Supplementation of *Bacillus subtilis* modified fecal microbiota of weaning pigs experimentally infected with pathogenic *E. coli*

**Abstract**

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Post-weaning Diarrhea

- Can be induced by
  - Stress
  - Sudden change of diet
  - Increased exposure to enterotoxigenic pathogens
    - F18 *E. coli*
- Often leads to weight loss and possibly death
- Economical loss
Probiotics

• Live microorganisms confer a health benefit on the host when administered in adequate amounts (UNFAO/WHO 2001)

• Potential alternatives to antibiotics

• *Bacillus subtilis*
  • Gram positive, aerobic bacteria
  • Stable in ambient temperature
  • Spores are resistance to harsh environment
Previous Studies (Kim et al., 2019)

- Supplementation of *B. subtilis* to weanling pigs challenged with F18 *E. coli*:
  - Improved growth rate
  - Reduced leaky gut
  - Enhanced gut barrier functions
Gut Microbiota

- **Gut microbiota** = collection of microbe living inside the gut
- **Gut microbiome** = totality of microbes, their genetic elements, and environmental interactions in the gut
- Microbes in the intestines play a role in keeping the host healthy
  - **Nutrition**: production of VFAs
  - **Physiology**: maintenance of gut structure and barrier function
  - **Immune system**: sIgA regulation
Gut Microbiota: Objective

**Feed additive:** *B. subtilis*

**Weaning stress**

**F18 E. coli**

**Diet**

**Stress**

**Disease**

**Time**

**Fecal microbiota altered?**
48 weanling pigs
- 6.73 ± 0.77 kg BW, 21 d old

RCBD (Blocks: BW x Sex)

4 treatments (12 pigs/treatment)

1. **Negative control** ← *E. coli* non-challenged
2. **Positive control**
3. **Single dose Bacillus subtilis**
   - $1.28 \times 10^9$ CFU/kg
4. **Double dose Bacillus subtilis**
   - $2.56 \times 10^9$ CFU/kg

*E. coli* challenged
• Oral inoculation of pathogenic F18 *E. coli* challenge with $10^{10}$ CFU/dose were given to pigs 3 times

• Fecal samples were collected on d 5 and d 11 post inoculation
16S rRNA Sequencing: Measurements

• Alpha diversity
  • **Observed**: measures richness
  • **Shannon**: measures richness and evenness

• Beta diversity
  • **Bray Curtis Dissimilarity**
    • Principal Coordinates Analysis (PCoA) plot

• Relative abundance
  • **Phylum**
  • **Family**: *Firmicutes & Proteobacteria*
16S rRNA Sequencing: Library Preparation

- DNA extraction
- PCR
  - Target V4 hypervariable region
  - Attached 8 bp barcoded Illumina adapter
- Illumina sequencing
  - MiSeq
  - Paired-end
  - 250 base reads
16S rRNA Sequencing: Bioinformatics

- **Fastq + Trimmomatic**
  - Raw reads evaluated for quality control and trimmed

- **QIIME2 (2018.6)**
  - `dada2`: quality control and feature table construction
  - `Greengenes`: database used to generate taxonomy file

- **R program**
  - `Phyloseq & ggplot2`: calculate and visualize beta diversity and relative abundance; calculate alpha diversity indices

- **SAS**
  - ANOVA using PROC MIXED of SAS for alpha diversity and relative abundance; significant difference at $P < 0.05$
Alpha Diversity: Shannon

- d 5
  - Negative control (b)
  - Positive control (a)
  - Single dose (ab)
  - Double dose

- d 11
  - Negative control (b)
  - Positive control (ab)
  - Single dose (a)
  - Double dose

Legend:
- Negative control
- Positive control
- Single dose
- Double dose
Beta Diversity: PCoA
Relative Abundance: Phylum

• **d 5:**
  • Lowest relative abundance of *Proteobacteria* in Positive control
  • Lowest relative abundance of *Firmicutes* in Single & Double doses
Relative Abundance: **Firmicutes Family**

- **d 5**
  - Highest relative abundance of *Lachnospiraceae* in Double dose
  - Low relative abundance of *Lactobacillaceae* Single & Double doses
Relative Abundance: 
Proteobacteria Family

- d 5: Lowest relative abundance of *Enterobacteriaceae* in Positive control
- d 11: Highest relative abundance of *Desulfovibrionaceae* in Double dose
Conclusions

• Supplementation of *Bacillus subtilis* could modify gut microbiota of weaned pigs challenged with F18 *E. coli*

• Further analysis
  • Quantify *Bacillus subtilis* and F18 *E. coli* in fecal microbiota
  • Evaluate metagenomics of fecal microbiota
Thank you!