

# New findings on mode of action of *Bacillus toyonensis*: disruption of Quorum sensing 35\_6\_Heuer

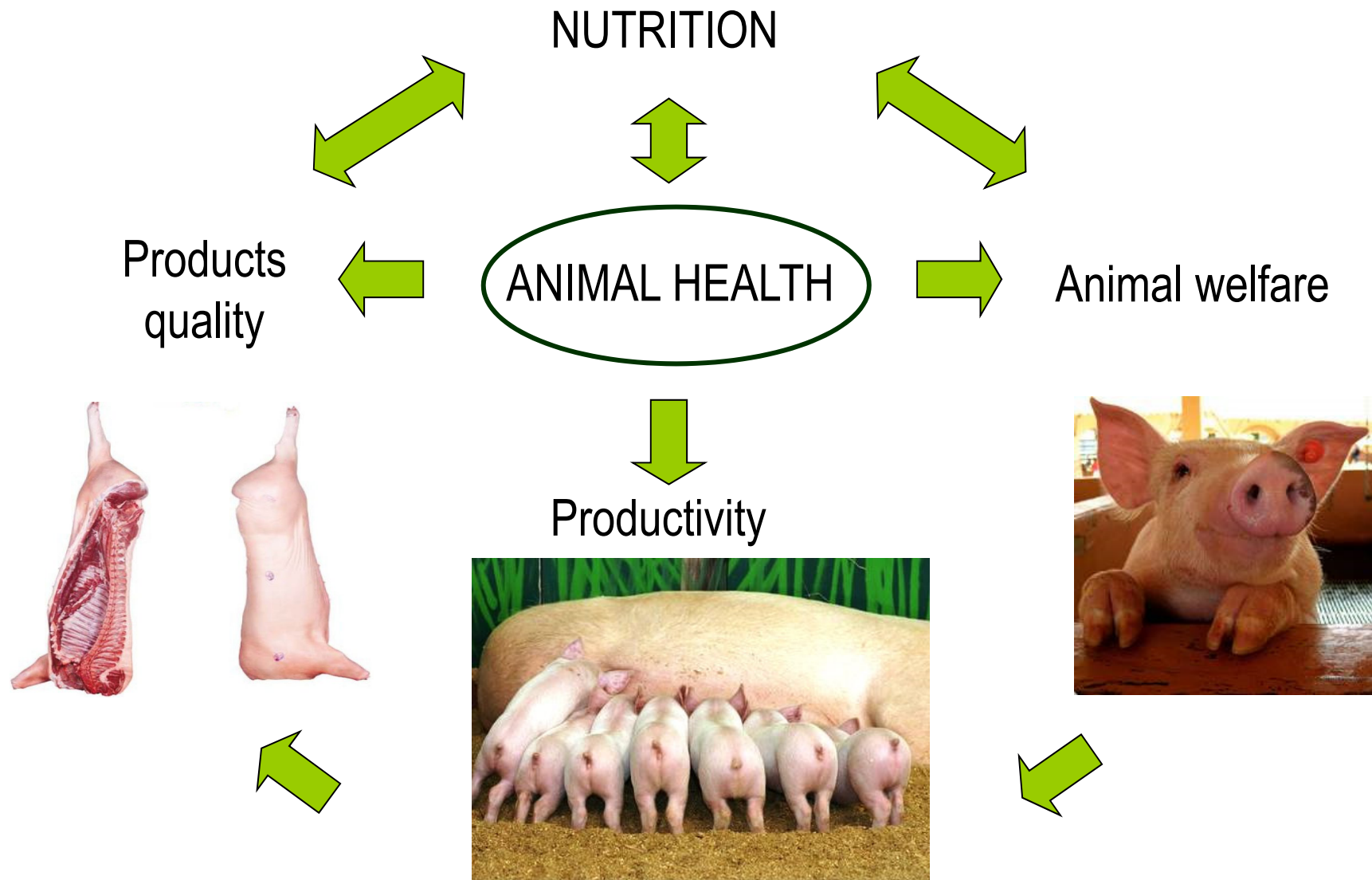
**G. González-Ortiz<sup>1</sup>, D. Solà-Oriol<sup>1</sup>, M. Castillo<sup>2</sup>, G. Jiménez<sup>2</sup>, S.M. Martín-Orúe<sup>1</sup>**

<sup>1</sup>*Departament de Ciència Animal i dels Aliments.*

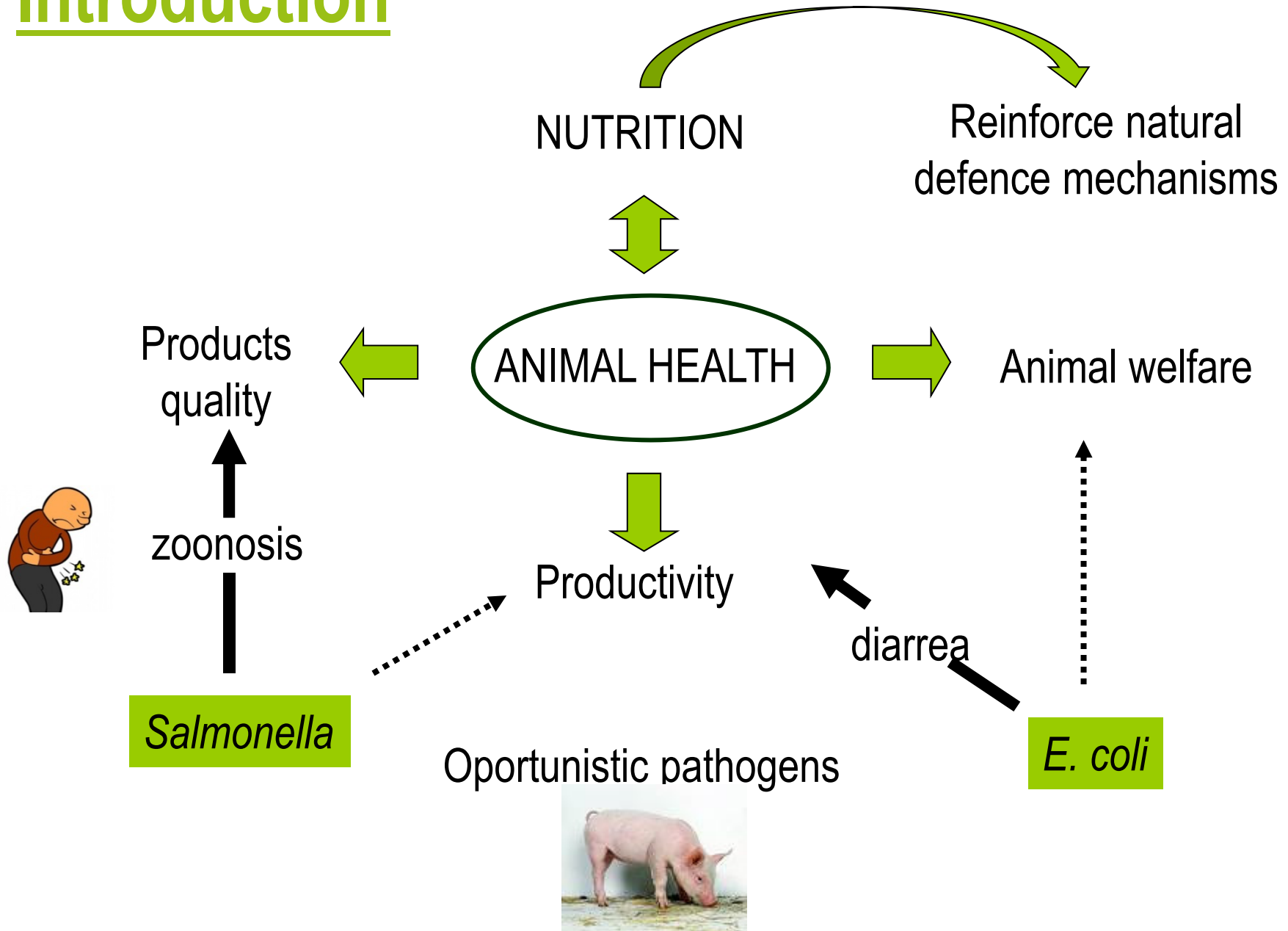
<sup>2</sup>*Rubinum S.A.*



# Introduction



# Introduction



# Introduction

*E. coli/Salmonella*

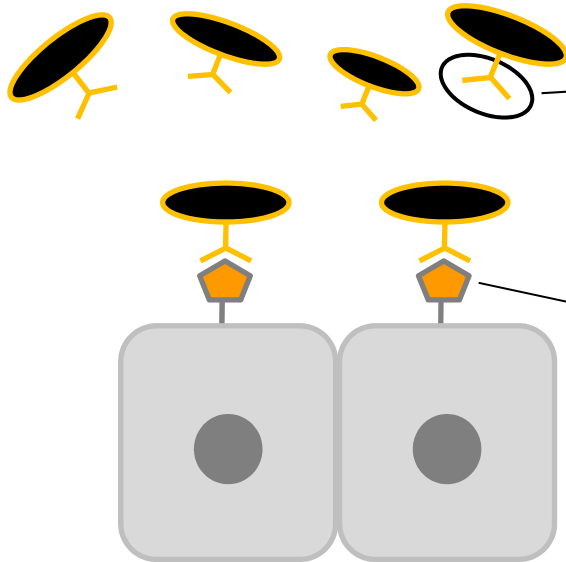
Environmental  
Opportunistic

Translocation

Adhesion

Colonization/Proliferation

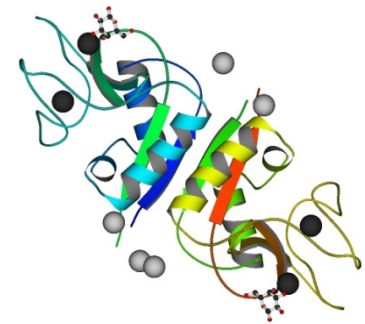
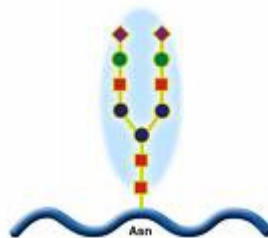
Toxins



Lectins

Fimbriae/adhesion factors

Glycoproteins



High diversity  
High specificity

# Introduction



Reinforce natural defence mechanisms

Symbiotics

Prebiotics

Probiotics

*Bacillus toyonensis*

# Introduction

## ***Bacillus toyonensis***

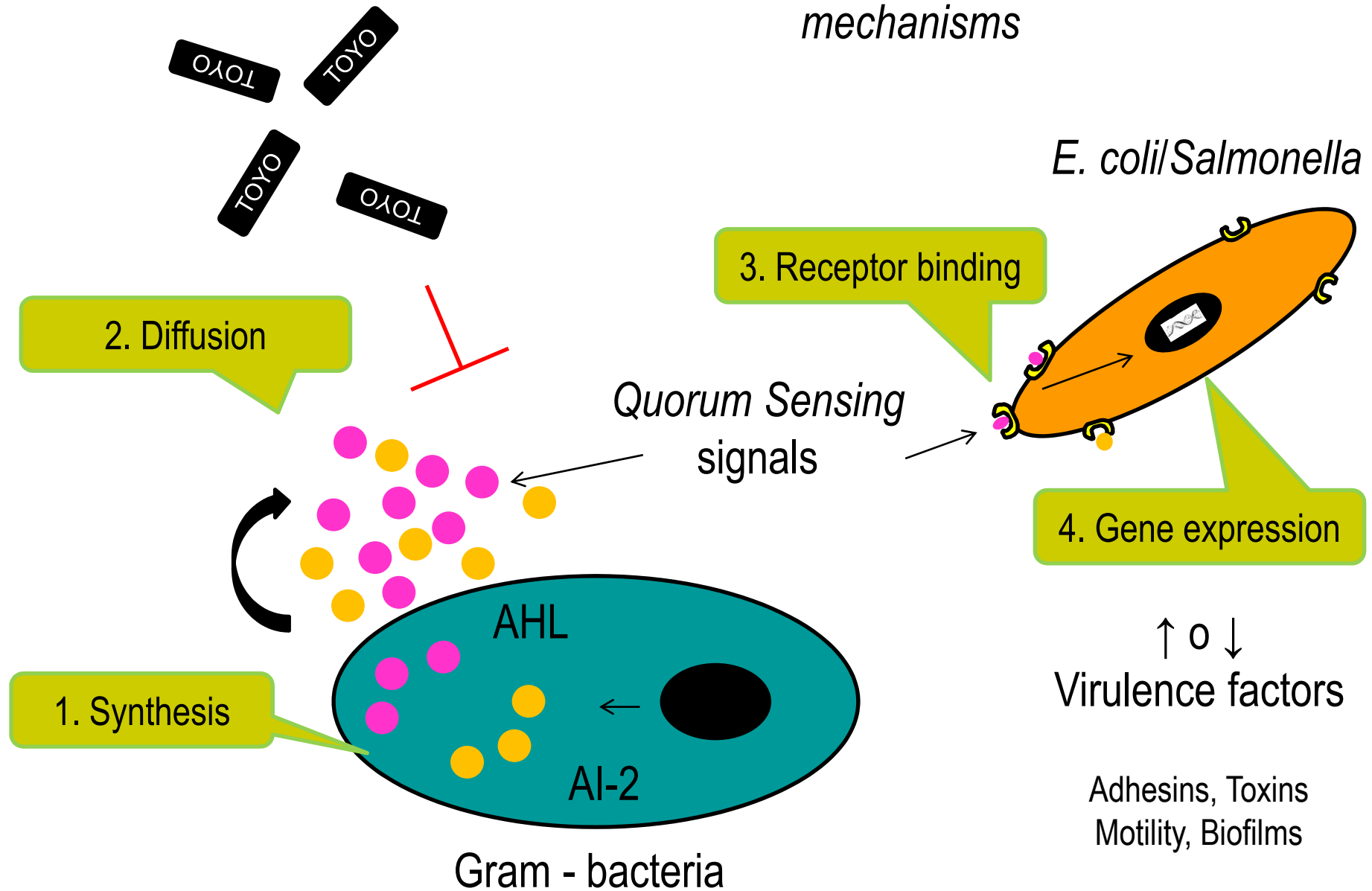
- ✓ Isolated from Japan soil
- ✓ Facultative anaerobic
- ✓ Spores
- ✓ No toxins producer (Jiménez et al., 2013)
- ✓ Starch, glucose, gelatin and propionate as nutrient sources
- ✓ Optimal growth temperature: 25-40°C
- ✓ Wide pH range
- ✓ ↑ resistance to critical T° and pH



***Enterobacteriaceae* spp. y *Enterococcus* spp.**

# Introduction

Interference of *B. toyonensis* on Quorum Sensing mechanisms



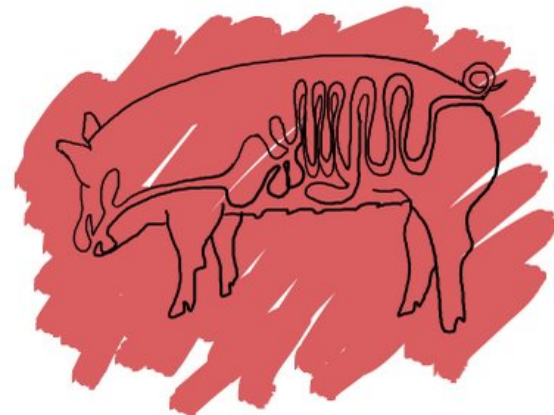
## Objective

The objective of this study was to evaluate the capacity of *Bacillus toyonensis* to disrupt the bacterial autoinduction mechanisms modifying *E. coli* invasiveness to porcine intestinal epithelial cells (IPEC-J2)

**Pure cultures**



**Natural digesta**





# Material and methods

## Strains



### *Escherichia coli* K88

(FV 12048)

(O149:K91:H10 [K-88]/ LT -/STb)

*E. coli* Reference Lab (Lugo, Spain)

**UAB**

Universitat Autònoma de Barcelona

### Non fimbriated (NF)

### *Escherichia coli*

(F4 -, F6 -, F18 -, LT1 -, ST1 -, ST2 +, Stx2e -)

Departamento de Sanidad y Anatomía Animal

**UAB**

Universitat Autònoma de Barcelona

### *Bacillus toyonensis*

Departamento de Sanidad y Anatomía Animal



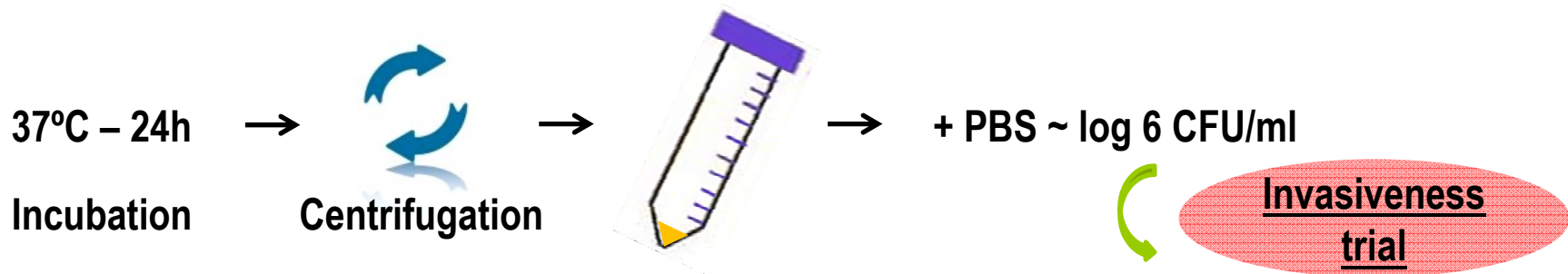
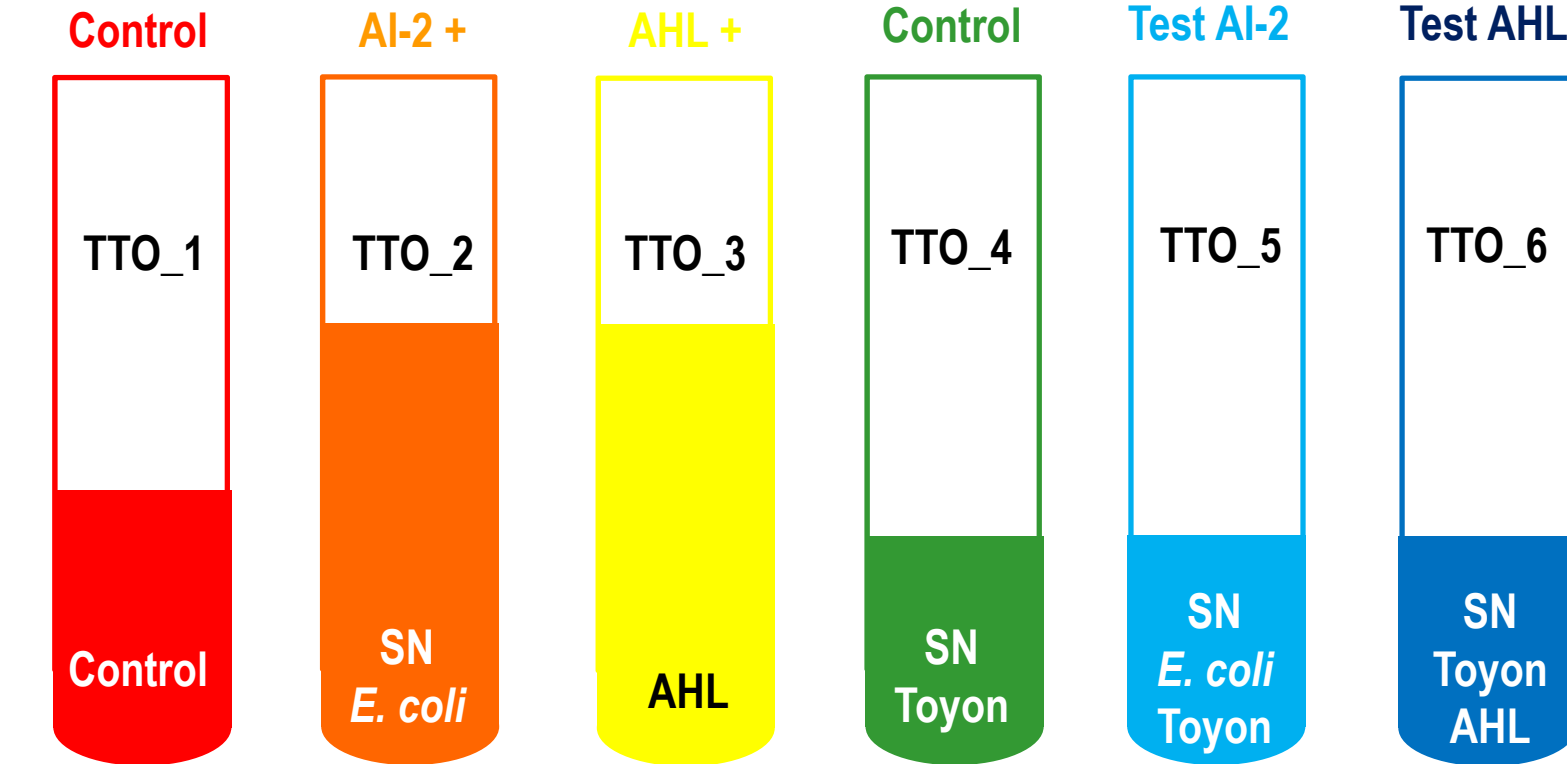
LURIA BROTH

37°C

3 PASSAGES/24h

# Material and methods

## Pure cultures



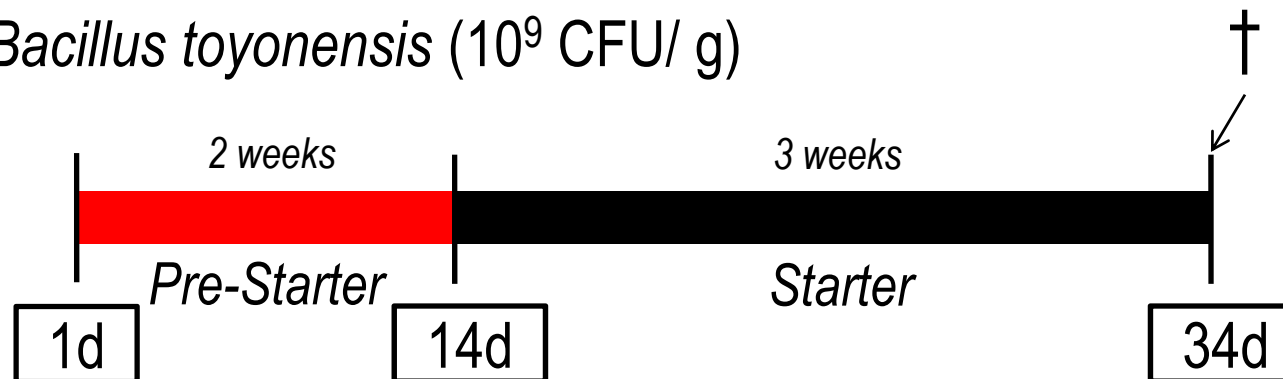
# Material and methods

## Animals

- ✓ 32 healthy weaned piglets
- ✓ 2 experimental groups
  - ✓ Control group (CTR)
  - ✓ Treatment group (TOY)



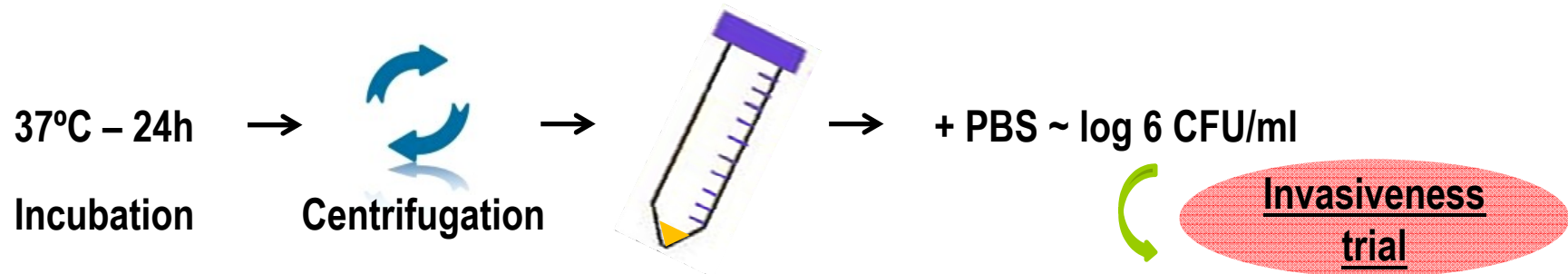
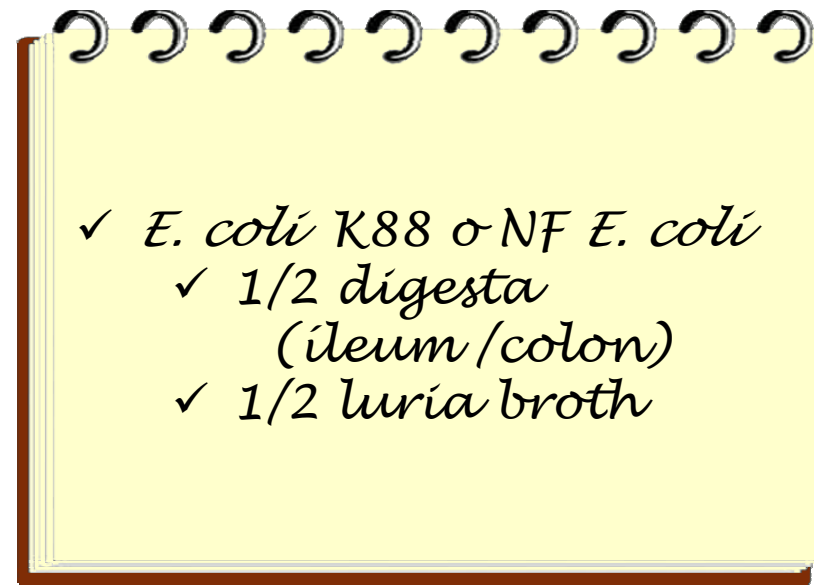
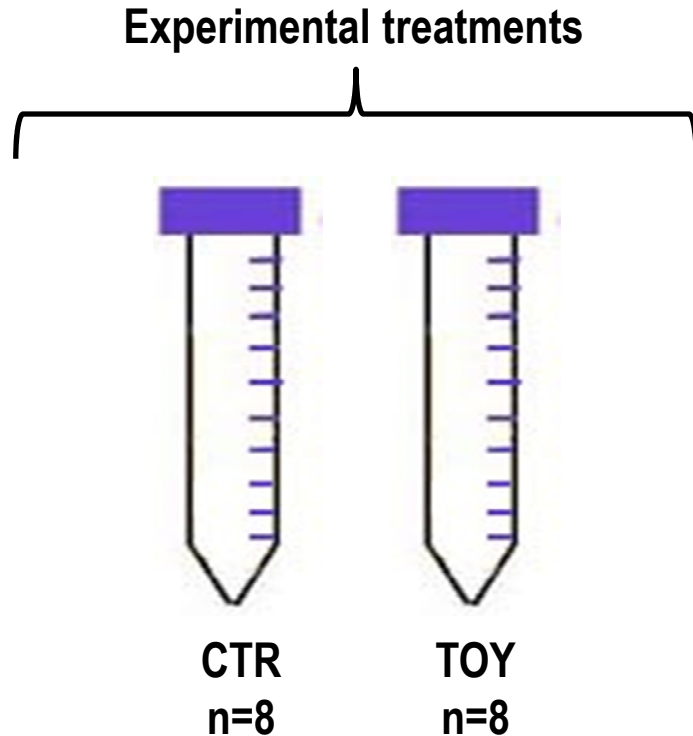
- ✓ *Bacillus toyonensis* ( $10^9$  CFU/ g)



- ✓ Ileal and colonic digesta
  - ✓ Pool 2 animals x pen
  - ✓ 2x Centrifugation (45,000 g) – filtration 0,2  $\mu$ m – supernatant (SN)

# Material and methods

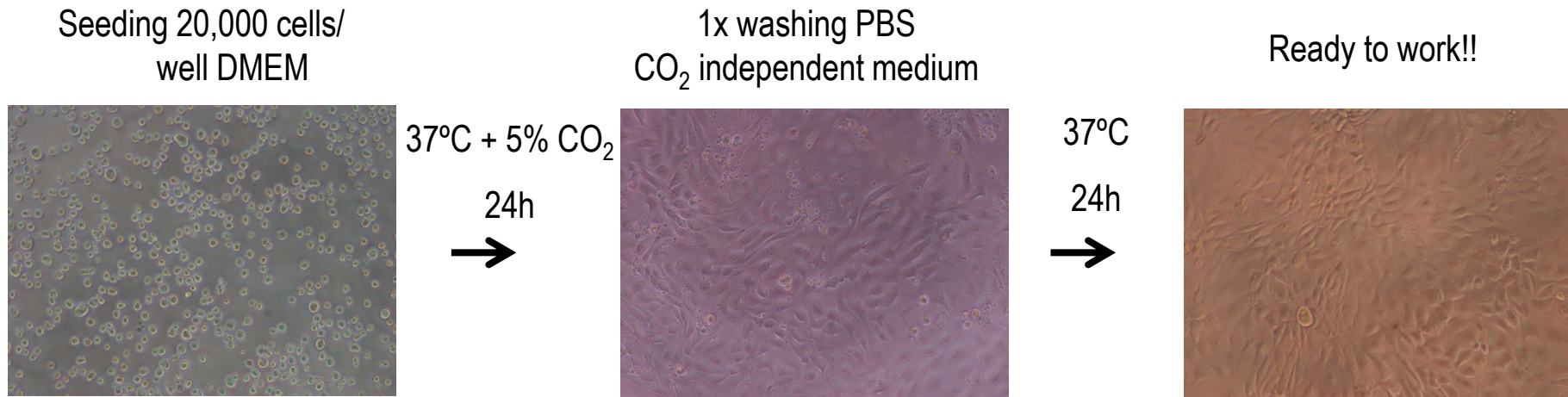
## Incubations with natural digesta



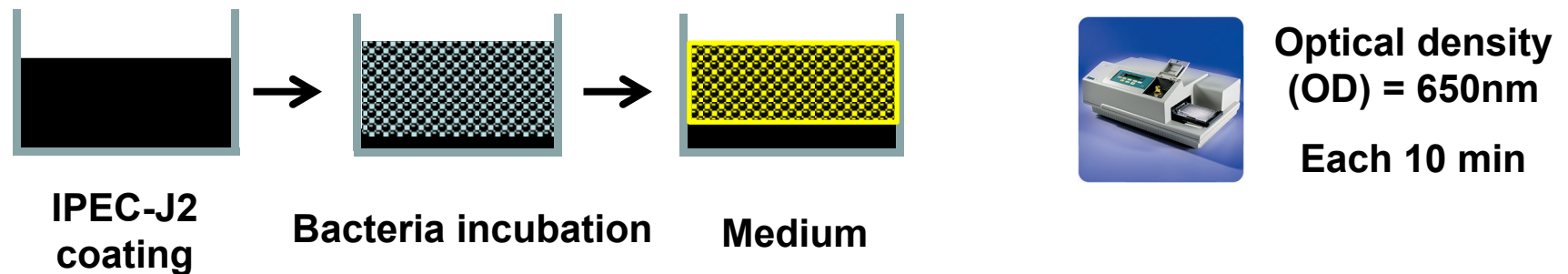
# Material and methods

## Invasiveness to intestinal porcine epithelial cells (IPEC-J2)

### - Monolayer preparation

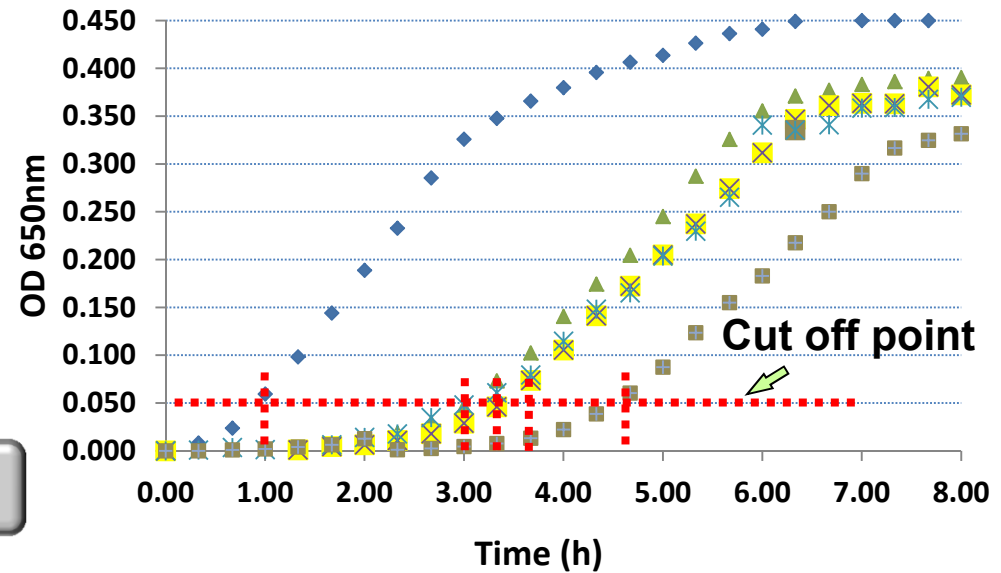
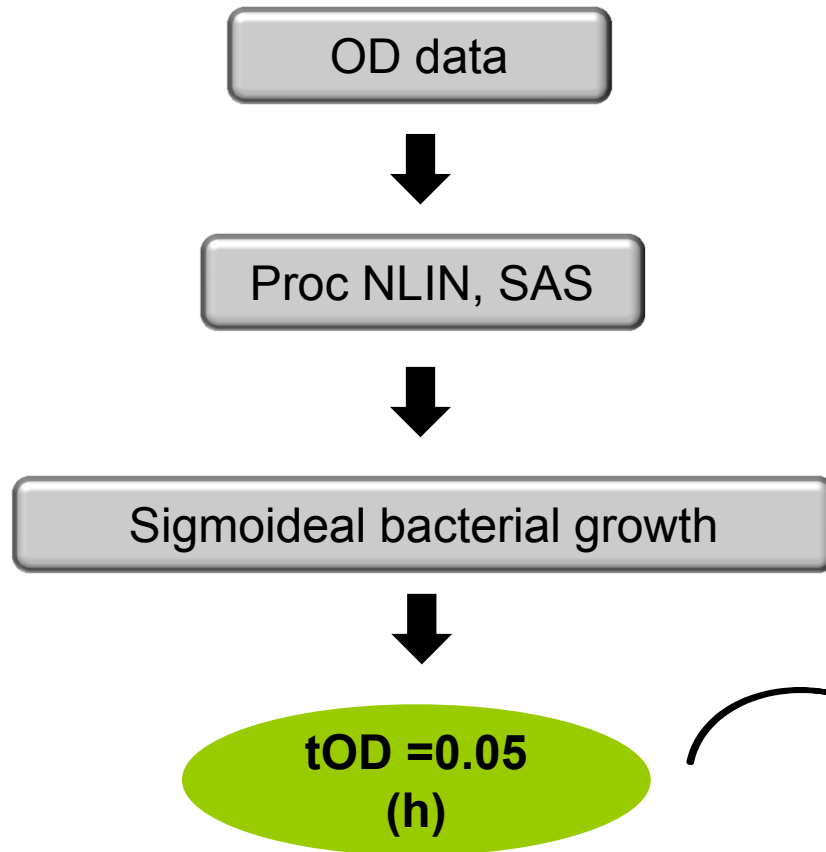


### - Invasiveness trial



# Material and methods

## Statistical analysis

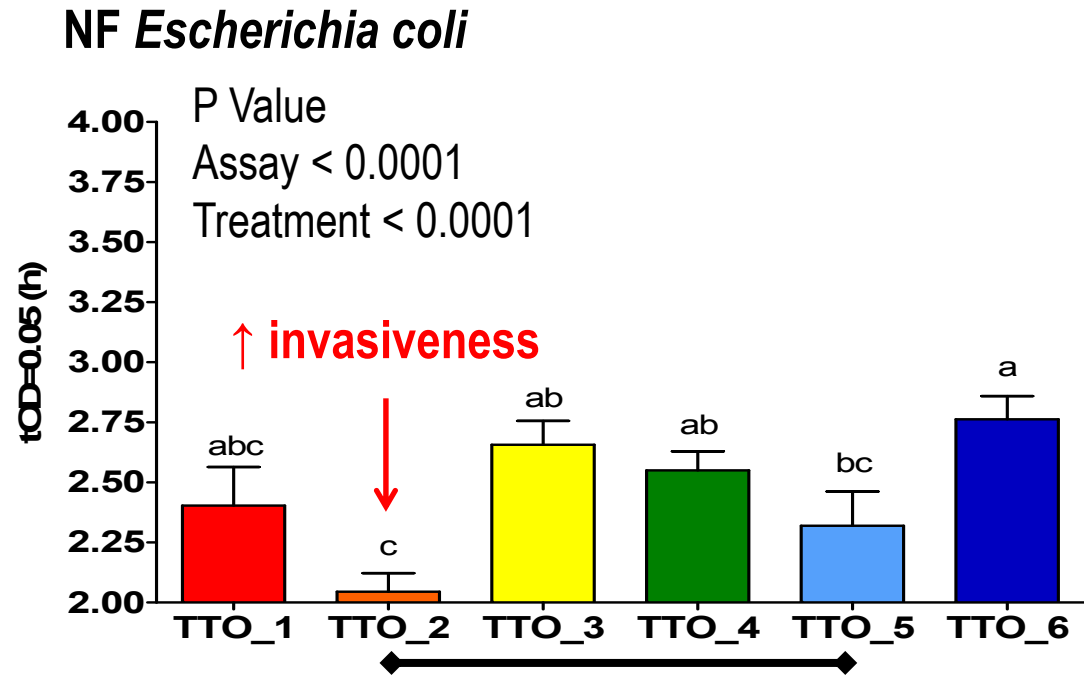
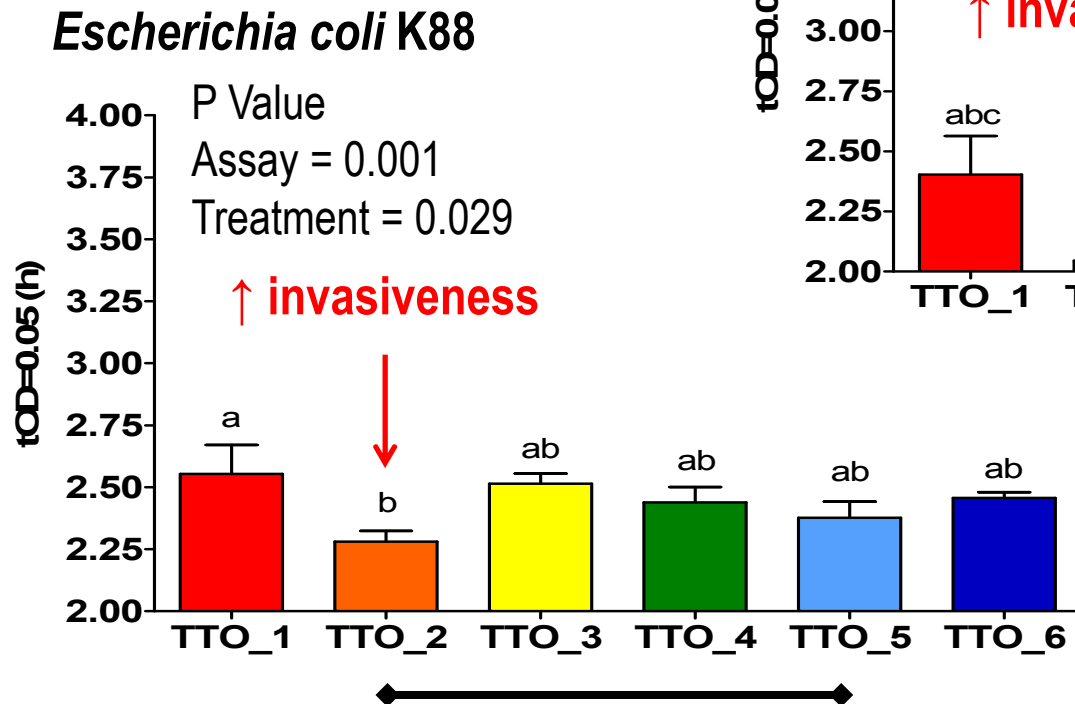


↑ tOD → ↓ Invasiveness

ANOVA, PROC GLM&MIXED,  
SAS ( $P \leq 0.05$ )

# Results

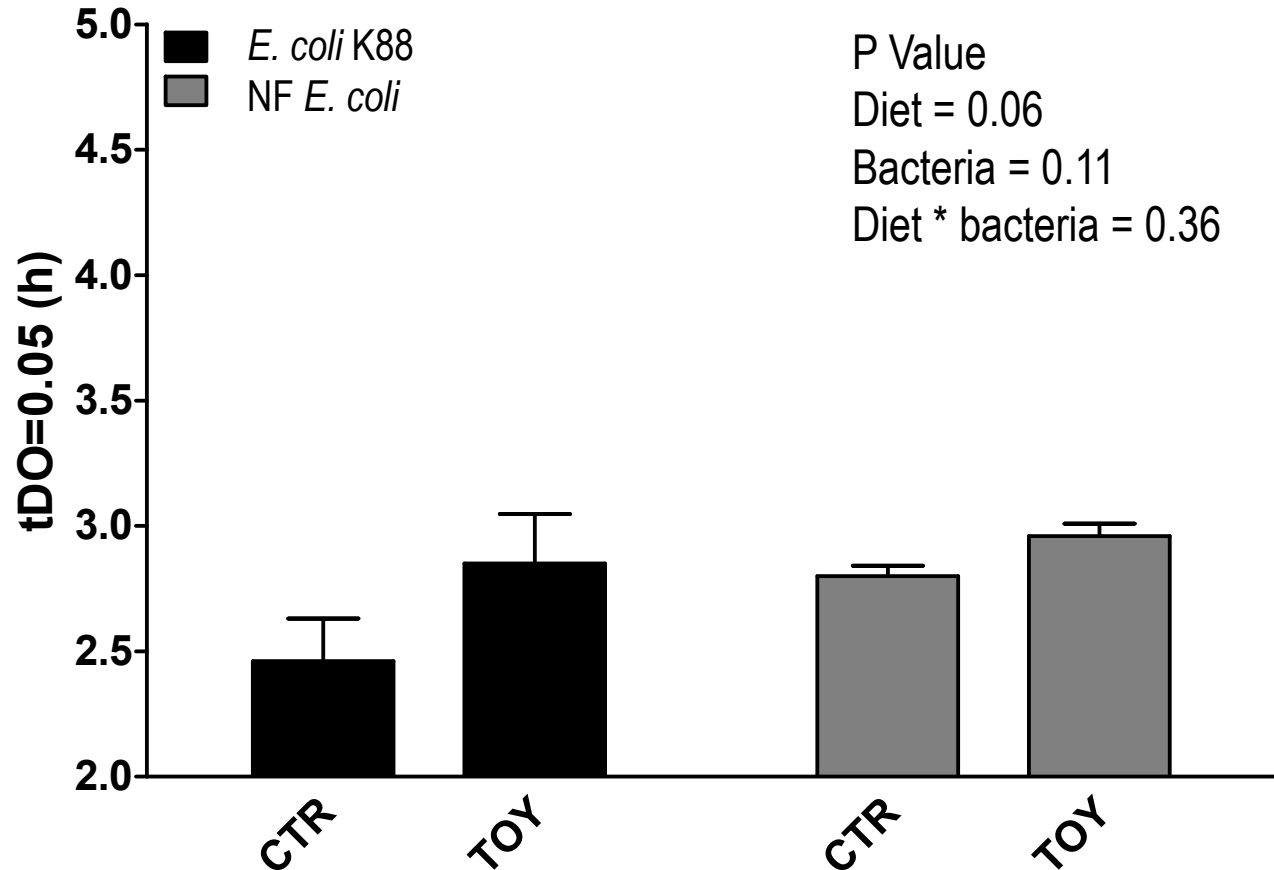
## Invasiveness of *E. coli* using pure cultures



↑ **AI-2 signals**

# Results

## Invasiveness of *E. coli* with ileal digesta

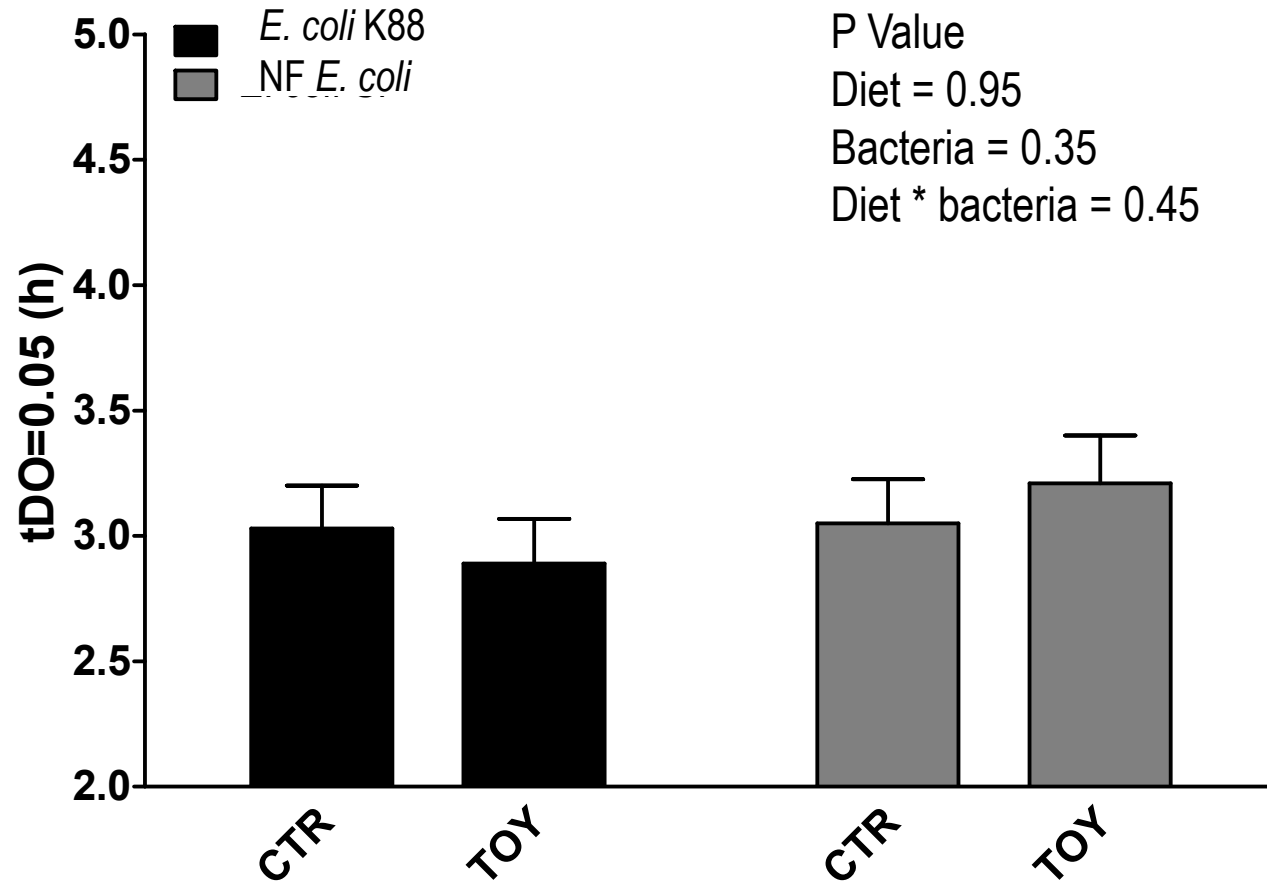


Ileal supernatant from animals supplemented with *Bacillus toyonensis* decreased the invasiveness of *E. coli*



# Results

## Invasiveness of *E. coli* with colon digesta



**Colon supernatant from animals supplemented with *Bacillus toyonensis* did not interfere in the invasiveness of *E. coli***

# Conclusions

1

The invasiveness response of *E. coli* in the current experimental conditions may be influenced by autoinducers type 2; however, *Bacillus toyonensis* did not decrease statistically its invasiveness.

2

The ileal supernatant from animals supplemented with the probiotic reduced the invasiveness of *E. coli* to IPEC-J2 cells.

3

The obtained results suggest that *Bacillus toyonensis*, may reduce the invasiveness of *E. coli* in the ileum compartment acting on the cell-to-cell communication mechanisms, by degrading or inhibiting the AI-2 signals.