New findings on mode of action of *Bacillus toyonensis*: disruption of Quorum sensing

G. González-Ortiz¹, D. Solà-Oriol¹, M. Castillo², G. Jiménez², S.M. Martín-Orúe⁴

¹Departament de Ciència Animal i dels Aliments.
²Rubinum S.A.
Introduction

ANIMAL HEALTH

NUTRITION

Animal welfare

Products quality

Productivity
Introduction

ANIMAL HEALTH

NUTRITION

Reinforce natural
defence mechanisms

Animal welfare

Productivity

Salmonella

Ouortunistic pathogens

E. coli

diarrea

zoonosis

Products quality

Reinforce natural
defence mechanisms
Introduction

**E. coli/Salmonella**

- **Environmental Oportunistic**
  - 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: 25-40ºC
- ✓ Wide pH range
- ✓ ↑ resistance to critical Tº and pH

Enterobacteriaceae spp. y Enterococcus spp.

Ortwin Simon, Anke Jadamus and Wilfred Vahjen, 2002
Interference of *B. toyonensis* on Quorum Sensing mechanisms

E. coli/Salmonella

Virulence factors
Adhesins, Toxins
Motility, Biofilms

1. Synthesis

2. Diffusion

3. Receptor binding

4. Gene expression

Gram - bacteria

Quorum Sensing signals

↑ o ↓

Introduction
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)
Material and methods

**Strains**

**Escherichia coli K88**  
(FV 12048)  
(O149:K91:H10 [K-88]/ LT −/STb)  
E. coli Reference Lab (Lugo, Spain)

**Non fimbriated (NF) Escherichia coli**  
(F4 -, F6 -, F18 -, LT1 -, ST1 -, ST2 +, Stx2e -)  
Departamento de Sanidad y Anatomía Animal

**Bacillus toyonensis**

Departamento de Sanidad y Anatomía Animal
Material and methods

<table>
<thead>
<tr>
<th>Pure cultures</th>
<th>Control</th>
<th>Al-2 +</th>
<th>AHL +</th>
<th>Control</th>
<th>Test Al-2</th>
<th>Test AHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTO_1</td>
<td>TTO_2</td>
<td>TTO_3</td>
<td>TTO_4</td>
<td>TTO_5</td>
<td>TTO_6</td>
<td></td>
</tr>
<tr>
<td>SN E. coli</td>
<td>AHL</td>
<td>SN Toyon</td>
<td>SN E. coli Toyon</td>
<td>SN Toyon AHL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

37°C – 24h → Incubation → Centrifugation → + PBS ~ log 6 CFU/ml

Invasiveness trial
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 µm – supernatant (SN)
Material and methods

Incubations with natural digesta

Experimental treatments

CTR  n=8
TOY  n=8

37°C – 24h
Incubation

Centrifugation

+ PBS ~ log 6 CFU/ml

Invasiveness trial

✓ E. coli K88 o NF E. coli
✓ 1/2 digesta (ileum/colon)
✓ 1/2 luria broth
Material and methods

Invasiveness to intestinal porcine epithelial cells (IPEC-J2)

- Monolayer preparation

Seeding 20,000 cells/well DMEM

37°C + 5% CO₂
24h

1x washing PBS
CO₂ independent medium

37°C
24h

Ready to work!!

- Invasiveness trial

IPEC-J2 coating → Bacteria incubation → Medium

Optical density (OD) = 650nm
Each 10 min
Material and methods

Statistical analysis

OD data → Proc NLIN, SAS → Sigmoidal bacterial growth → tOD = 0.05 (h)

↑ tOD → ↓ Invasiveness

ANOVA, PROC GLM&MIXED, SAS (P ≤ 0.05)
Results

Invasiveness of *E. coli* using pure cultures

*Escherichia coli* K88

P Value
Assay = 0.001
Treatment = 0.029

↑ invasiveness

NF *Escherichia coli*

P Value
Assay < 0.0001
Treatment < 0.0001

↑ invasiveness

↑ 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.