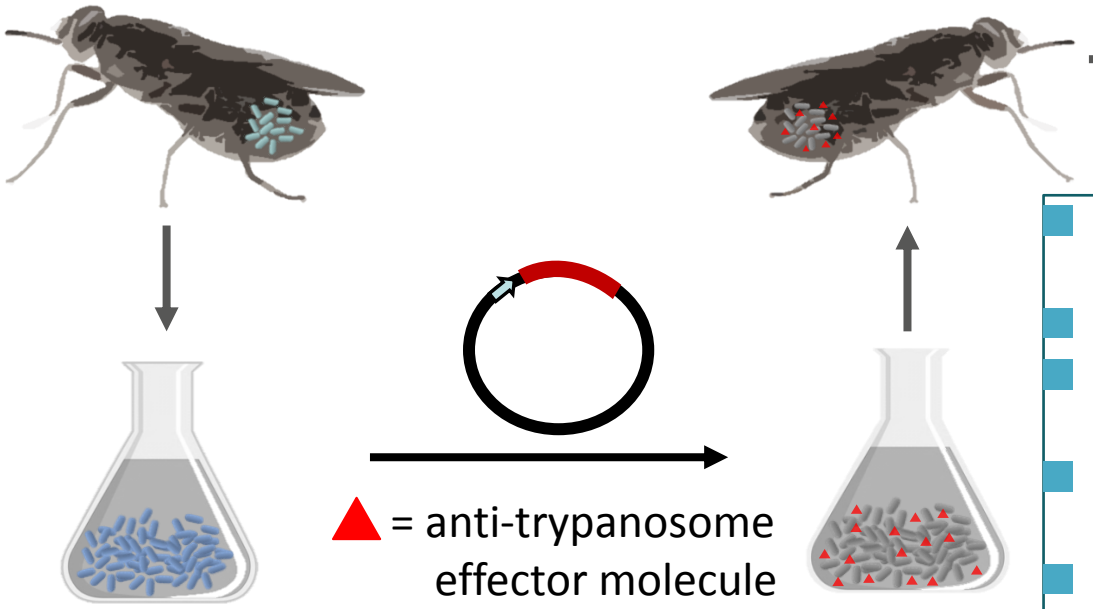


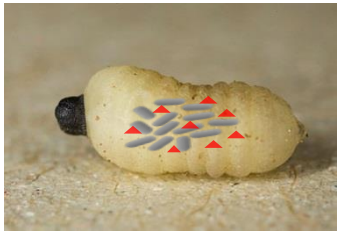
Dale & Maudlin, 1999



Toh et al., 2006

Tsetse – *Sodalis*: paratransgenesis



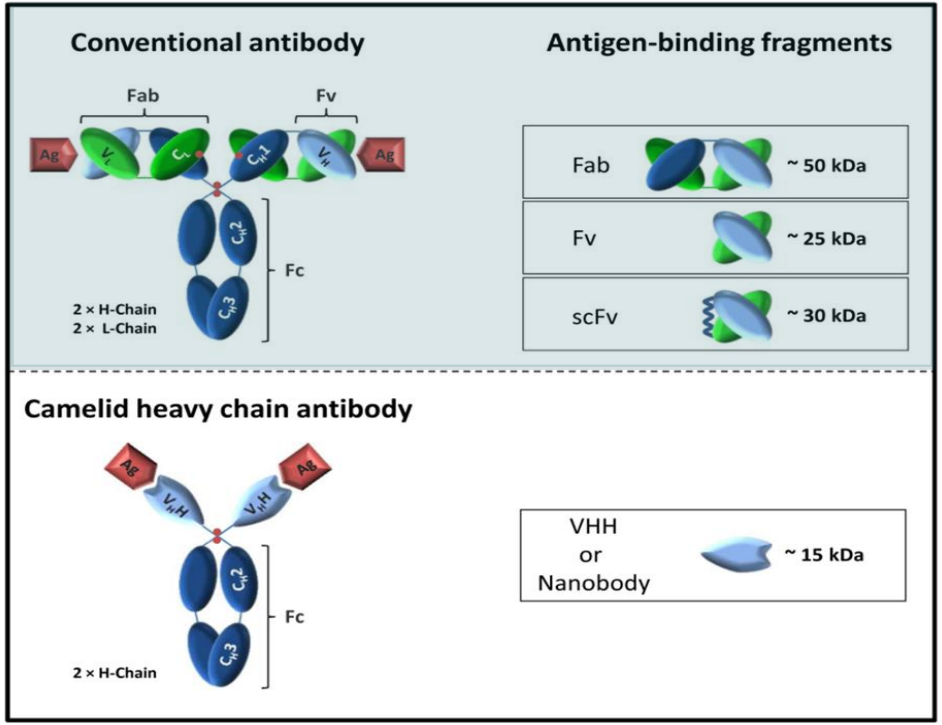


R. Wilson

- Suitable and stable anti-trypanosome molecule;
- Stable genetic modification;
- Stable expression and extracellular release;
- GM-*Sodalis* is competitive versus WT *Sodalis*;
- Mother to offspring transfer:

→ ability to build up a GM-*Sodalis* tsetse fly colony

Anti-trypanosome molecule: Nanobodies (Nb)



Nanobody:

- Single-domain antigen-binding fragments derived from camel heavy-chain antibodies
- Selection through phage display and panning
- Unique epitope repertoire targeting

Advantages:

- Small size (~15 kDa)
- High solubility/stability
- Easy to express; high production yields in *E. coli*
- Nanobodies can be modified: binding affinity; protease resistance,…

Sodalis expression and extracellular release of functional Nb in the tsetse fly

De Vooght *et al. Microbial Cell Factories* 2012, **11**:23
http://www.microbialcellfactories.com/content/11/1/23

MICROBIAL CELL FACTORIES

RESEARCH Open Access

Expression and extracellular release of a functional anti-trypanosome Nanobody® in *Sodalis glossinidius*, a bacterial symbiont of the tsetse fly

Linda De Vooght[1,2], Guy Caljon[2,3,4], Benoit Stijlemans[3,4], Patrick De Baetselier[3,4], Marc Coosemans[1] and Jan Van Den Abbeele[2*]

De Vooght *et al. Microbial Cell Factories* 2014, **13**:156
http://www.microbialcellfactories.com/content/13/1/156

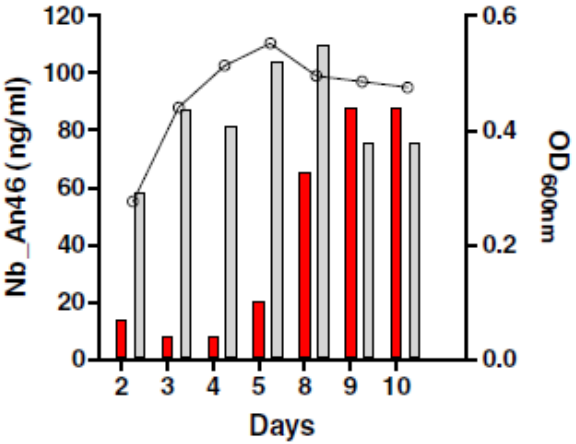
MICROBIAL CELL FACTORIES

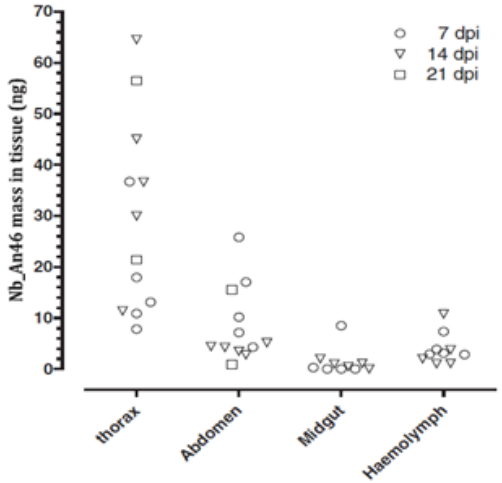
RESEARCH Open Access

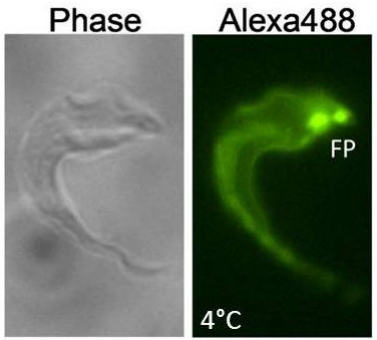
Delivery of a functional anti-trypanosome Nanobody in different tsetse fly tissues via a bacterial symbiont, *Sodalis glossinidius*

Linda De Vooght[1*], Guy Caljon[1,2,3], Karin De Ridder[1] and Jan Van Den Abbeele[1,4*]

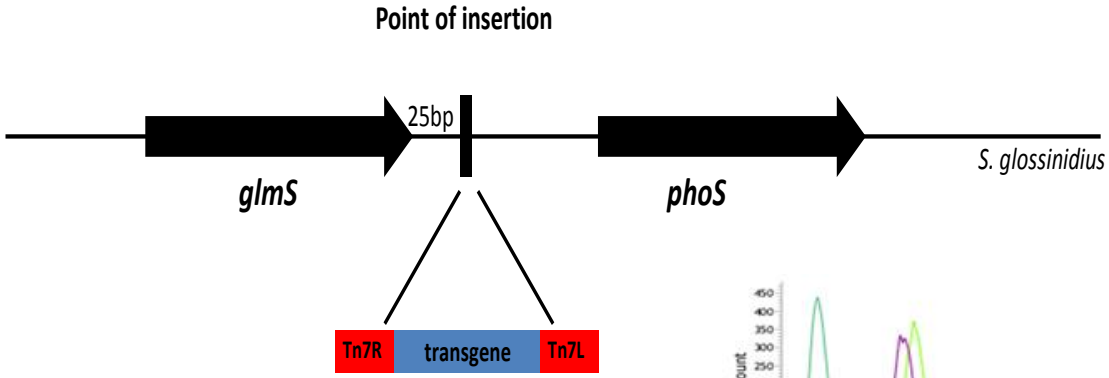
Stable expression of functional Nb at the 'ng' level; midgut < level (ELISA problem; midgut proteases)

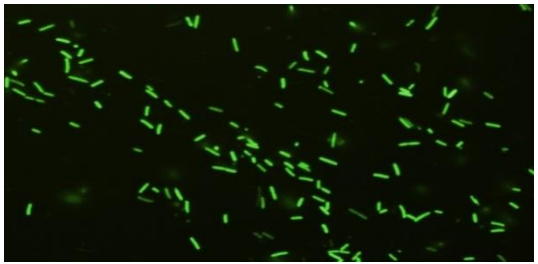


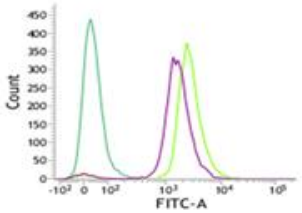


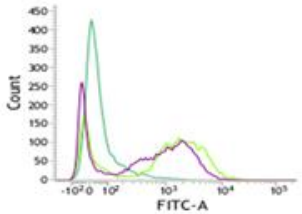


Stable integration in *Sodalis* genome





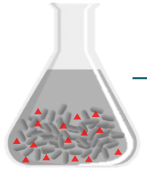




Transposon-based system (Tn7) for the site-specific insertion of Nb genes into the chromosome of *Sodalis:*

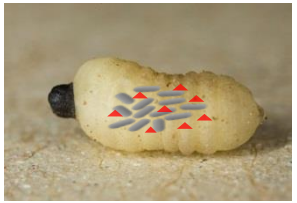
→ **Stable expression of the Nb in the tsetse fly**

Mother to offspring transfer of GM-*Sodalis*



Needle injection of fresh-born larvae

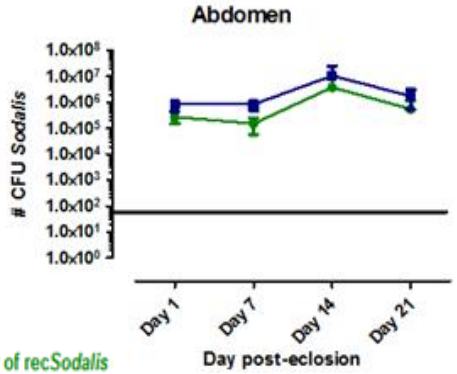
10^6 GM-*Sodalis*

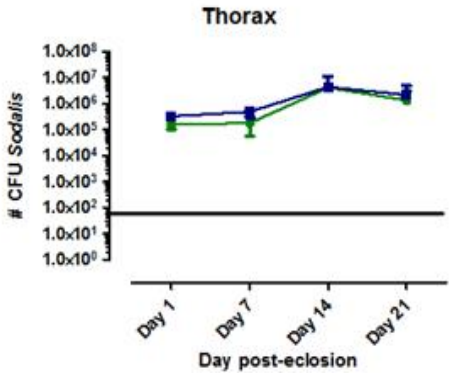


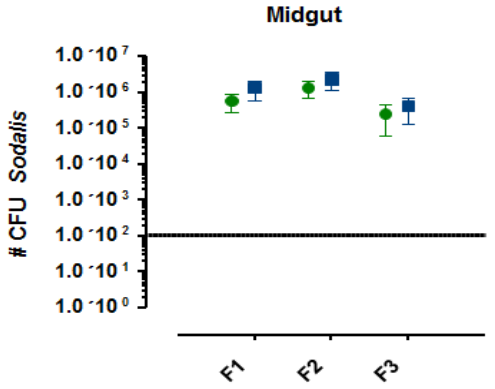
- Intralarval microinjection of GM-*Sodalis* results in **stably colonised adult flies** over **multiple generations;**
- GM-*Sodalis* is **competitive** vs. WT-*Sodalis*

Adult F_0 female

F_1, F_2, F_3 females



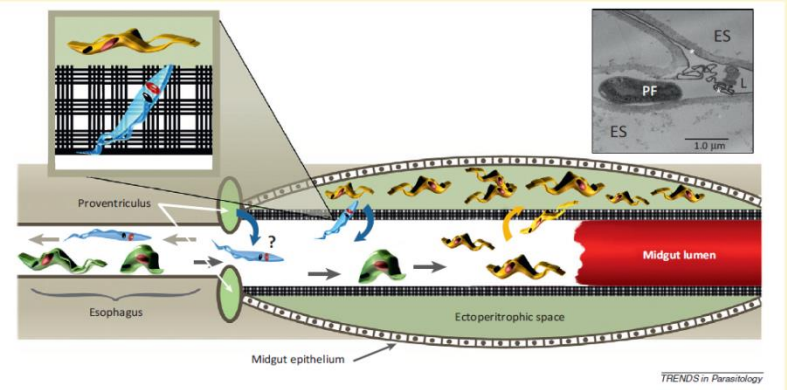


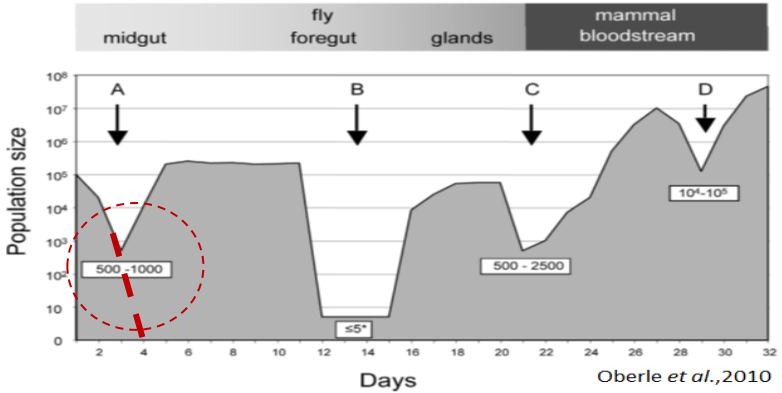


Total number of recSodalis

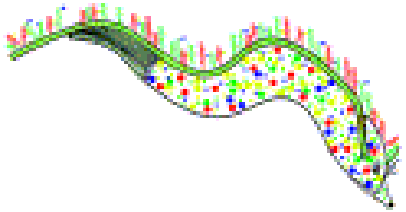
Total number of *Sodalis*

Blocking trypanosome development in the tsetse midgut?





T.brucei



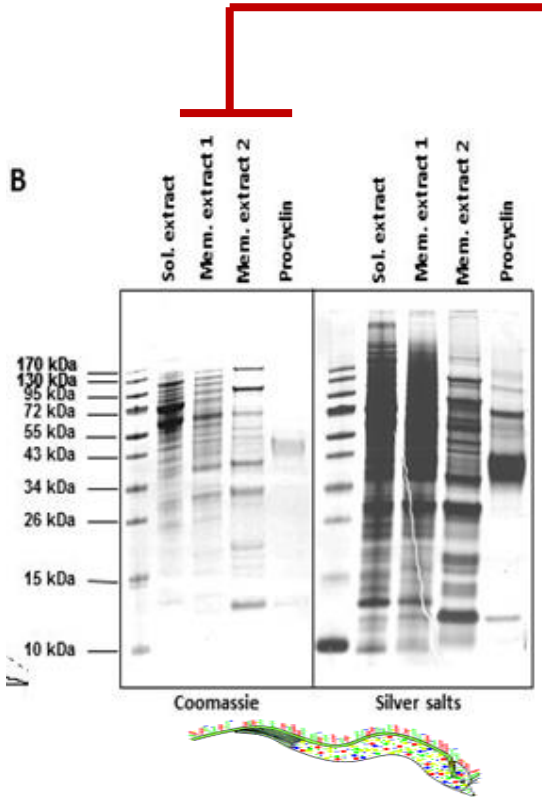
Pearson, 2001

Procyclic stage surface:

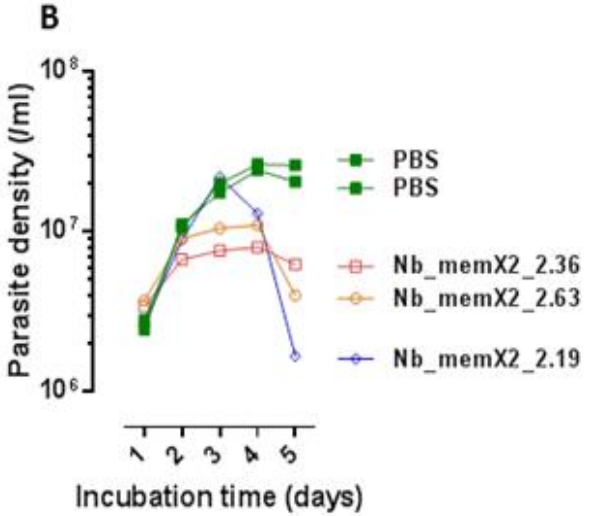
- GPEET-procyclin
- EP-procyclin
- Other membrane-bound components?

→ **Anti-procyclic nanobodies**

Selection of anti-procyclic Nbs



Immunisation; Lymphocyte collection; RNA extraction + VHH cDNA; Nb-phage library construction; screening of Nbs for activity on procyclic trypanosomes



Strong trypanolytic effect on *in vitro* cultured procyclics

GM-*Sodalis*/anti-procyclic Nbs in the *T.brucei* infected tsetse

But: *in vivo* in the tsetse fly

Sodalis strain	Infected/total # flies Midgut	Salivary gland
WT *Sodalis*	17/50	7/17
Sodalis:Nb88	27/53	11/27
Sodalis:Nb63	19/53	9/19
Sodalis:Nb36	21/45	10/21
Sodalis:Nb19	29/43 **	14/29

Enhancement of trypanosome midgut establishment!?

Conclusions

- *Sodalis* is highly suitable for tsetse paratrangenesis;
- Stable genomic integration; expression and release of functional effector molecule in the tsetse fly;
- Efficient transfer to offspring /subsequent generations → possibility to build a tsetse colony with GM-*Sodalis;*
- CURRENT BOTTLENECKs:
  - **highly potent and stable trypanocidal peptide/protein** that blocks trypanosome midgut development?; ideally targeting both *T. brucei sp.* and *T. congolense.*
  - **Impact of irradiation** (sterile males/SIT) on the GM-*Sodalis?*

Acknowledgments

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