Effects of *Bacillus subtilis* on colon digesta metabolomic profiles in weanling pigs experimentally infected with a pathogenic *E. coli*

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Abstract

Our previous studies have shown that supplementation of *Bacillus subtilis* enhanced growth rate, improved gut barrier function, and modified colon microbiome of weaned pigs infected with pathogenic *Escherichia coli* (*E. coli*). The objective of this experiment was to investigate the effects of *Bacillus subtilis* on colon digesta metabolomic profiles of weaned pigs experimentally infected with F18 *E. coli*. Forty-eight pigs (6.73 ± 0.77 kg BW) were individually housed in disease containment rooms and randomly allotted to one of the four treatments (12 pigs/treatment). Four treatments included negative control (NC), positive control (PC), low-dose (1.28 × 10⁹ CFU *Bacillus subtilis*/kg feed), and high-dose (2.56 × 10⁹ CFU *Bacillus subtilis*/kg feed). The experiment lasted 18 d [7 d before and 11 d after first inoculation (d 0)]. The F18 *E. coli* inoculum was orally provided to all pigs with the dose of 10¹⁰ cfu/3 mL for 3 consecutive days, except NC. Twenty-four pigs (6 pigs/treatment) were euthanized on d 5 post-inoculation (PI) and the remained pigs were euthanized on d 11 PI to collect colon digesta for the analysis of metabolomic profiles by gas chromatography time of flight-mass spectrometer (GCTOF-MS). All processed data were statistically analyzed and evaluated by online MetaboAnalyst tool. No significant differences were observed in the metabolites between NC and PC on d 5 and 11 PI. Compared with PC, low- and high-dose *Bacillus subtilis* reduced (Fold change > 1.5; FDR < 0.20) four metabolites (proline, 2-hydroxyglutaric acid, lysine, and glutamic acid) and two metabolites (ribose, and D-xylulose) in colon digesta on d 5 PI, respectively. These metabolites were related to aminoacyl-tRNA-biosynthesis, arginine and proline metabolism, and lysine degradation. In conclusion, supplementation of *Bacillus subtilis* modified the levels of microbial metabolites associated with amino acid metabolism in colon digesta of pigs.
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**Introduction**

- Direct fed microbials are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001).

- Supplementation of *Bacillus subtilis* (DSM 25841) enhanced the growth rate of F18 *E. coli* infected pigs by enhancing gut integrity and decreasing gut permeability (Kim et al., 2019).

- Supplementation of *Bacillus subtilis* reduced the abundance of fecal *Firmicutes*, and within this phylum, the abundance of *Lachnospiraceae* was increased, but the abundance of *Lactobacillaceae* and *streptococcaeae* was decreased (Jinno et al., 2019).

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**Preliminary data**

- **Average daily gain**
- **Transcellular permeability**
- **Paracellular permeability**
- **Jejunal mucosa gene expression**
- **Ileal mucosa gene expression**

(Kim et al., 2019)
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**Objective & Methods**

Investigate the effect of *Bacillus subtilis* on colon digesta metabolomic profiles of weaned pigs experimentally infected with F18 *E. coli*.

- Colon digesta samples were collected on d 5 and 11 PI.
- Colon digesta metabolomics were analyzed by gas chromatography time of flight-mass spectrometer (GCTOF-MS).
- All processed data were analyzed by MetaboAnalyst (http://www.metaboanalyst.ca) (Chong et al., 2018).

- **E. coli** challenge
  - Statistical analysis
    - Fold change > 1.5
    - FDR (adjusted P-value) < 0.2
  - Enrichment & pathway analysis
    - *P* < 0.05

- Experimental design: RCBD (Blocks: BW x Sex)
- 48 weanling pigs (6.73 ± 0.77 kg, 21 d old)
- Treatments: 4 dietary treatments (12 pigs/treatment)

<table>
<thead>
<tr>
<th>Negative control (NC)</th>
<th>Positive control (PC)</th>
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<tbody>
<tr>
<td>CON + 1.28 × 10^9 CFU <em>Bacillus subtilis</em>/kg feed (LOW)</td>
<td></td>
</tr>
<tr>
<td>CON + 2.56 × 10^9 CFU <em>Bacillus subtilis</em>/kg feed (HIGH)</td>
<td></td>
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</tbody>
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*PI= post-inoculation*
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**Results**

**Metabolite sets enrichment, d 5 PI**

- Aminoacyl-tRNA Biosynthesis
- Arginine and Proline Metabolism
- Lysine Degradation
- Biotin Metabolism
- Pentose Phosphate Pathway

**Synchronized 3D plots:** Partial least squares – discriminant analysis (PLSDA), d 5 PI

**Heatmap and hierarchical clustering, d 5 PI**

- PC
- LOW
- HIGH

- 2-hydroxyglutaric acid
- Proline
- Glutamic acid
- Lysine
- Ribose
- Shikimic acid
- D-xylulose
- Pyruvic acid
**Most affected metabolic pathways**

- Supplementation of *Bacillus subtilis* modified
  - Aminoacyl-tRNA biosynthesis
  - Arginine and proline metabolism
  - Lysin degradation

Compared with positive control on d 5 PI.

- No differences were observed in colon digesta metabolites among dietary treatments on d 11 PI.

**Results**

- Supplementation of *Bacillus subtilis* modified the colon digesta metabolites and associated metabolic pathways of weaned pigs infected with F18 *E. coli* during the peak infection period.

- Modification of amino acid metabolic pathways may account for the higher growth rate and improved gut health of pigs fed *Bacillus subtilis*.

**Conclusions**


- World Health Organization (WHO). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. FAO/WHO.