MARRY TIM & SHIME

for an in-depth understanding of the anti-infectious properties of probiotic *S. cerevisiae* CNCM I-3856 against ETEC food-borne pathogens in the human gut

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Paris - Adebiotech - MBIO 2018

Physiopathology of ETEC infections & Probiotic strategy

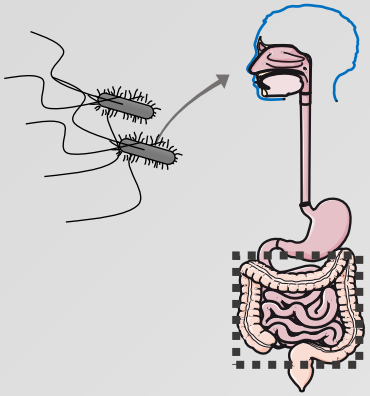
Simulation of the GIT to serve innovation

TIM & M-SHIME: Experimental design & Results

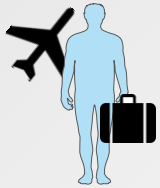
Conclusions & Future directions

PHYSIOPATHOLOGY OF ETEC INFECTIONS

Enterotoxigenic *E. coli*



Traveler's diarrhea



Travellers

Route of transmission: Contaminated water and food

At-risk populations

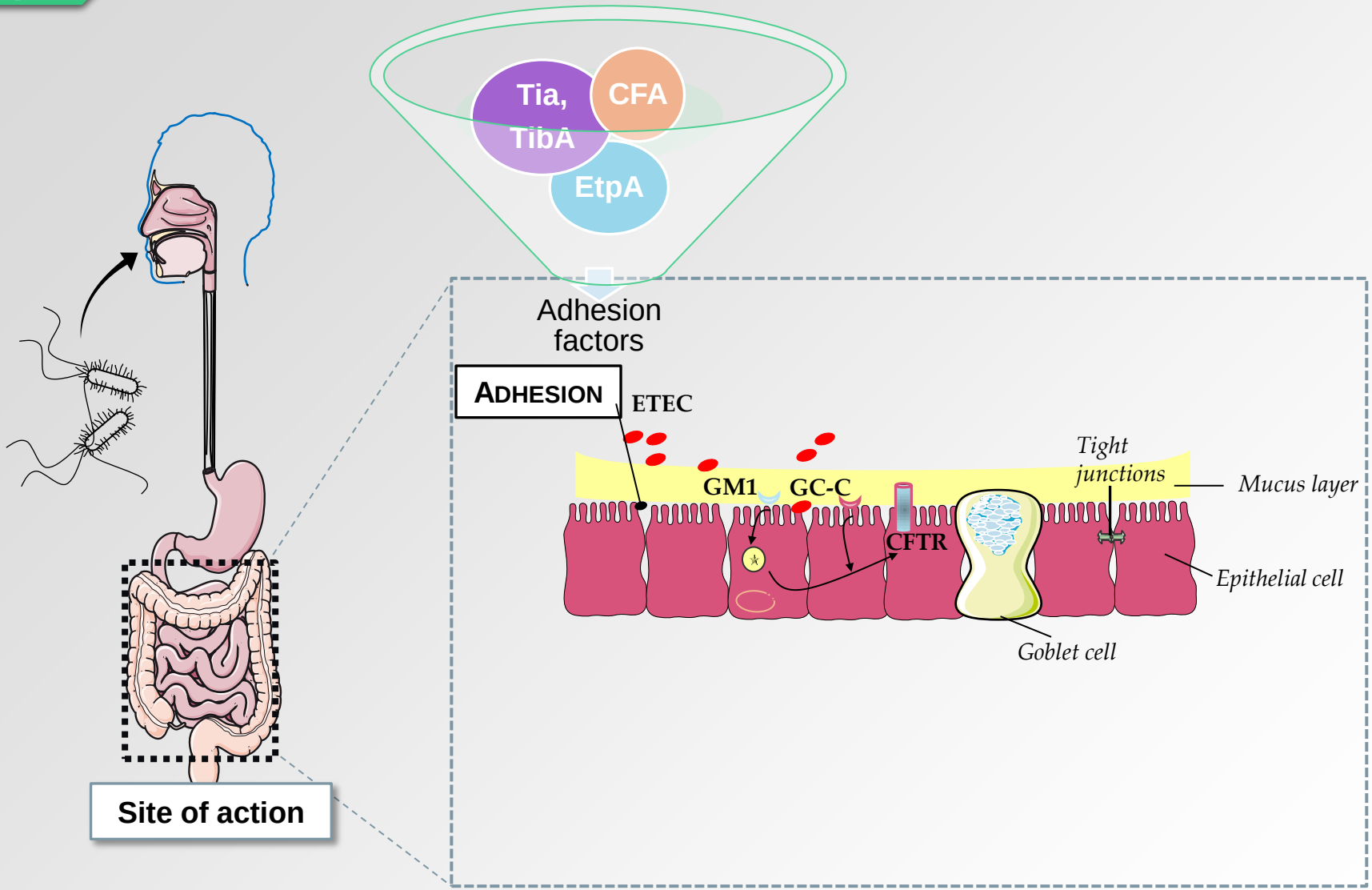
280 million diarrheal episodes worldwide/year

Infant diarrhea

Young children < 5 years old

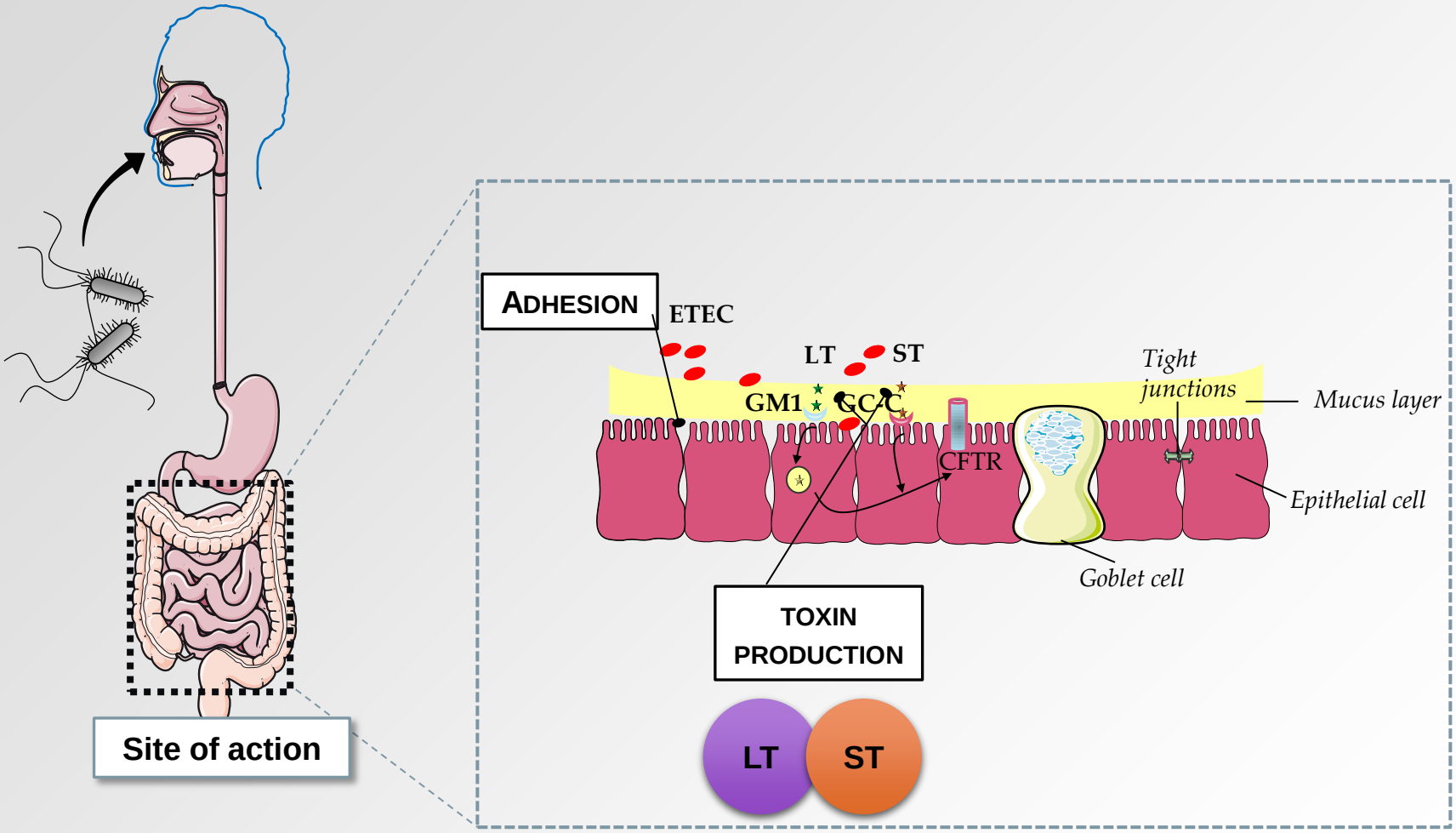
Liu et al., Lancet 2012; Boschi-Pinto et al., Bull WHO 2008
Kotloff et al., Lancet 2013; Lanata et al., PLoS ONE 2013

PHYSIOPATHOLOGY OF ETEC INFECTIONS



Roussel et al., Future Microbiology 2017
Roussel et al., Book chapter: Escherichia coli (ISBN 978-953-51-5008-4) 2017

PHYSIOPATHOLOGY OF ETEC INFECTIONS



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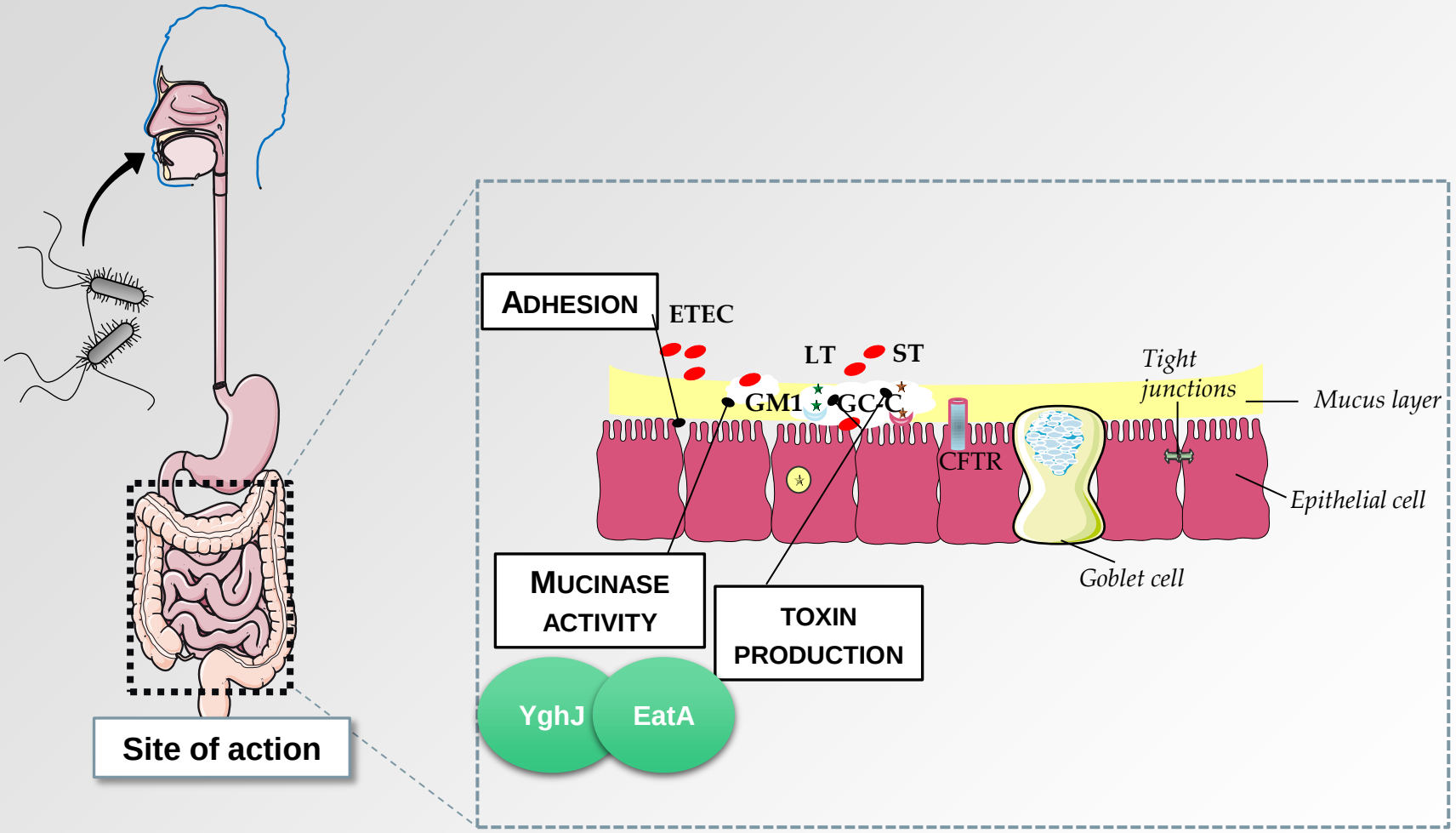

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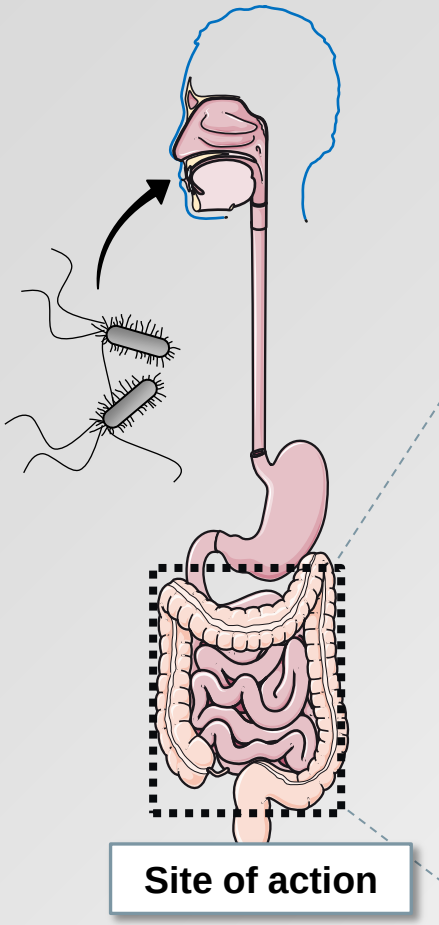
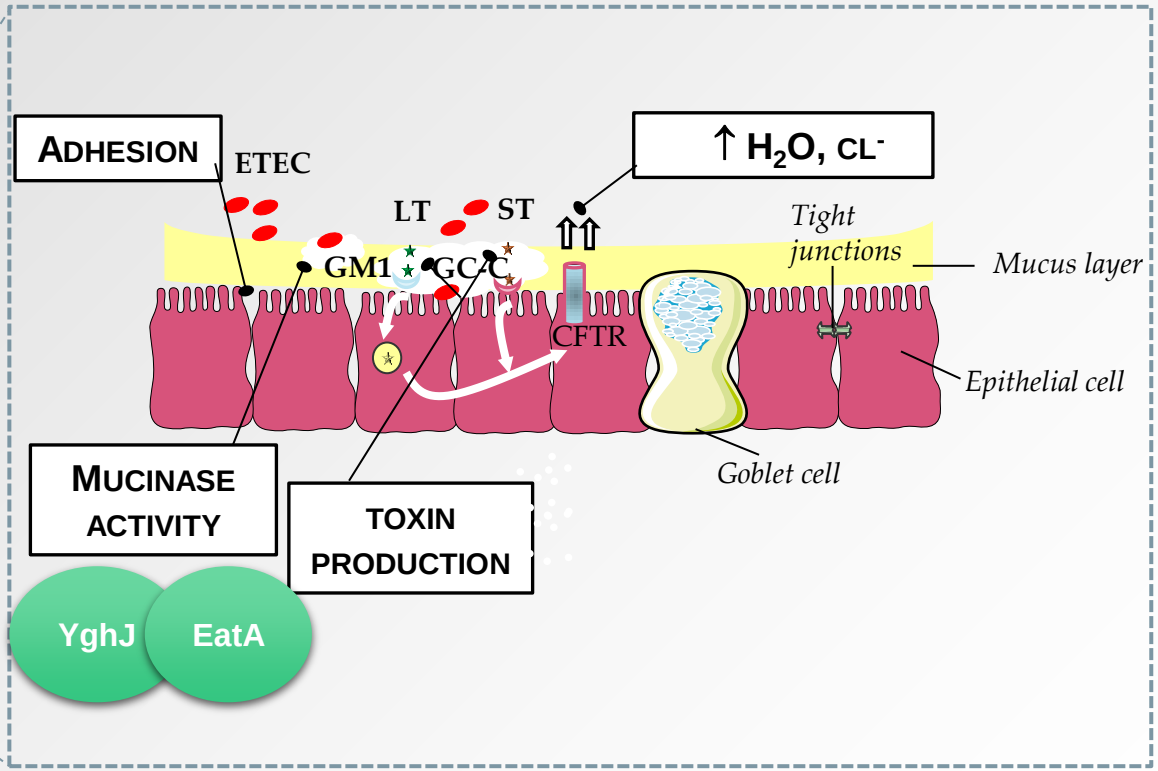


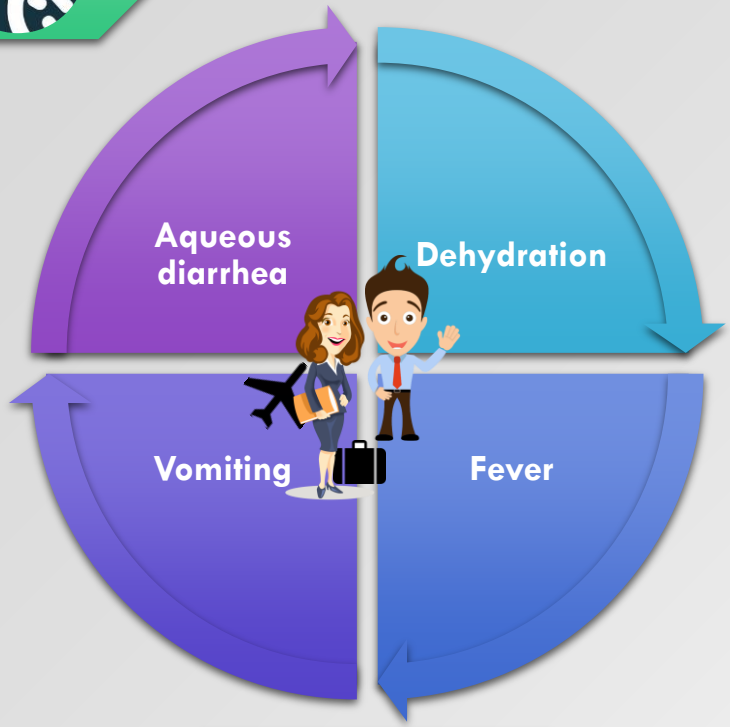
AQUEOUS DIARRHEA

Close to cholera syndrome (*Vibrio cholerae*)



Roussel et al., Future Microbiology 2017
Roussel et al., Book chapter: Escherichia coli (ISBN 978-953-51-5008-4) 2017

PHYSIOPATHOLOGY OF ETEC INFECTIONS



Symptoms

Chronic Complications

Post-infectious irritable bowel syndrome (PI-IBS)

risk ↑ x 6 (after diarrheal episode)

Quadri et al., Clin. Microbiol. Rev. 2005
CDC, 2014
Thabane et al., World J Gastroenterol 2007

PHYSIOPATHOLOGY OF ETEC INFECTIONS

Symptomatic treatments (rehydration, anti-diarrheal)

Use of antibiotics
BUT ↑ antibioresistance worldwide

Looking for preventive alternative strategies

Nutrition | **Vaccine** | **Probiotic**

Quadri et al., Clin. Microbiol. Rev. 2005
CDC, 2014
Thabane et al., World J Gastroenterol 2007

PROBIOTICS AS AN ALTERNATIVE IN THE FIGHT OF ETEC

LHC
LESAFFRE HUMAN CARE

Saccharomyces cerevisiae
CNCM I-3856

Mouse model / AIEC

Reduces inflammation

Improves gut barrier function

1

in vitro models of human gut / EHEC

Reduces ileal bacterial growth

Inhibits toxin gene expression

2
3

Human (clinical study)

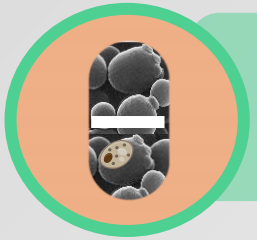
Reduces IBS symptoms

4
5
6

ibSium®

1 *Sivignon et al., Inflamm. Bowel. Dis. 2015;* **3** *Etienne-Mesmin et al., Appl. Environ. Microbiol. 2011 ;* **5** *Pineton De Chambrun et al., Dig. Liver Dis. 2015*
2*Thévenot et al., Appl. Microbiol. Biotechnol. 2015 ;* **4** *Cayzeele-Decherf et al., World J Gastroenterol. 2016 ;* **6** *Spiller et al., United European Gastro J. 2016*

PROBIOTICS AS AN ALTERNATIVE IN THE FIGHT OF ETEC



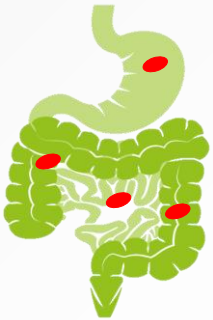
Saccharomyces cerevisiae
CNCM I-3856

LHC
LESAFFRE HUMAN CARE

Interest of the probiotic yeast in the prevention of ETEC infection?

In the simulated human upper & lower GIT

- **Dynamics of ETEC survival?**
- **Regulation of ETEC virulence function?**



Physiopathology of ETEC infections & Probiotic strategy

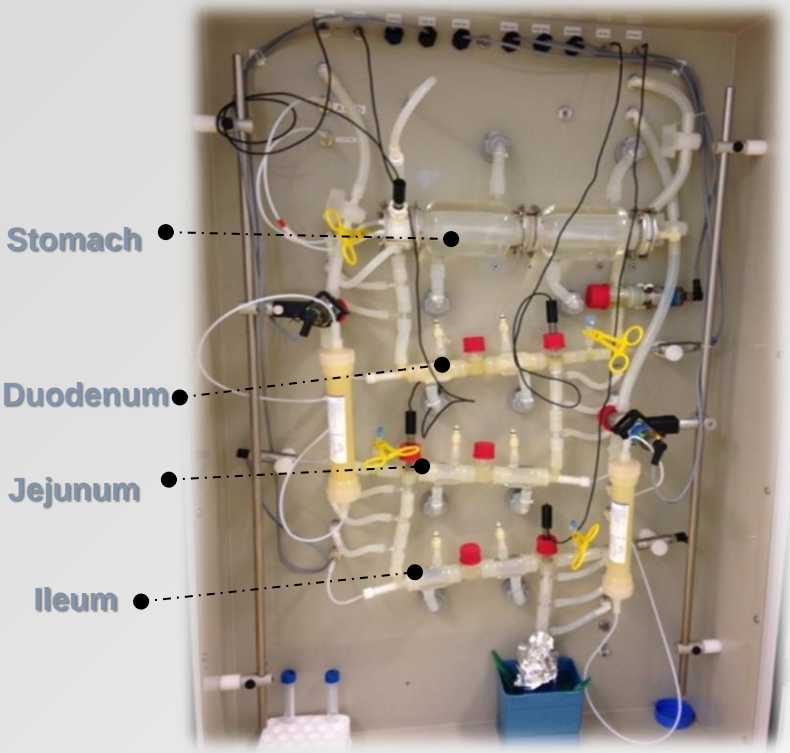
Simulation of the GIT to serve innovation

TIM & M-SHIME: Experimental design & Results

Conclusions & Future directions

TIM & SHIME: innovative well-controlled and bio-regionalized simulators of the human gut

TIM (TNO gastrointestinal model)



Parameters simulated

- Body temperature
- Temporal and longitunal changes in gastric and intestinal pH
- Transit time
- Digestive secretions (pepsin, lipase, pancreatic juice, bile)
- Chyme mixing
- Gastric and ileal deliveries
- Passive absorption (dialysis)

Guerra et al., Trends in Biotcehnol. 2012
Cordonnier et al., Microorganisms 2015

TIM & SHIME: innovative well-controlled and bio-regionalized simulators of the human gut

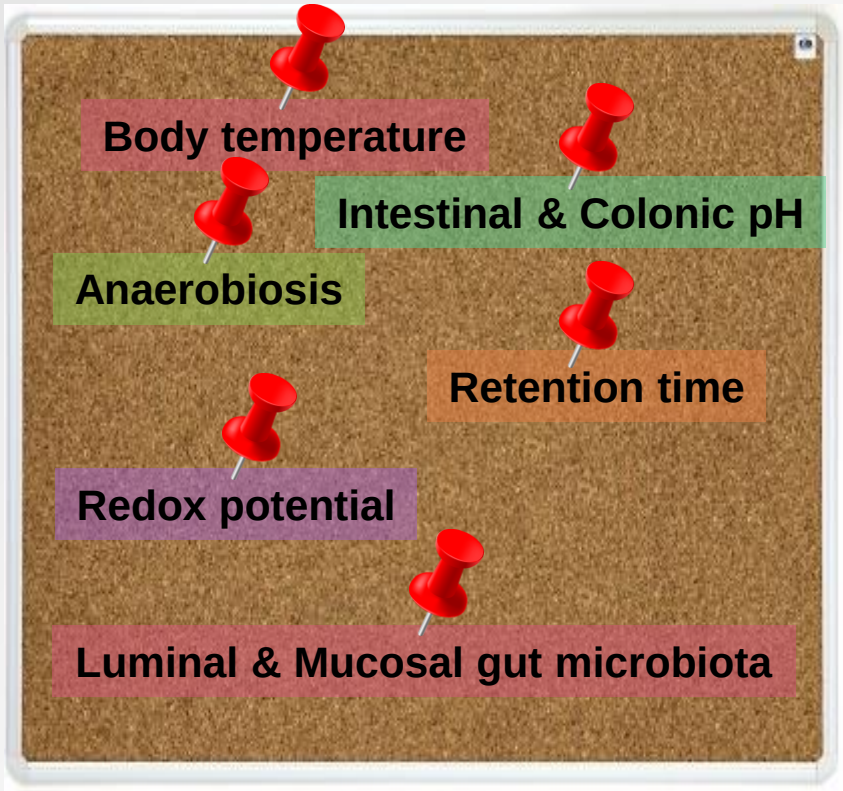
M-SHIME (Mucosal Simulator of the Human Microbial Ecosystem)

Inoculation with fresh human feces

Gut regions:

- **Proximal / distal**
- **Luminal / mucosal**

Parameters simulated



Microcosms coated with type III mucin-agar = Mucosal phase

Possemiers et al., J Nutr. 2006
Van den Abbeele et al., The ISME Joiurnal 2013

Physiopathology of ETEC infections & Probiotic strategy

Simulation of the GIT to serve innovation

TIM & M-SHIME: Experimental design & Results

Conclusions & Future directions

TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

CONDITIONS

Adult protocol

N=4

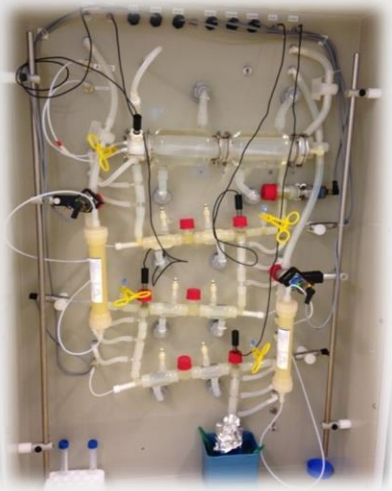
Yeast

ETEC H10407

ETEC + Yeast

SAMPLING

TIM



T0 → T300 min

Stomach | Duodenum | Jejunum | Ileum

Gastric effluents

Ileal effluents

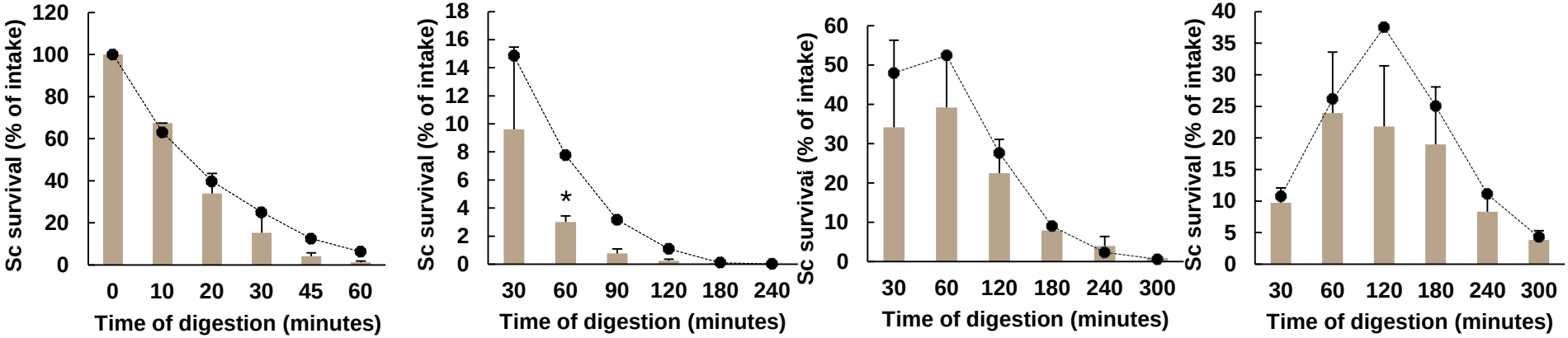
ANALYSIS

- **ETEC / Yeast survival** — Plating
- **ETEC physiological state** — Flow cytometry
- ***eltB* gene expression** — qRT-PCR
- **LT toxin production** — ELISA

TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

Q1: Does the digestive environment affect *S. cerevisiae* CNCM I-3856 survival?

STOMACH | **DUODENUM** | **JEJUNUM** | **ILEUM**



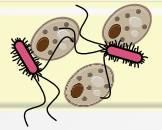
Global survival ~ 100%

Transit marker

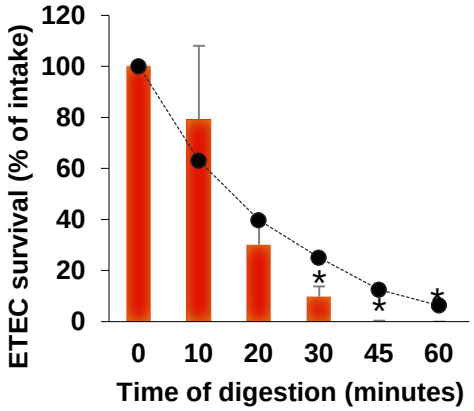
High resistance of the probiotic yeast to
- **low gastric pH**
- **gastric and small intestinal secretions (enzymes, bile salts)**

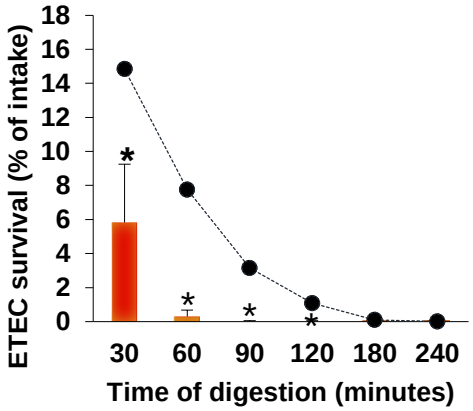
TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

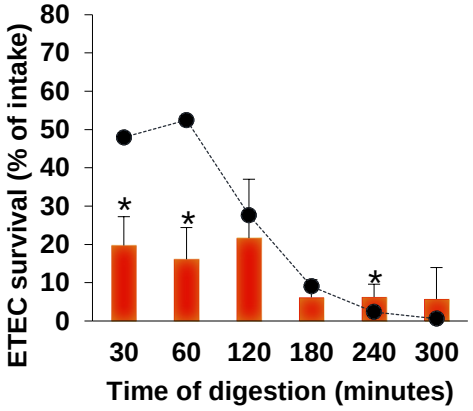
Q2: Does the digestive environment affect ETEC survival?

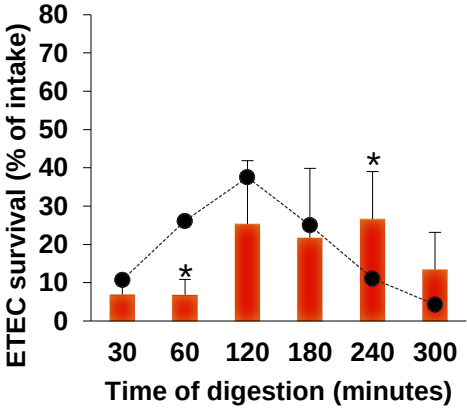


STOMACH | **DUODENUM** | **JEJUNUM** | **ILEUM**









Control

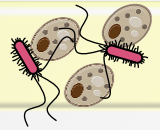
Transit marker

ETEC mortality in the stomach & duodenum

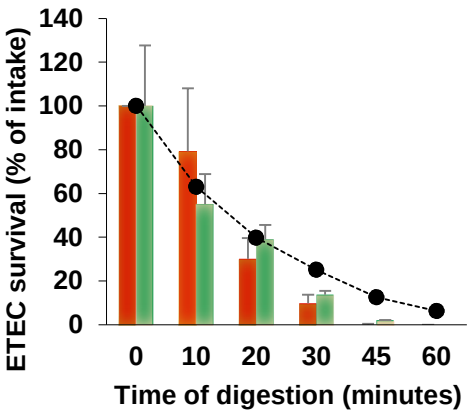
ETEC growth renewal in the jejunum / ileum at the end of digestion

TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

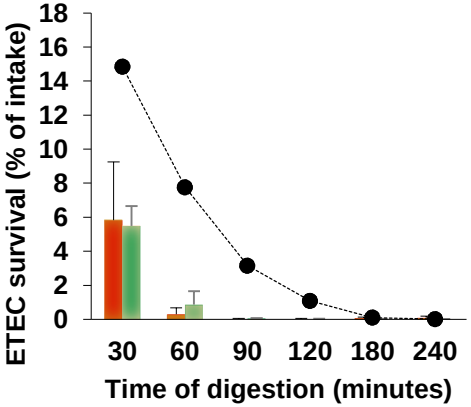
Q3: Does *S. cerevisiae* CNCM I-3856 have an effect on ETEC survival?



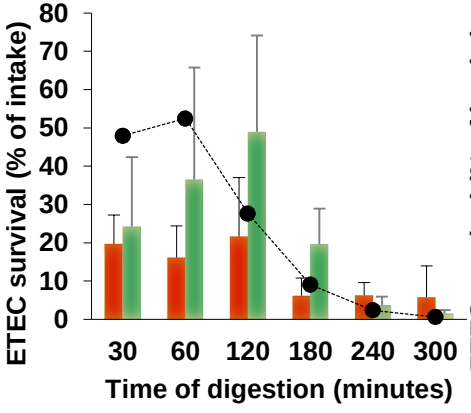
STOMACH



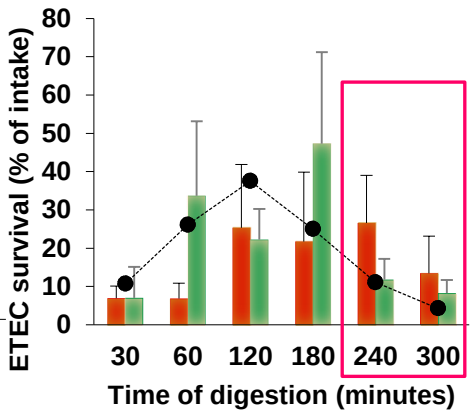
DUODENUM



JEJUNUM



ILEUM

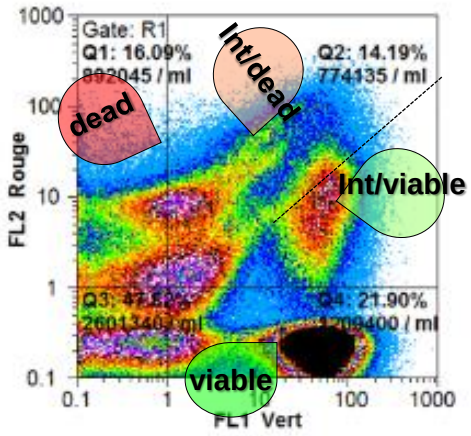


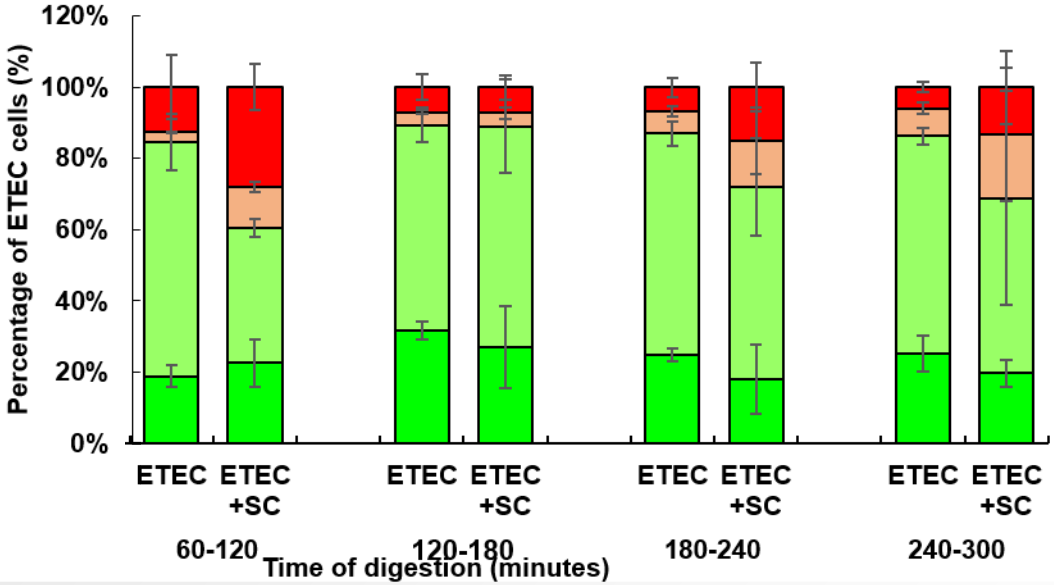
***S. cerevisiae* has no effect on ETEC survival**

Control
Probiotic
Transit marker

TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

Q4: Does *S. cerevisiae* CNCM I-3856 influence ETEC physiological state?

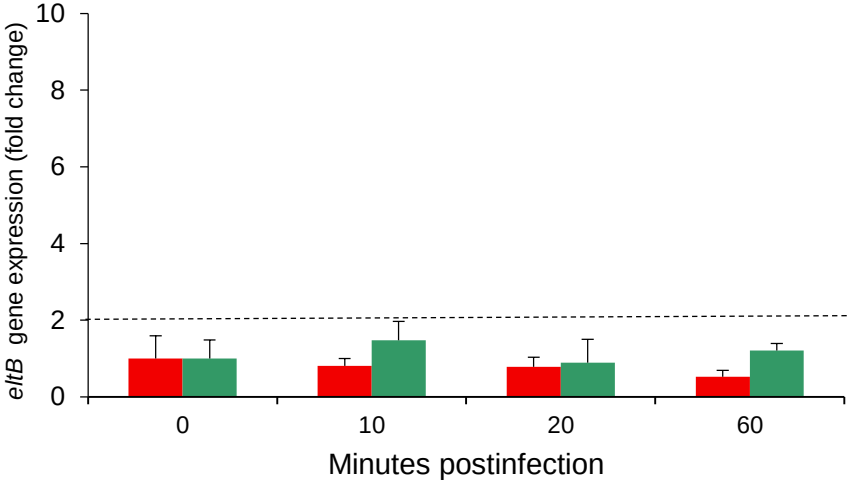




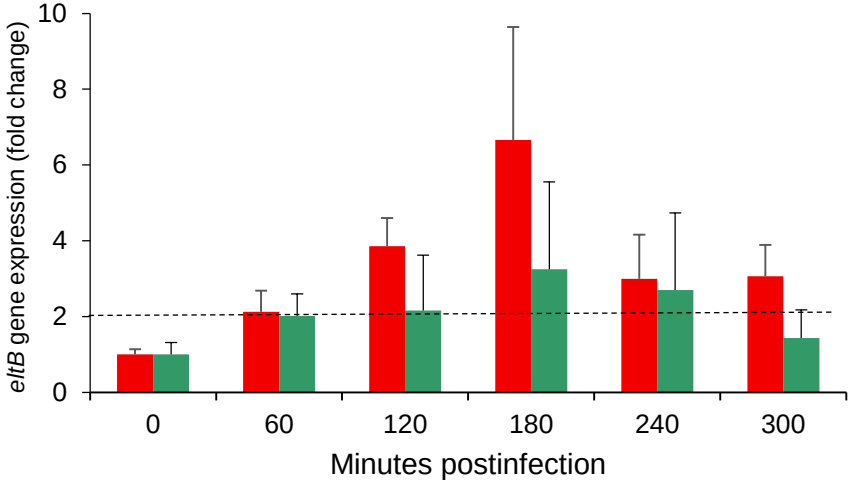
The yeast seems to increase the number of dead / damaged ETEC cells

Q5: Does *S. cerevisiae* CNCM I-3856 have an effect on ETEC virulence toxin genes expression?

GASTRIC EFFLUENTS



ILEAL EFFLUENTS



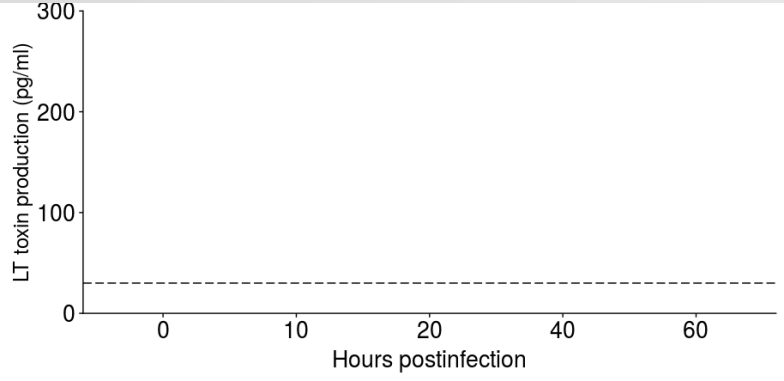
Control
Probiotic

The yeast tends to reduce *eltB* gene expression in the ileal effluents

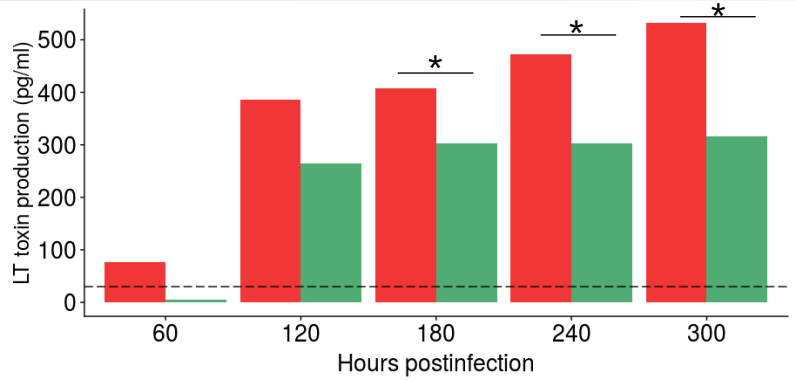
TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

Q6: Does *S. cerevisiae* CNCM I-3856 have an effect on enterotoxin production?

GASTRIC EFFLUENTS



ILEAL EFFLUENTS

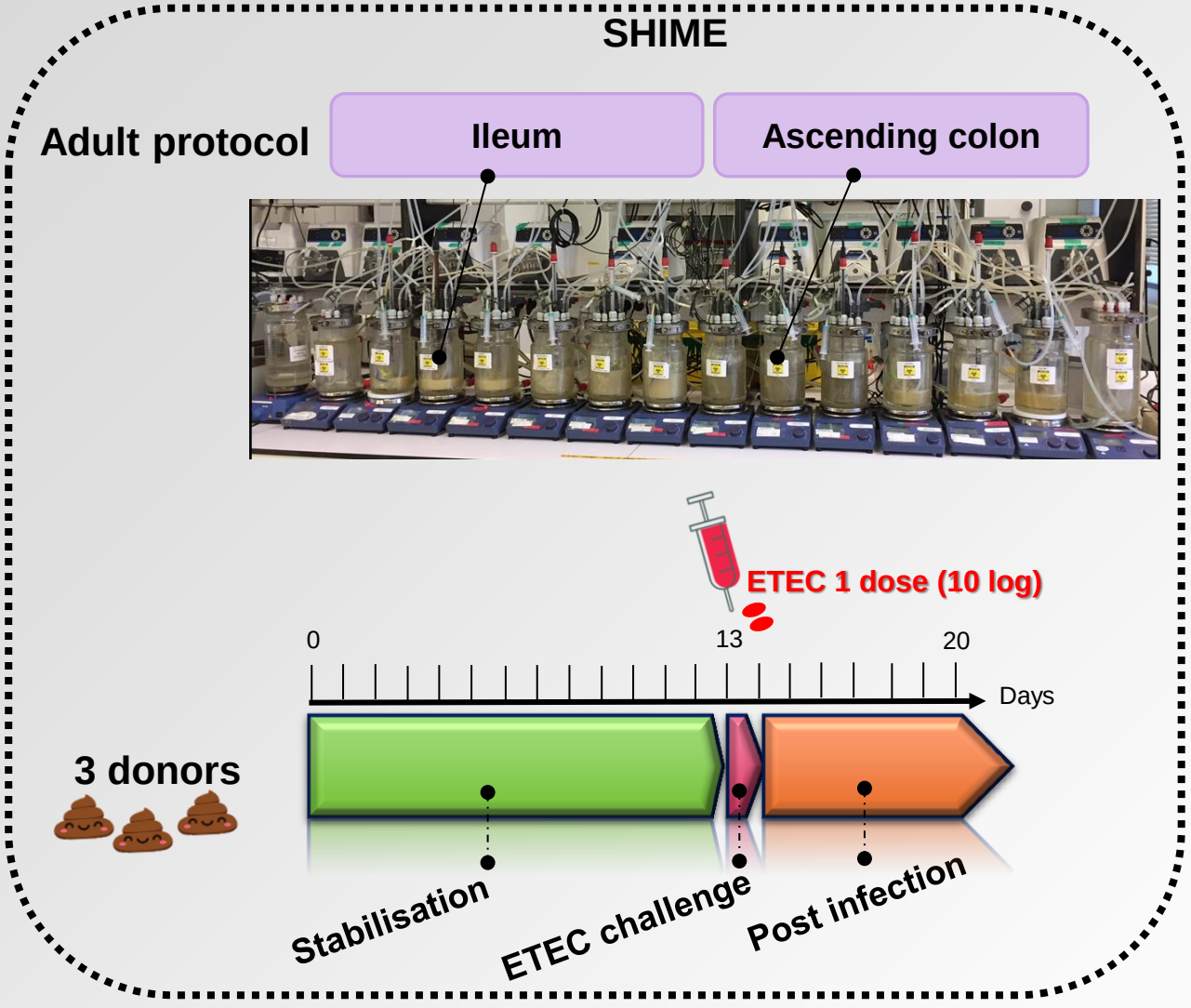


Control
Probiotic

The yeast significantly decreases LT toxin production

TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

CONDITIONS & SAMPLING



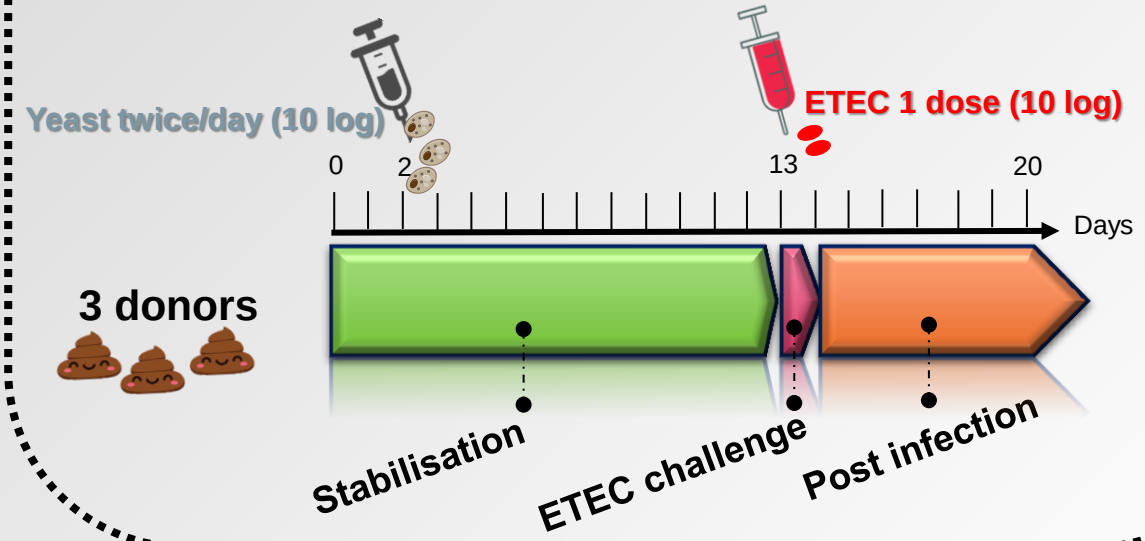
TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

CONDITIONS & SAMPLING

SHIME

Adult protocol

Ileum | **Ascending colon**

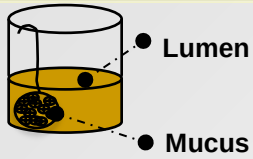


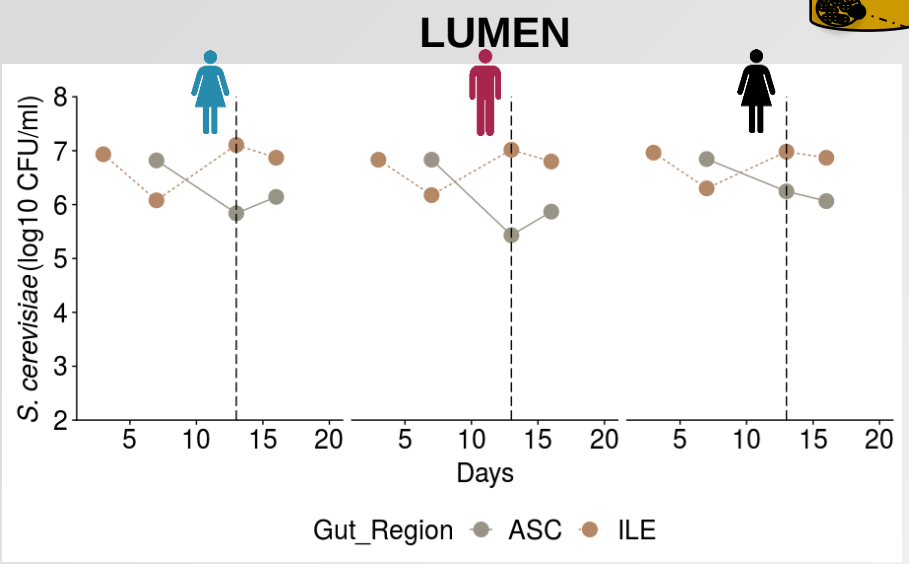
ANALYSIS

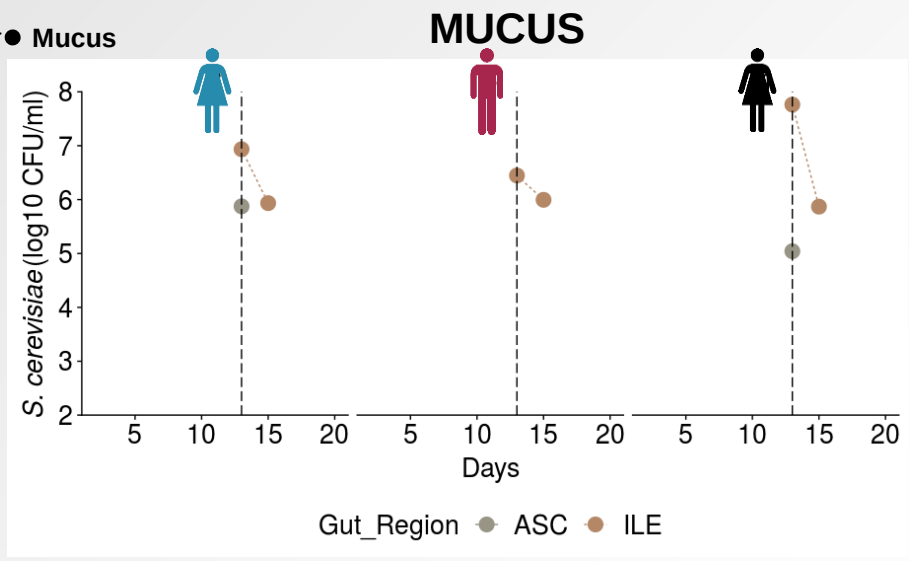
- **ETEC / Yeast survival**
  - qPCR
  - Plating
  - PMA-qPCR
- ***eltB* genes expression**
  - qRT-PCR
- **LT toxin production**
  - ELISA
- **Microbiota activity/composition**
  - SCFA
  - Illumina seq

TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

Q1: Does *S. cerevisiae* CNCM I-3856 survive in the regionalized SHIME?



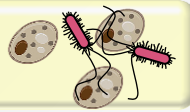


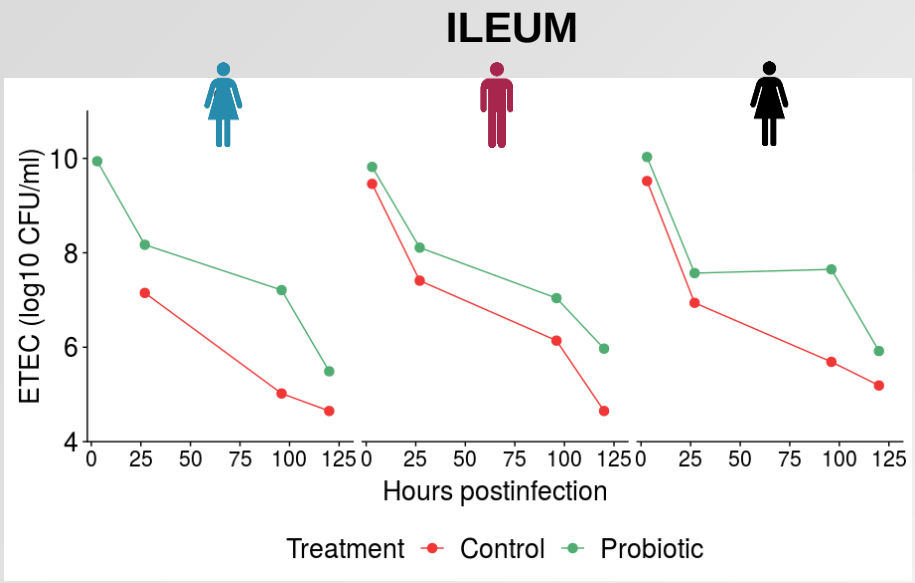


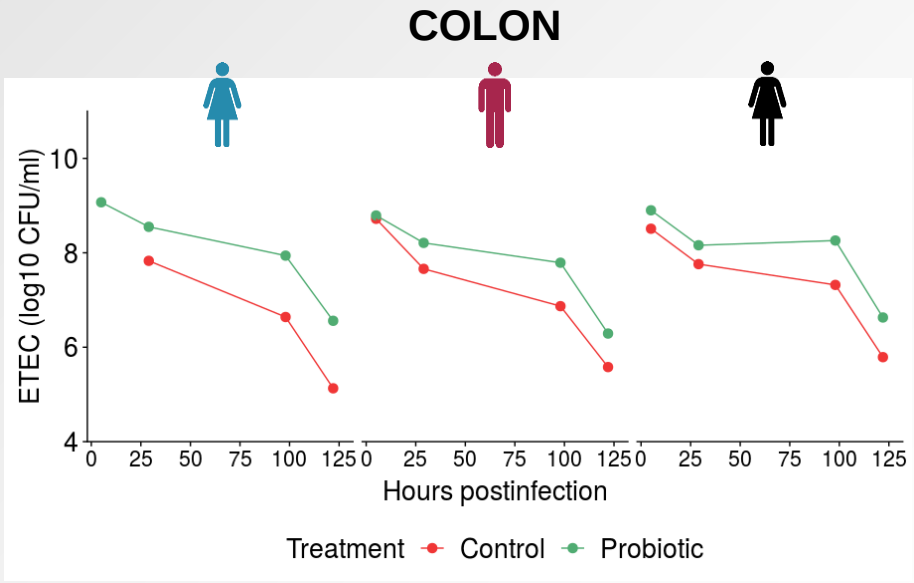
The yeast achieves steady-state concentrations in the L-SHIME

TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

Q2: Does *S. cerevisiae* CNCM I-3856 have an effect on ETEC survival?







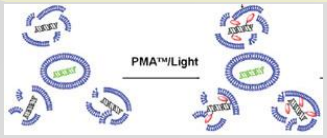
Measured by qPCR=
total number of ETEC cells

↓

Reflect ETEC biomass NOT VIABILITY

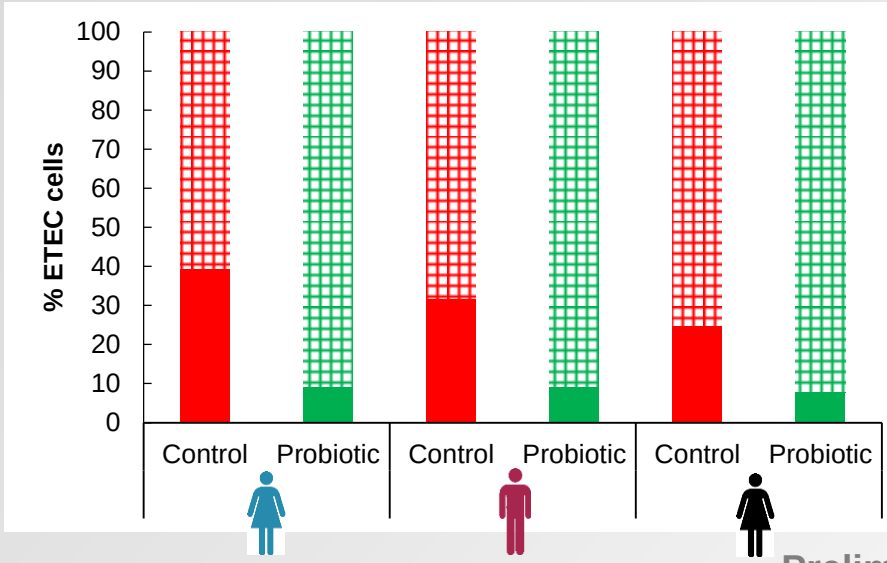
TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

Q2: Does *S. cerevisiae* CNCM I-3856 have an effect on ETEC survival?

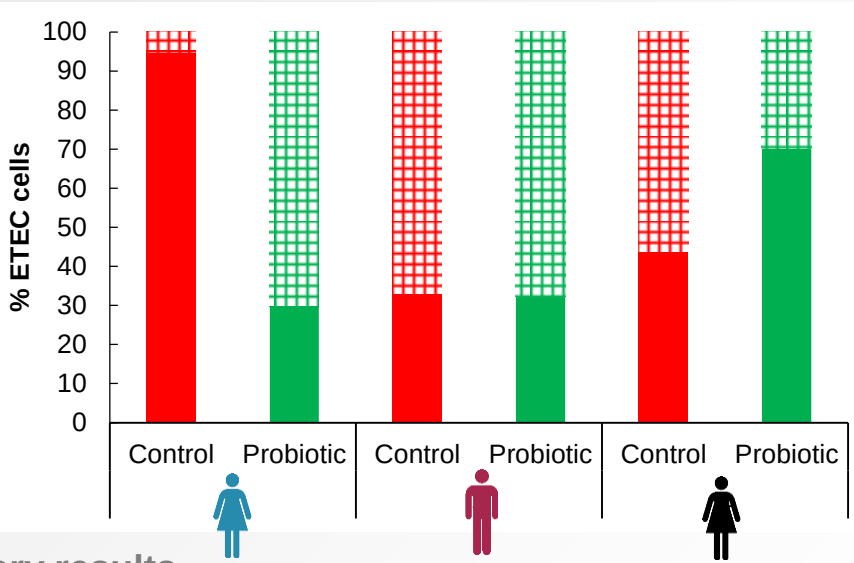


PMA-qPCR

ILEUM (3h PI)



COLON (5h PI)

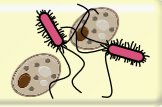


Preliminary results

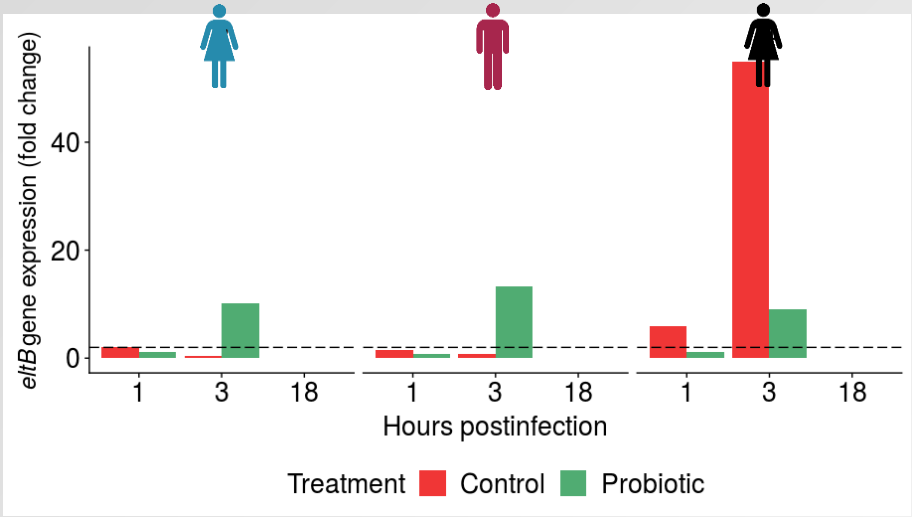
Control viable
Control altered
Probiotic viable
Probiotic altered

The yeast decreases the number of viable ETEC cells in the Ileum

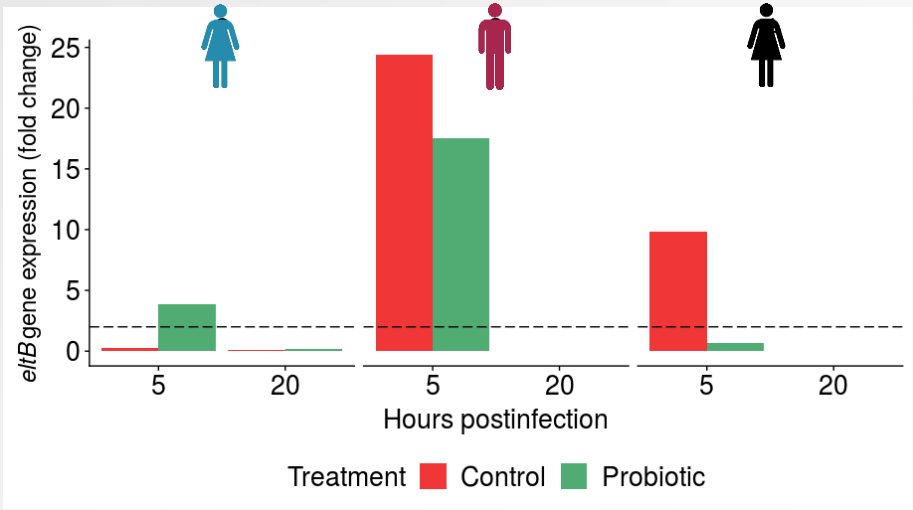
Q3: Does *S. cerevisiae* CNCM I-3856 have an effect on *eltB* gene expression?



ILEUM



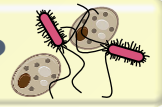
COLON

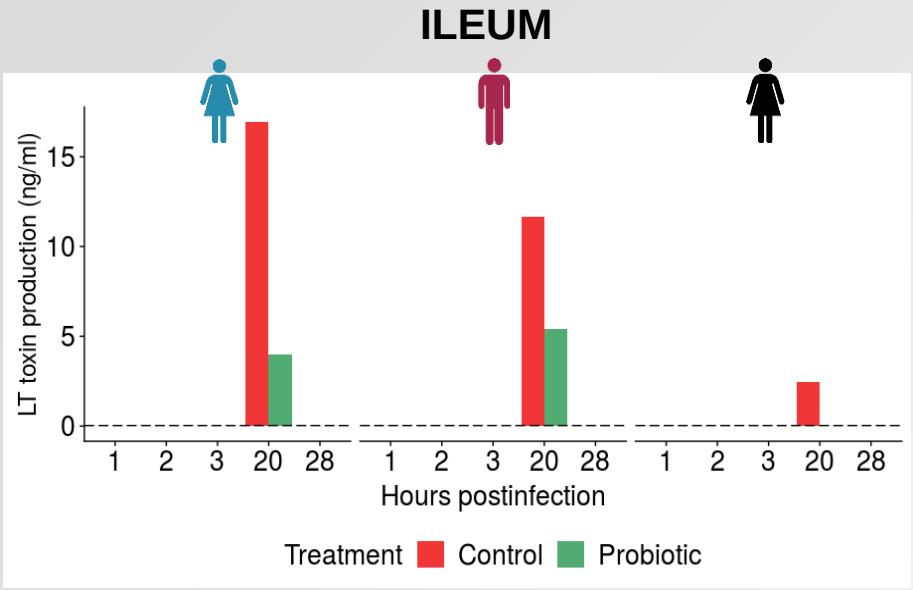


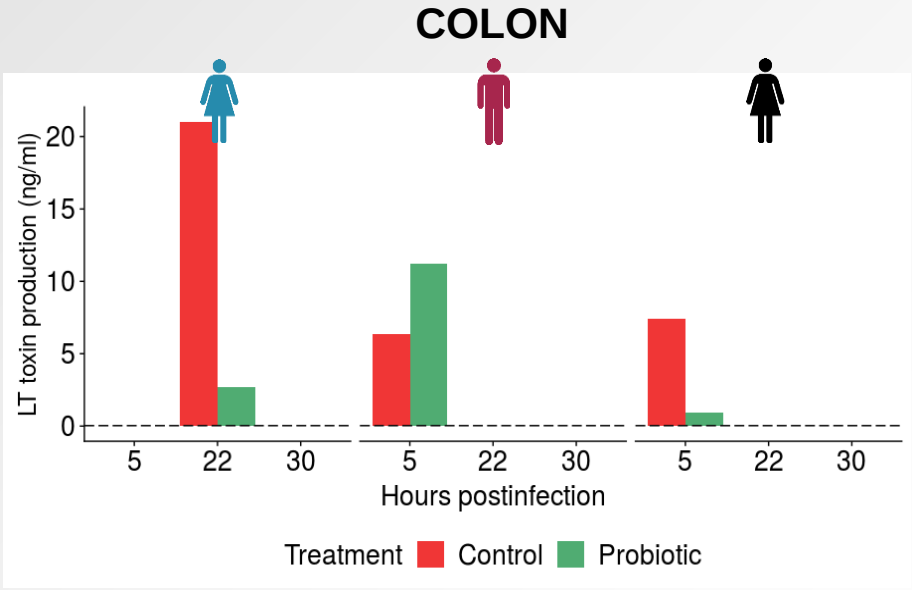
The yeast tends to downregulate *eltB* gene expression mostly in the colon

TIM & M-SHIME: EXPERIMENTAL DESIGN & RESULTS

Q4: Does *S. cerevisiae* CNCM I-3856 have an effect on enterotoxin production?







The yeast tends to decrease LT toxin production, in both ileum & colon

Physiopathology of ETEC infections & Probiotic strategy

Simulation of the GIT to serve innovation

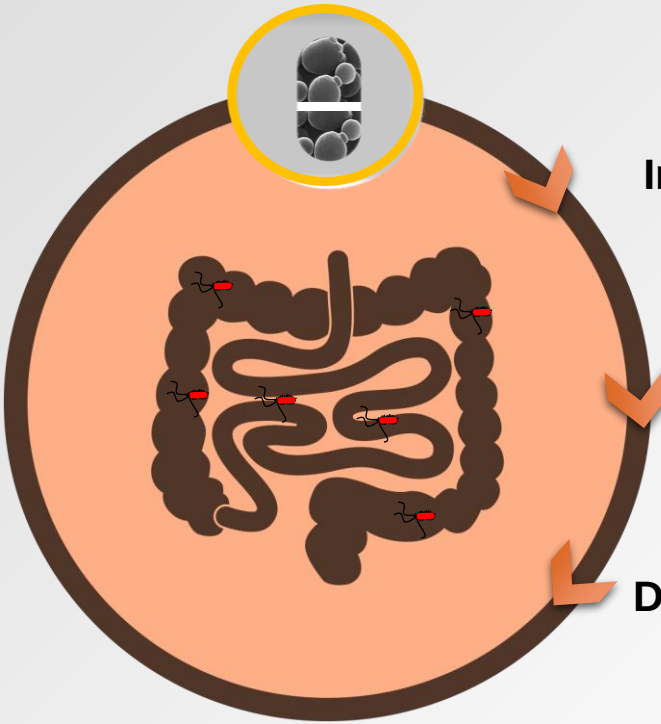
TIM & M-SHIME: Experimental design & Results

Conclusions & Future directions

CONCLUSIONS & FUTURE DIRECTIONS

Saccharomyces cerevisiae
CNCM I-3856

LHC
LESAFFRE HUMAN CARE

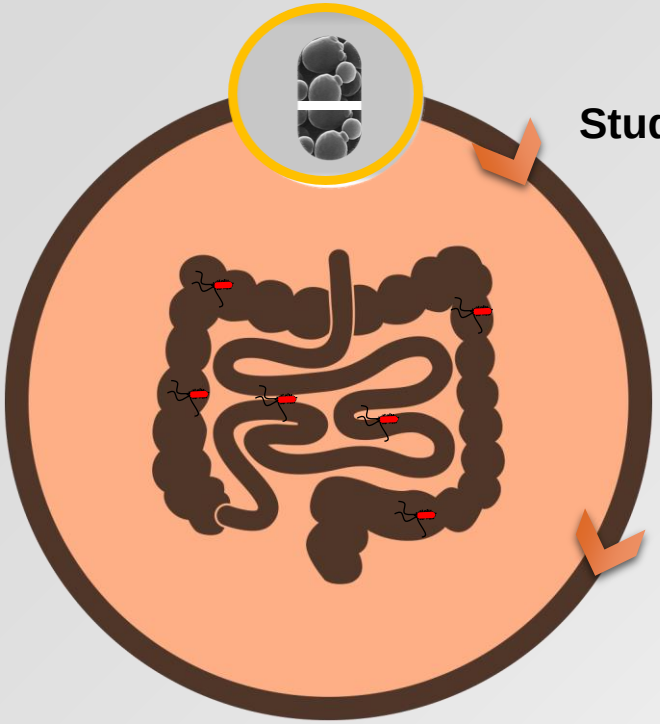


Increases ETEC damaged cells

Inhibits *eltB* gene encoding LT toxin

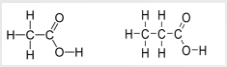
Decreases LT toxin production

FUTURE DIRECTIONS



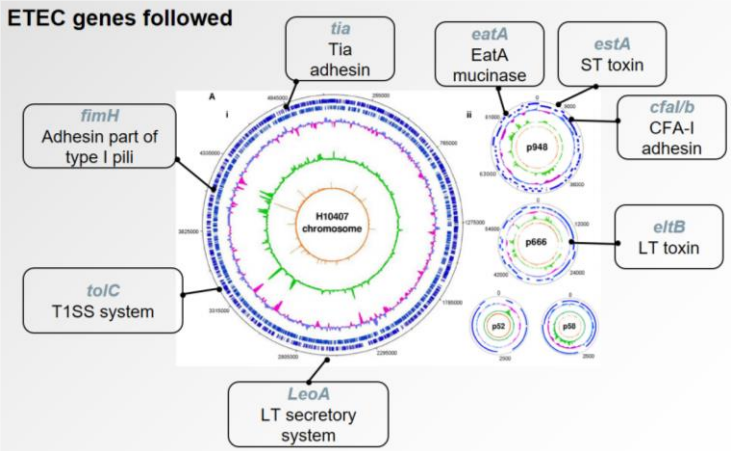
Study the impact on gut microbiome composition / activity

Illumina seq



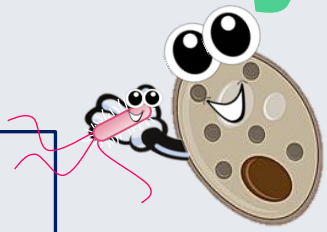
SCFA

Provide a more complete view of ETEC virulence function



RTq-PCR

Thank you for your attention



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