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# Development of nanocarrier for engineered *L. lactis* delivery as edible vaccine against swine *E. coli*


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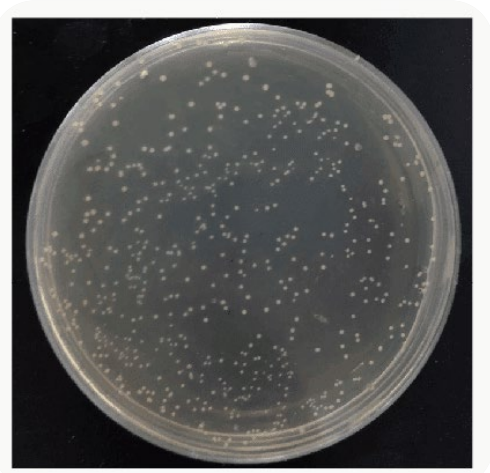


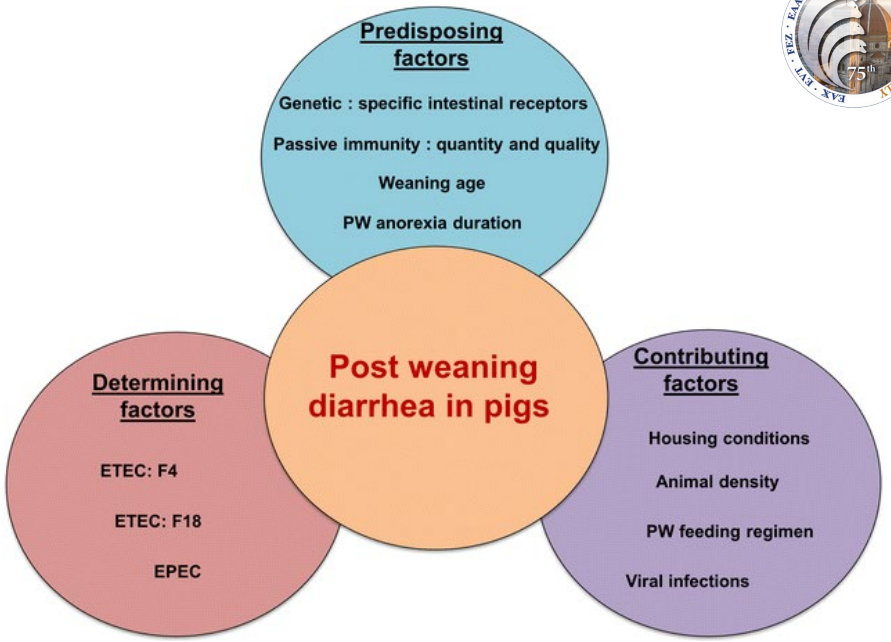
# Post-weaning diarrhoea

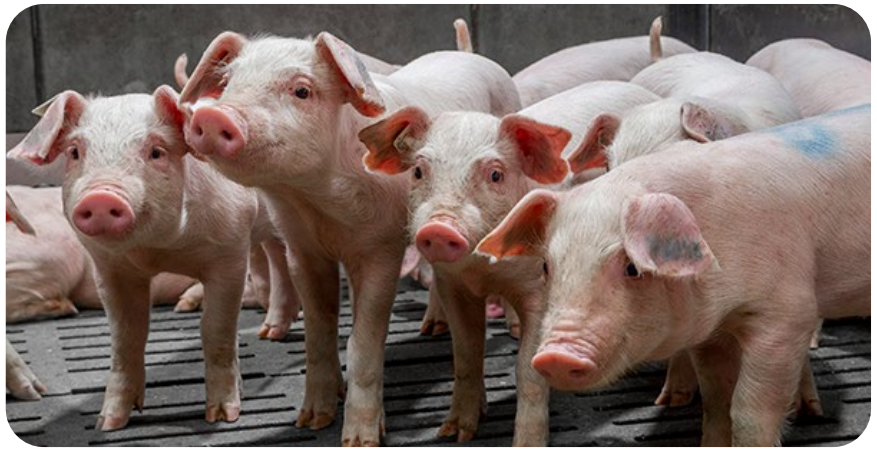
- In swine farming, the weaning is one of the most critical period where young animals are exposed to several stressors (Rossi et al., 2023).

- ETEC and VTEC *E. coli* strains are cause of high morbidity and mortality rate and often veterinarian and farmers resort to antibiotics for limiting the detrimental effect during the weaning phase (Reggi et al., 2024).

- However, AMR is one of the major concern worldwide and alternative actions have to be pursued. *E. coli* is often considered as environmental indicator of AMR genes spreading (EFSA & ECDC, 2023)

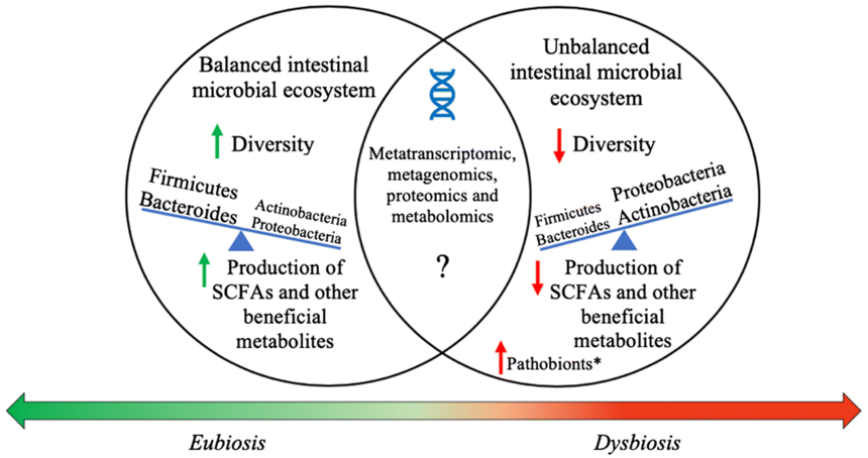
- Even though an important reduction in antimicrobials for livestock has been achieved, more alternative solutions are still necessary…



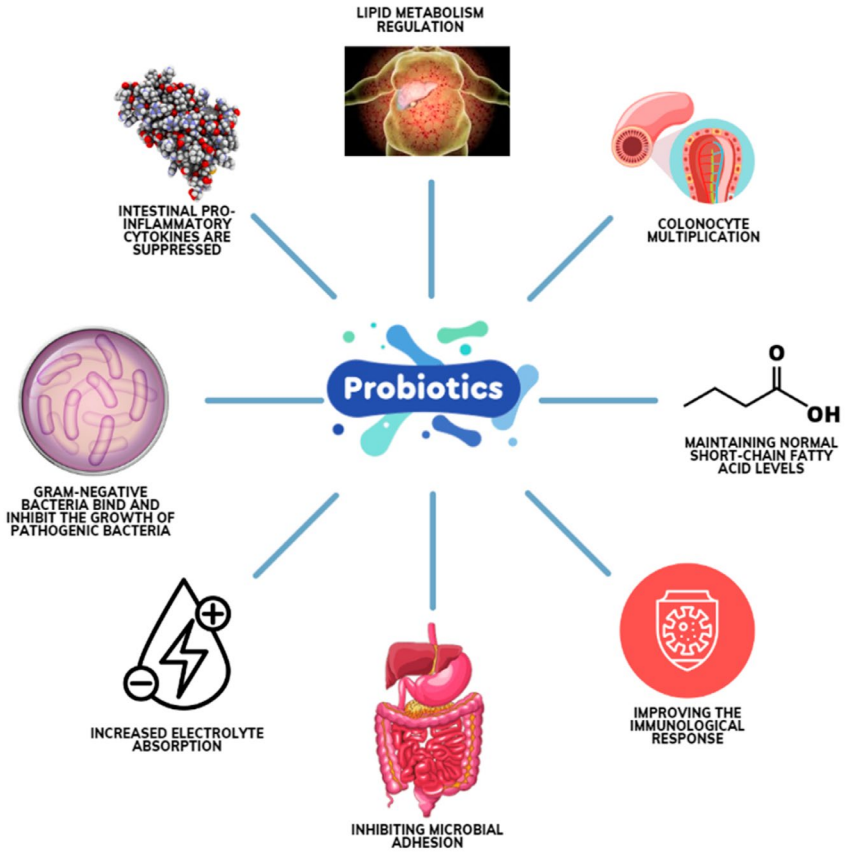



# Probiotics

- Probiotics are known to positively shape the gut microbiota competing with different mechanisms with pathogens.
- They have been proposed by EFSA as alternatives to antibiotics (EFSA, 2013).
- They play a positive role for the gut health is a crucial key for reducing the occurrence of gastrointestinal and systemic disorders.



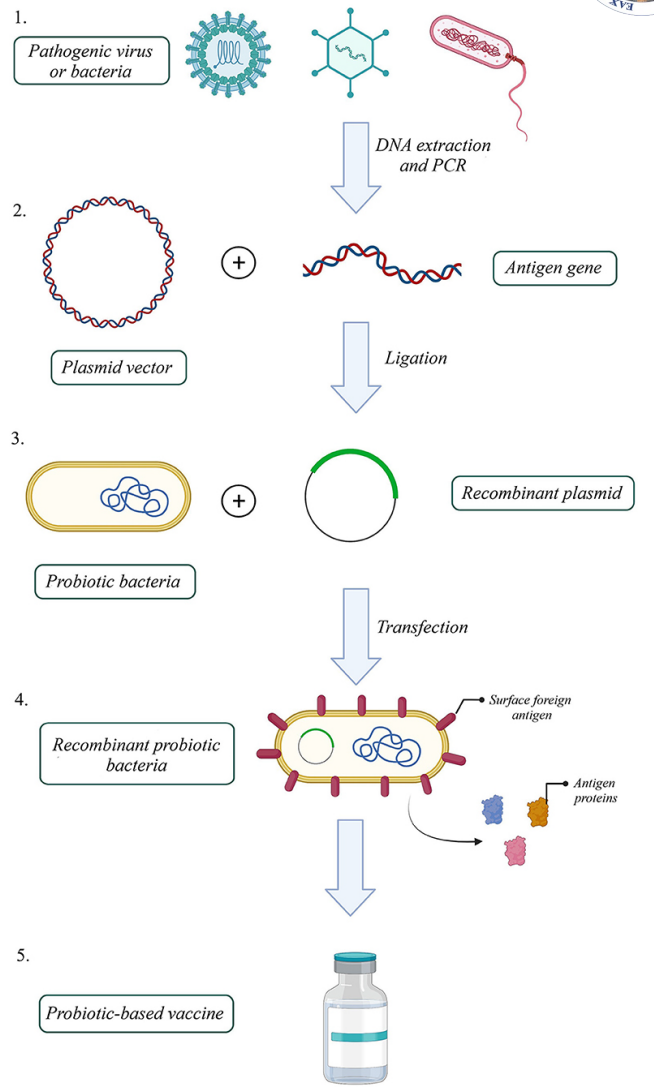

Rodriguez et al. 2024




Maftei et al. 2024

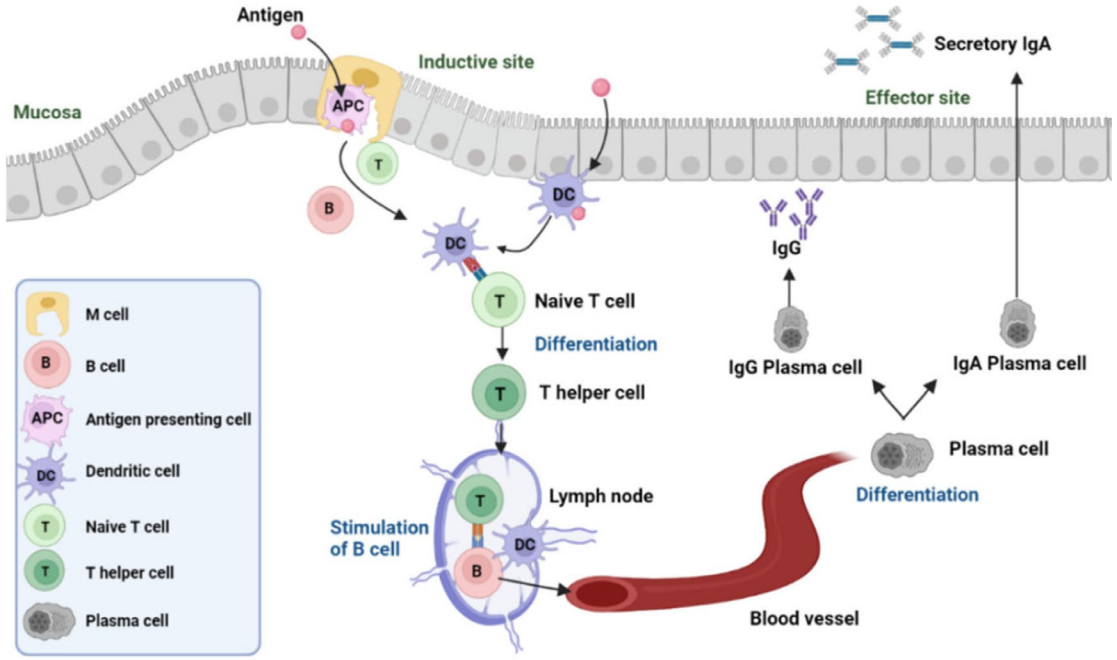
# *L. lactis* as expression platform

- *Lactococcus lactis* is a well-known probiotic species commonly used in the food industry.
- Recently *L. lactis* strain renewed its interest in research for genetic engineering and protein expression (Zhai et al., 2023)
- Edible vaccination present several advantages to the traditional injectable, particularly in the livestock sector where cost-opportunity has always to been considered (Reggi et al., 2023)
- The production of SIgA allows the "immune exclusion", characterized by agglutination, entrapment and clearance of the pathogen (Van der Weken et al., 2020).



# Antigens' survival for receptor targeting

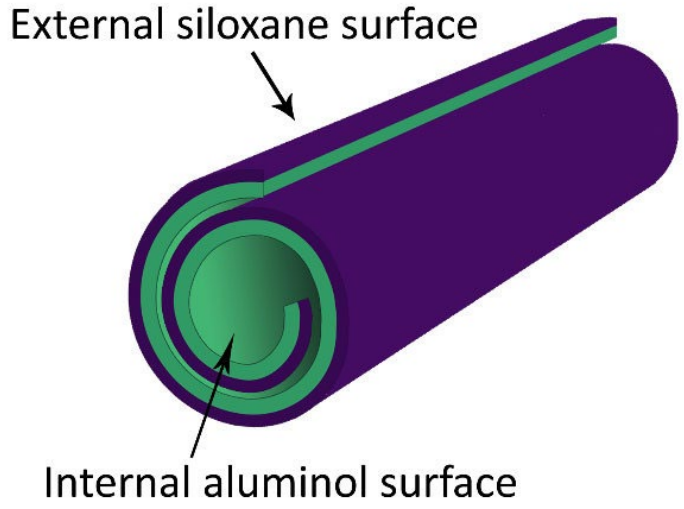
- Even if probiotics are designed to resist during the gastrointestinal transit, concern among the preservation of expressed antigens still persist (Kwong et al. 2023)
- Thus, it is fundamental to ensure that orally-delivered antigen could arrive in the specific sites integer for eliciting the immune response (Aryamvally et al., 2017)
- Several methods of encapsulation/protection have been proposed.

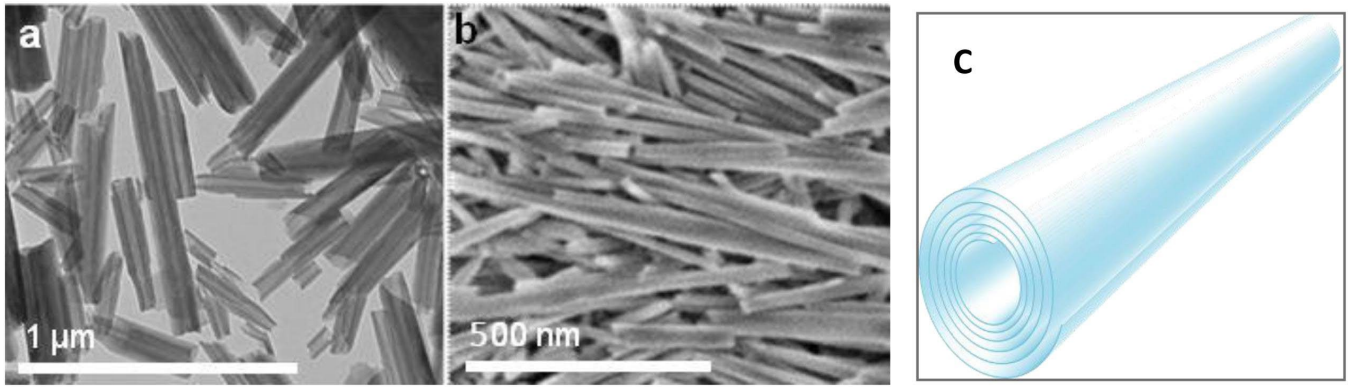



Kwong et al., 2023

# Halloysites nanotubes

- Natural aluminosilicates nanotubes with hollow tubular structure.
- 50-70 nm diameter and 1-2 µm length.
- They demonstrated loading capacity for drug delivery and a controlled release pH sensitive (Lei et al., 2023)
- They are promising in the protection of drug delivery (Hanfi et al., 2018).



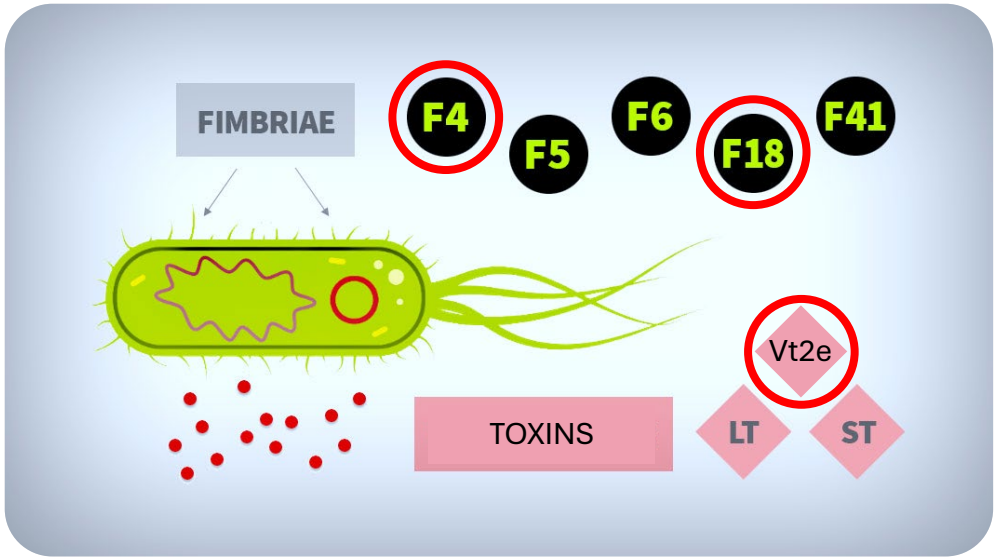



# AIM

The aim of this study was to develop and combine with halloysite nanotubes different strains of engineered *L. lactis* for the expression of antigens of major *E. coli* pathotypes of swine, as a model of multivalent edible vaccines against VTEC and ETEC strains.

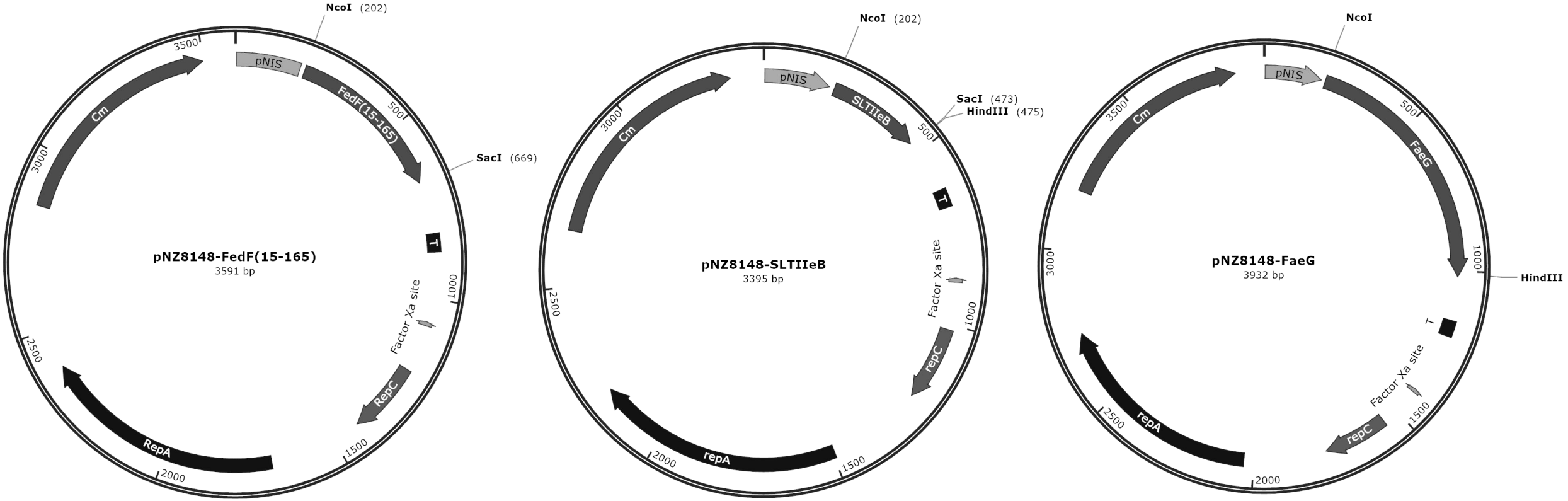
Immunogenic antigens selected:

- FedF (the minor subunit highly conserved of F18 fimbria)
- FaeG (the major structural subunit of F4 fimbria)
- B subunit of the verocytotoxin-2e (VT2e-B)



# A double approach

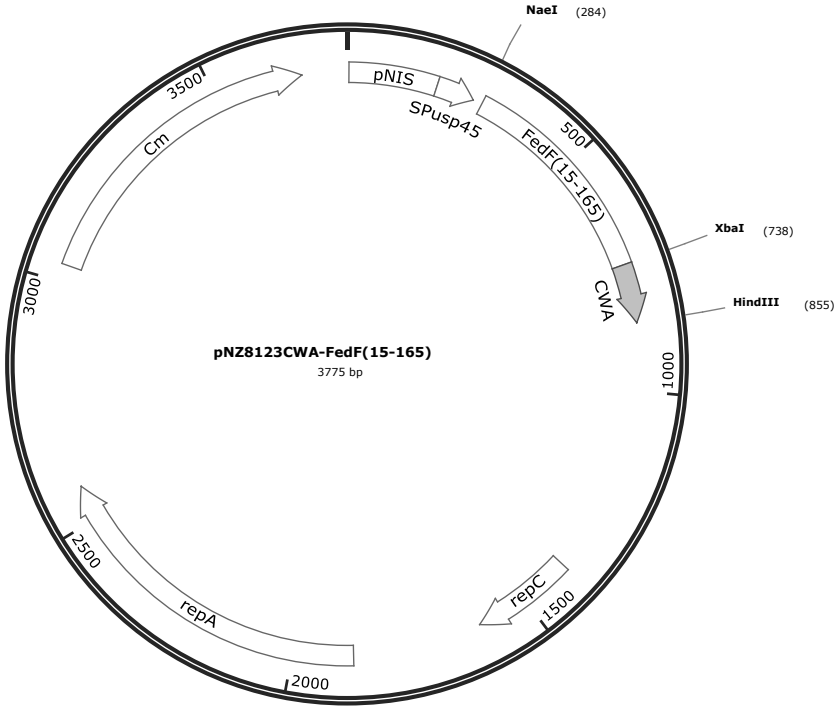
FedF, VT2eB, FaeG were cloned in the NICE vector pNZ8148 plasmid under the control of nisin-inducible promoter NisA for expression in *L. lactis* NZ9000

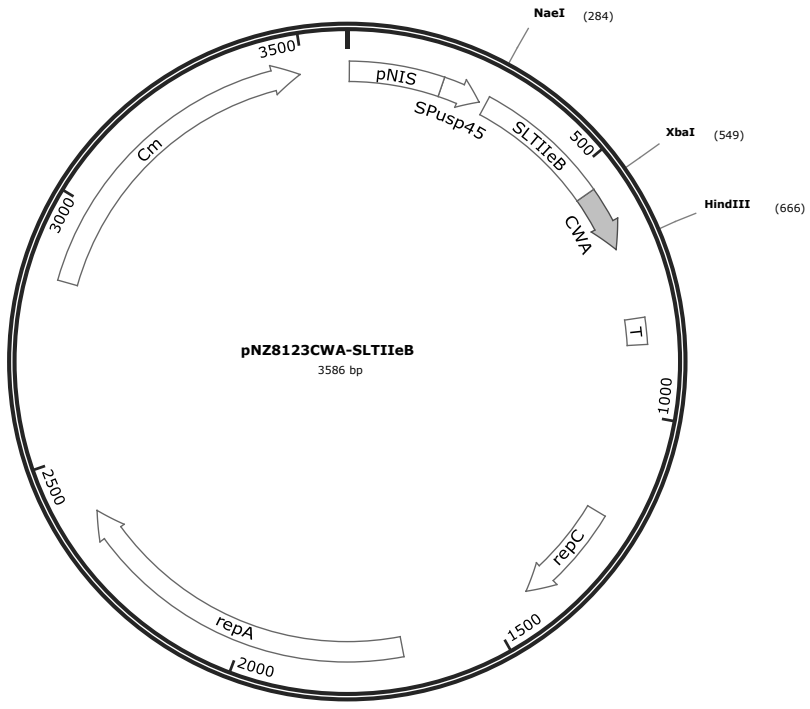


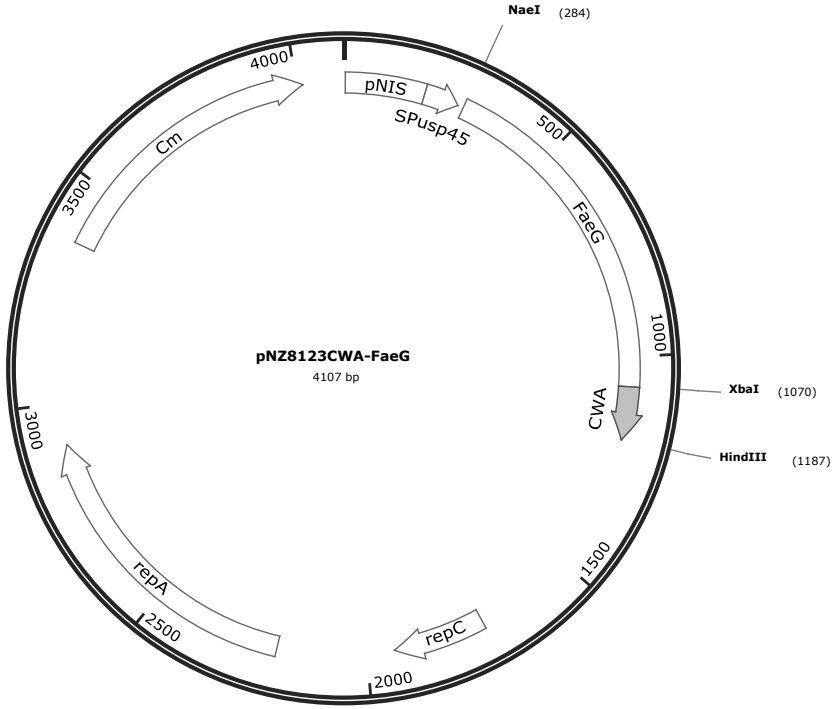

Chimeric constructs for intracellular expression using pNZ8148 plasmid

# A double approach

Cell wall anchor (CWA) was amplified form *Streptococcus pyogenes* genomic DNA with unique restriction sites.



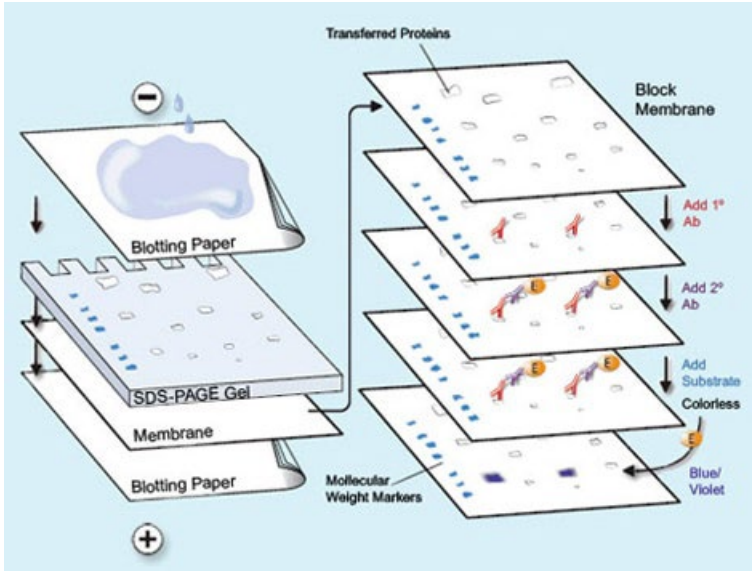




Chimeric constructs for extracellular expression using pNZ8148 plasmid

# Plasmid integration and expression

- After electroporation PCR reaction was used to confirm the stable integration of *E. coli* genes.

- Protein were extracted from cytoplasm or cell-wall → SDS page and WB analysis were used to verify the expression of recombinant protein after nisin induction (OD = 0.4; 3 ng/mL nisin).




| Primer | Sequence 5'-3' |
|---|---|
| SLTIIeB-F | **CCATGG**AGAAGATGTTTATAGCGGTTTTATTTGC |
| SLTTIIeB-R | **GAGCTCTTA**GTTAAACTTCACCTGGGCAAAGC |
| FedF-F | **CCATGG**ATTCTAGTGCGAGTAGTGCTCAAGTC |
| FedF-R | **GAGCTCTTA**TTTGCAATCGCAGGAACATAGATTGAAG |
| FaeG-F | **CCATGG**GGATGACTGGTGATTTCAATGGTTCGG |
| FaeG-R | **TCTAGATTA**GTAATAAGTTATTGCTACGTTCAGCG |

# Freeze-drying and HNTs immobilization

- A 48h culture of *L. lactis* was centrifuged and resuspended in cryoprotection solution for freeze-drying (-56°C for 12 hours).

- Freeze-dried culture was plate counted on MRS for the enumeration of viable colonies.

- Freeze-dried culture was resuspended with HNTs at different concentration for measuring the immobilization efficiency:

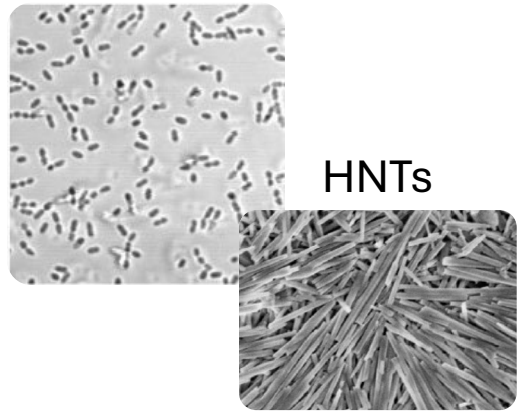
1 mg/mL

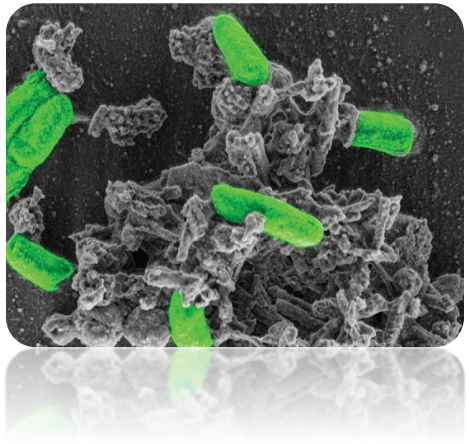
0.5 mg/mL

- The absorption was verified trough chemical oxygen demand (COD) after separation of the solution of HNTs and *L. lactis*

- Number of viable colonies were enumerated after air-drying the pellet.

*L. lactis*



# Simulated *in vitro* digestion resistance

Oral phase: Electrolyte solution + amylase

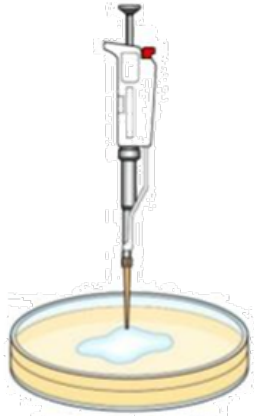
Gastric phase: pH was adjusted to 3.0 incubated with pepsin for 90' at 37 °C

Intestinal phase: bile and pancreatin incubated at pH 7 for 240' at 37 °C

1st sampling

2nd sampling

Plate counting on MRS agar



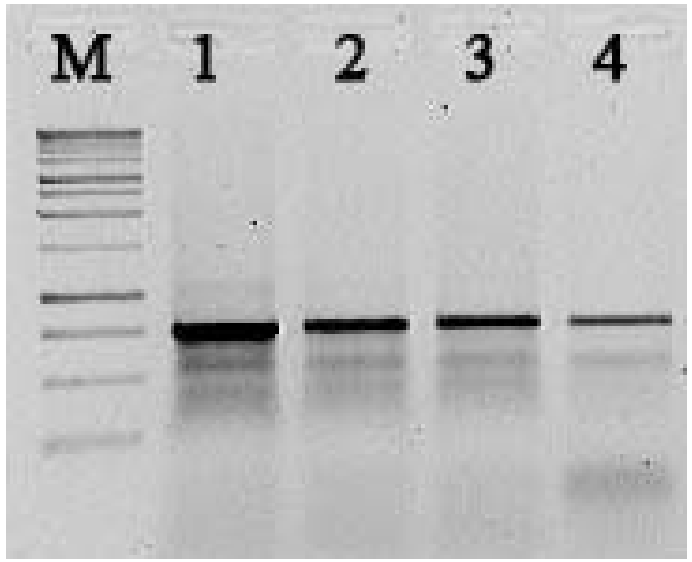
INFOGEST - Ayyash et al., 2021

# PCR amplification

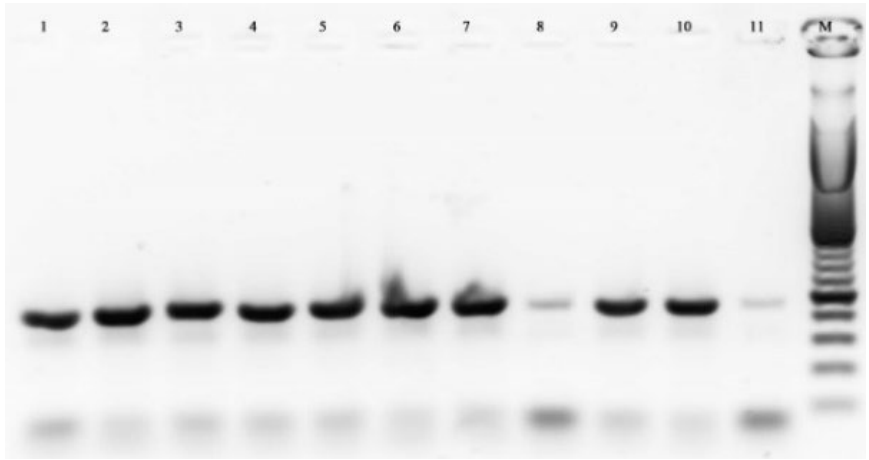
## Results

All *L. lactis* strain showed stable integration of heterologous genes

**FedF**

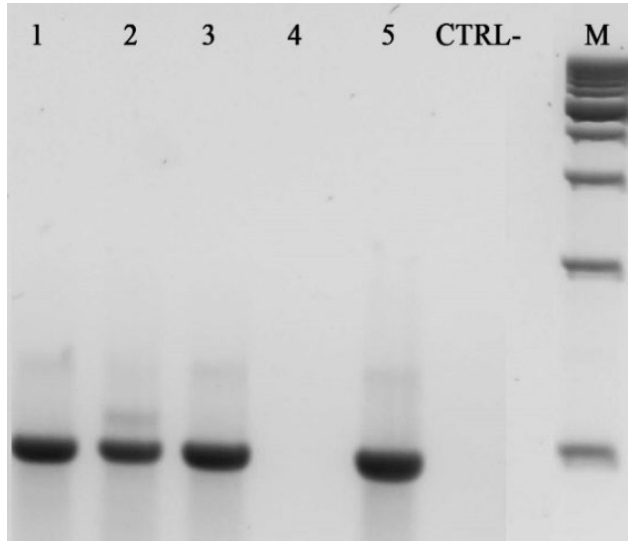



**VT2eB**




500 bp

**FaeG**




1000 bp

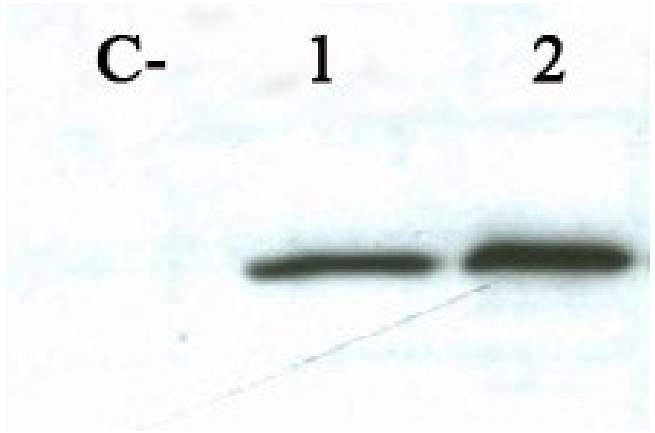
# Protein expression

## Results

Positive signals for polyclonal hybridization were detected at WB analysis

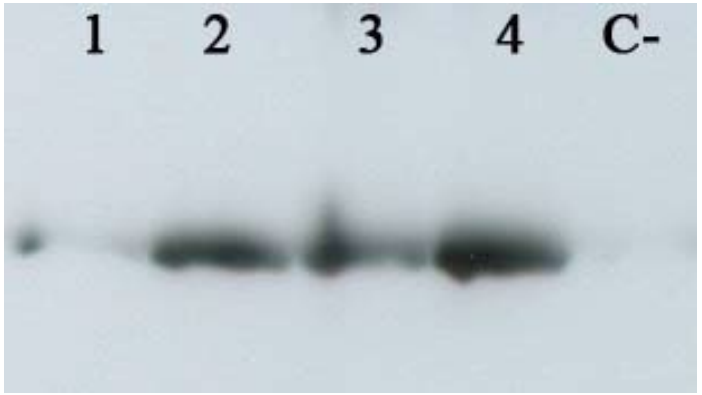
Intracellular

20 kDa




**FedF**

14 kDa




**VT2eB**

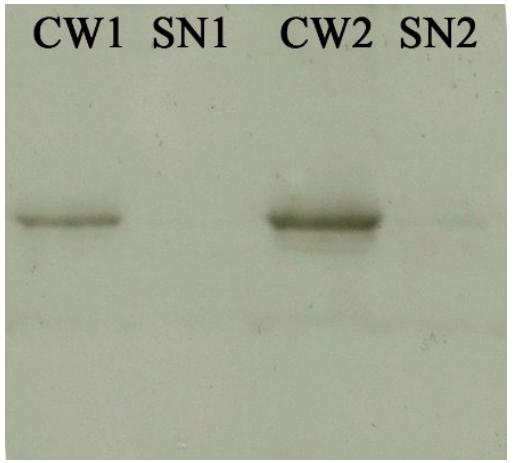
30 kDa


**FaeG**

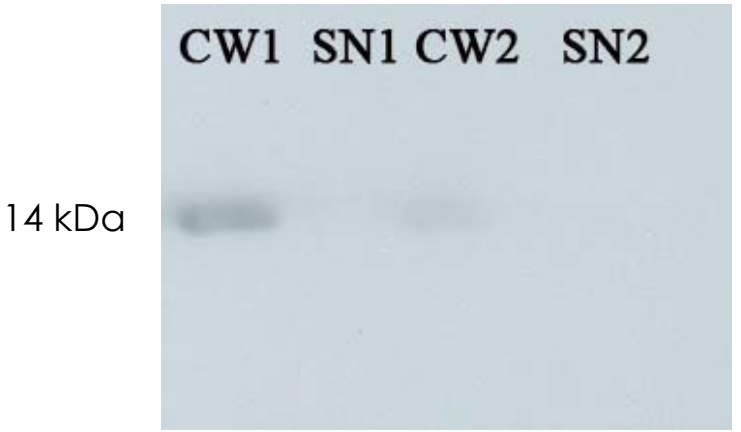
# Protein expression

## Results

CWA expression




**FedF**




**VT2eB**

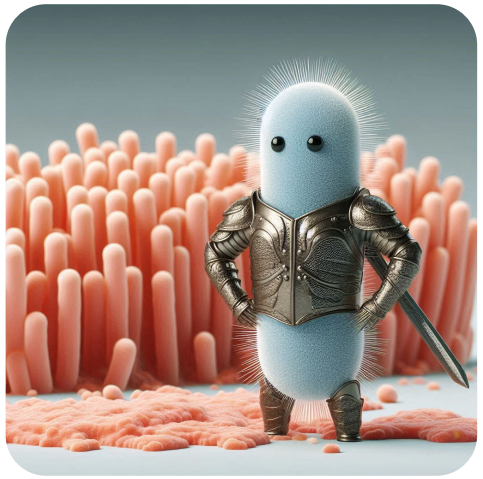

**FaeG**

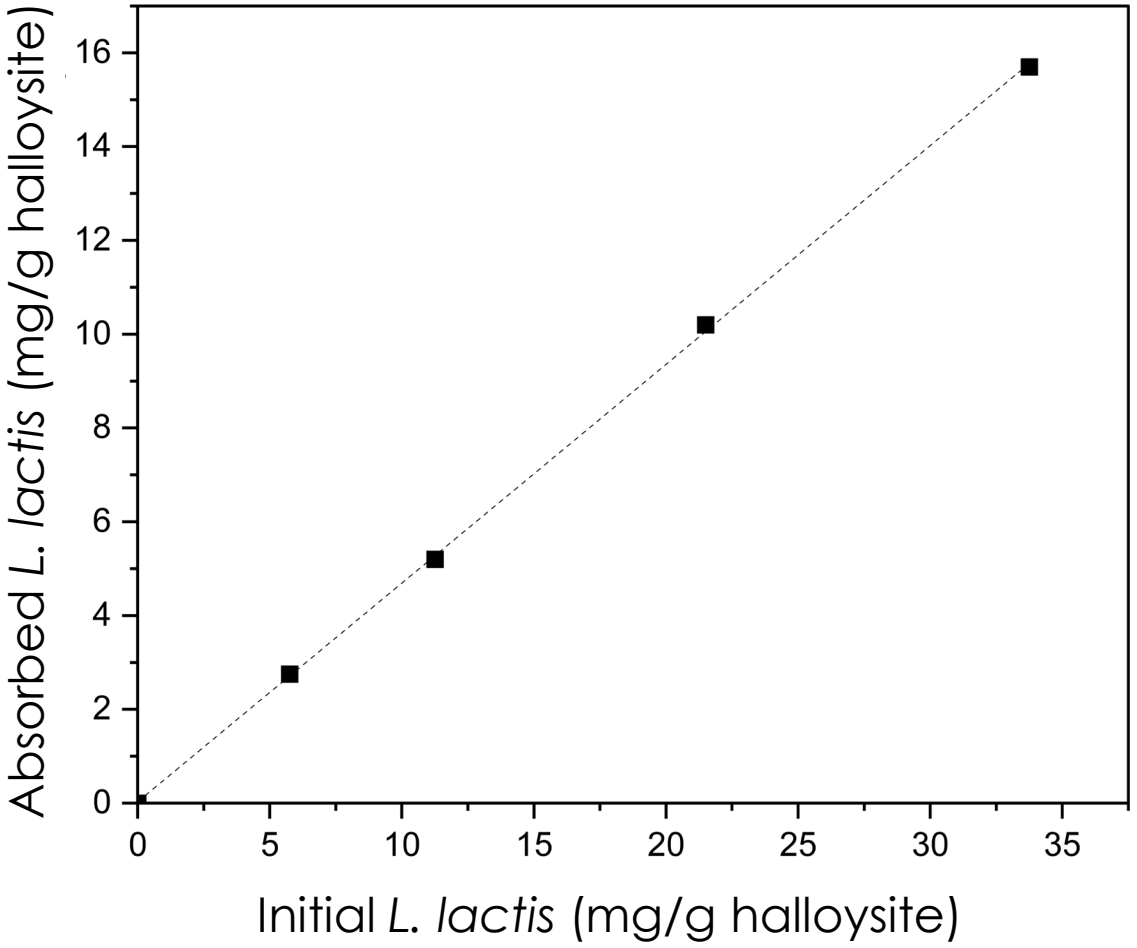
**Italian Patent n° 102021000006506**
**by Prof. Luciana Rossi and Dr. Serena Reggi**

# HNTs immobilization

## Results

- Direct dose-adsorption response
- Efficiency 46.53±1.52

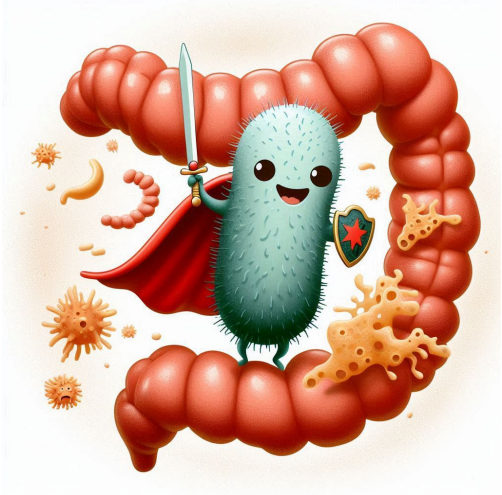


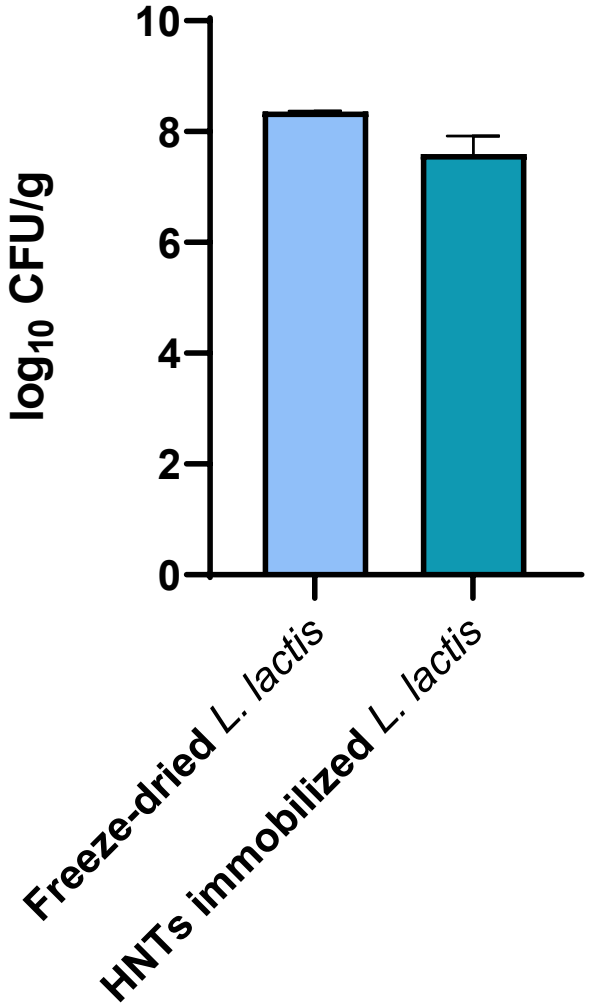


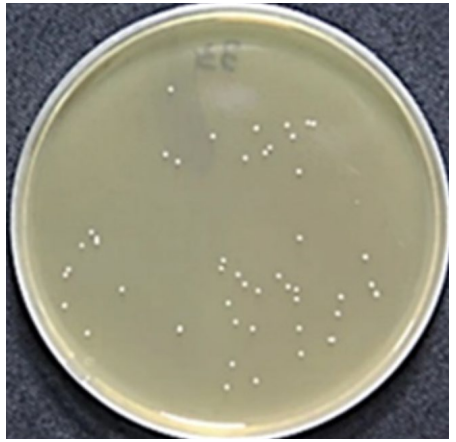
# HNTs immobilization - survival

## Results

Small reduction of survival after the process of immobilization was registered (-12%)



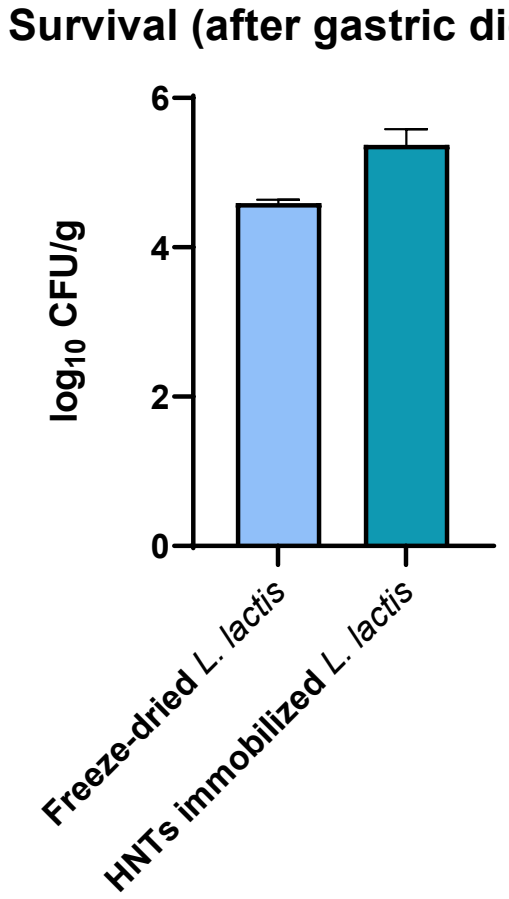


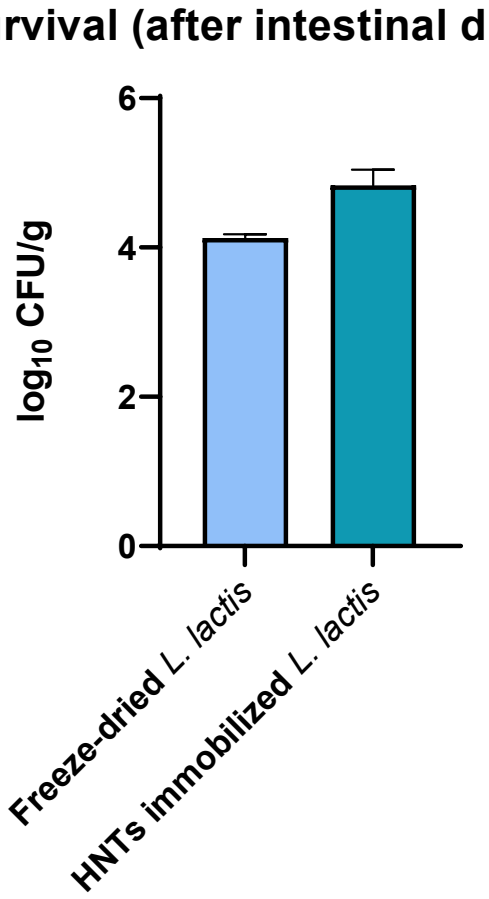



# HNTs immobilization – survival digestion

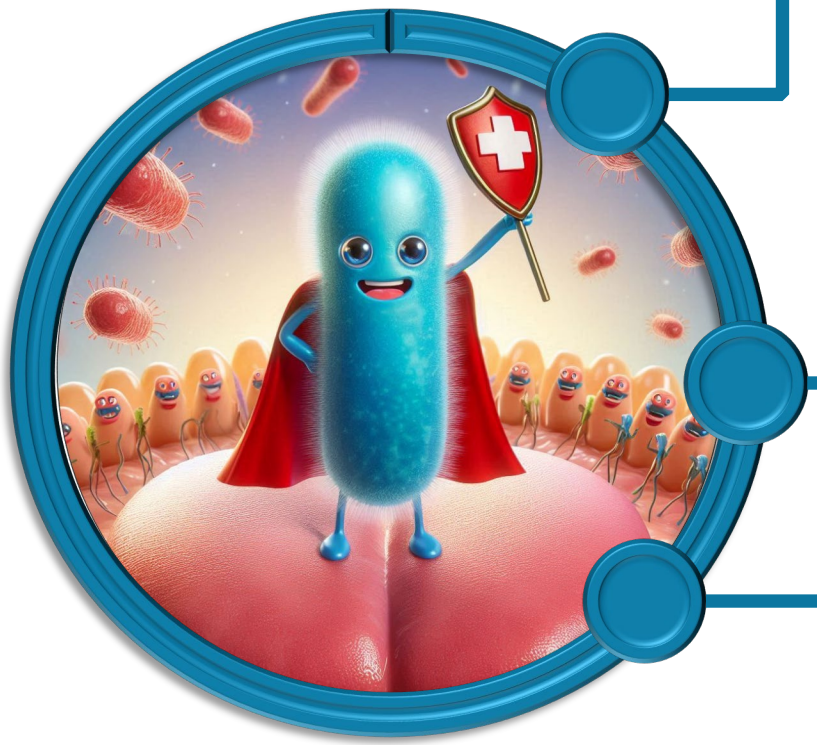
## Results

- *L. lactis* showed a significant drop in viability after gastric digestion (-46%)
- HNTs protected *L. lactis* during the simulated gastrointestinal transit **(+15% log10 CFU)**

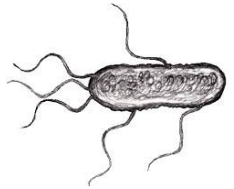


# Conclusions



*L. Lactis* strains showed successful expression for all *E. coli* selected antigens



Halloysite immobilization allowed to increase the bacterial survival during the gastrointestinal transit

*L. lactis* combined with nanomaterials are a promising strategy for multiepitope vaccination against major *E. coli* pathotypes in swine

reduce
replace
rethink

# Thanks

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DIPARTIMENTO DI MEDICINA VETERINARIA
E SCIENZE ANIMALI

The innovation of this study is under patent n°
102021000006461 (18/03/2021, Italy)