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 (1.28×10^9 CFU *Bacillus subtilis*/kg feed), and high-dose (2.56×10^9 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 10^{10} 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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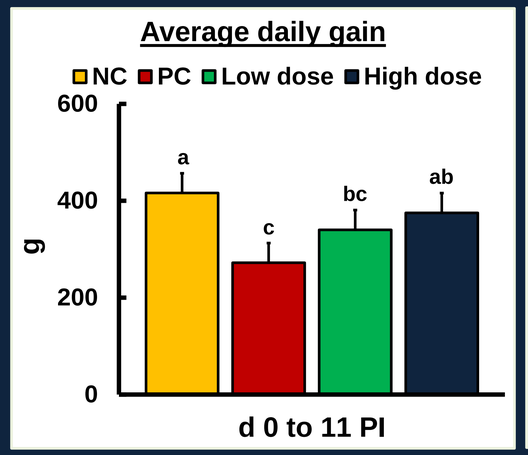
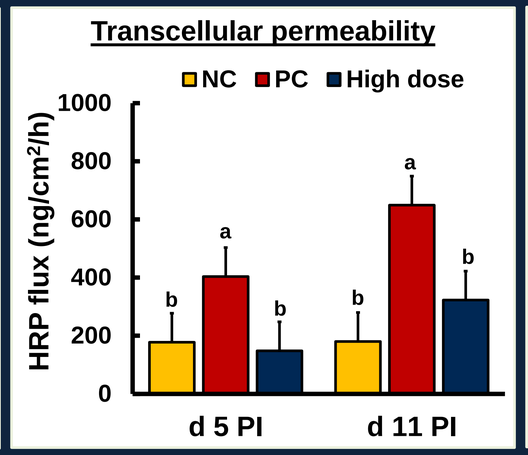
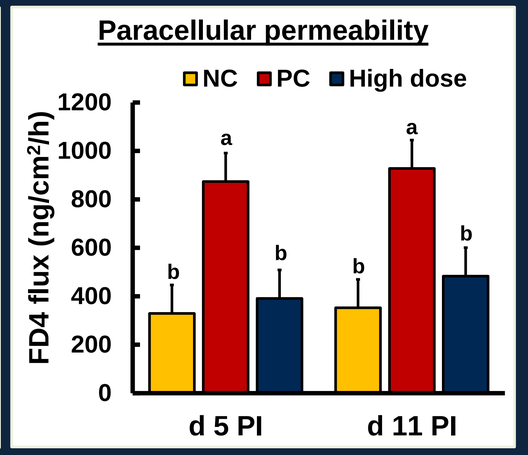
Introduction

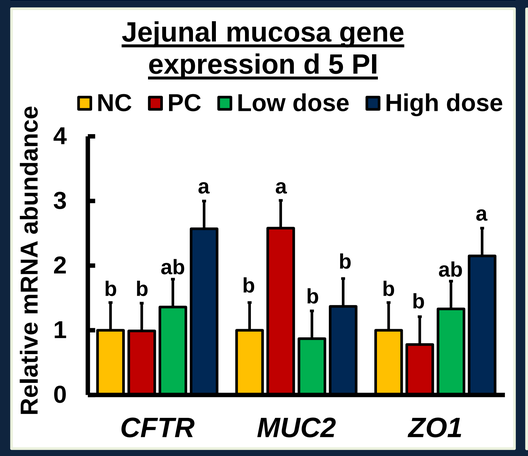
- Direct fed microbials are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001).
- Supplementation of *Bacillus subtilis* (DSM 25841) enhanced the growth rate of F18 *E. coli* infected pigs by enhancing gut integrity and decreasing gut 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 *streptococcaceae* was decreased (Jinno et al., 2019).

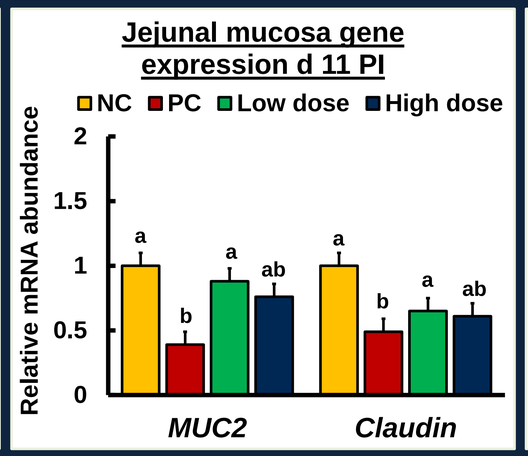
Preliminary data

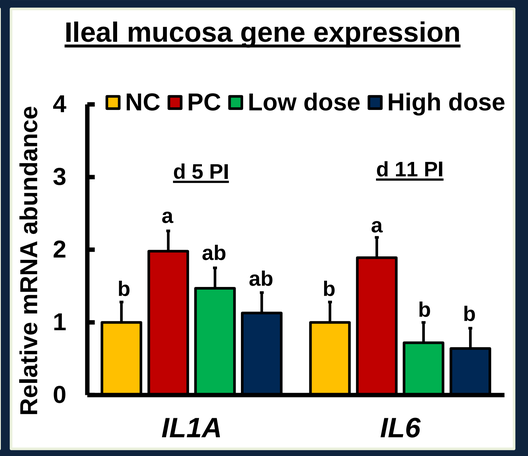












(Kim et al., 2019)

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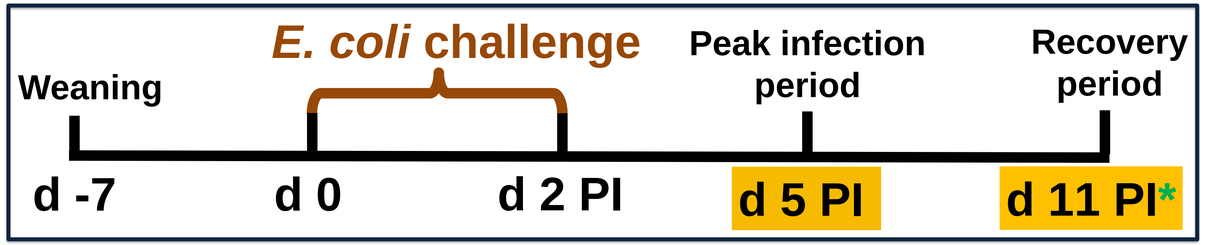
Objective & Methods

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



- 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)

Negative control (NC)	
Positive control (PC)	*E. coli* challenge
CON + 1.28 × 10^9 CFU *Bacillus subtilis*/kg feed (LOW)	*E. coli* challenge
CON + 2.56 × 10^9 CFU *Bacillus subtilis*/kg feed (HIGH)	*E. coli* challenge

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

*PI= post-inoculation

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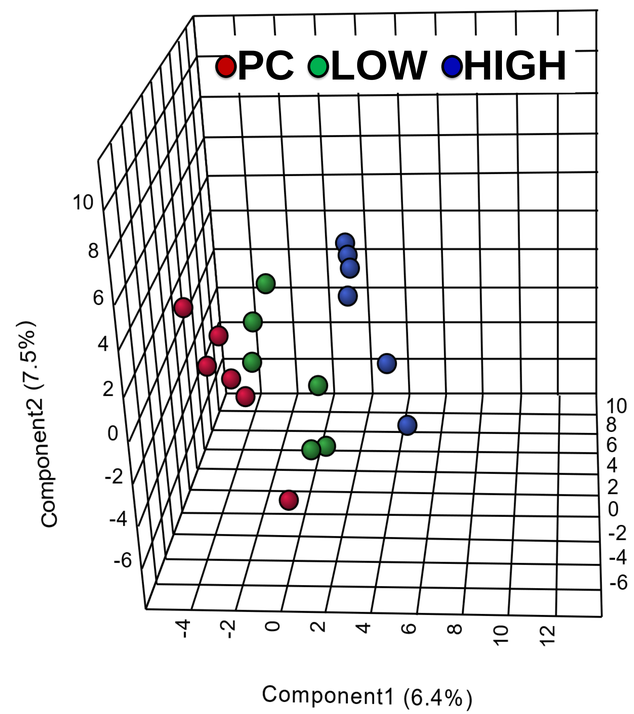
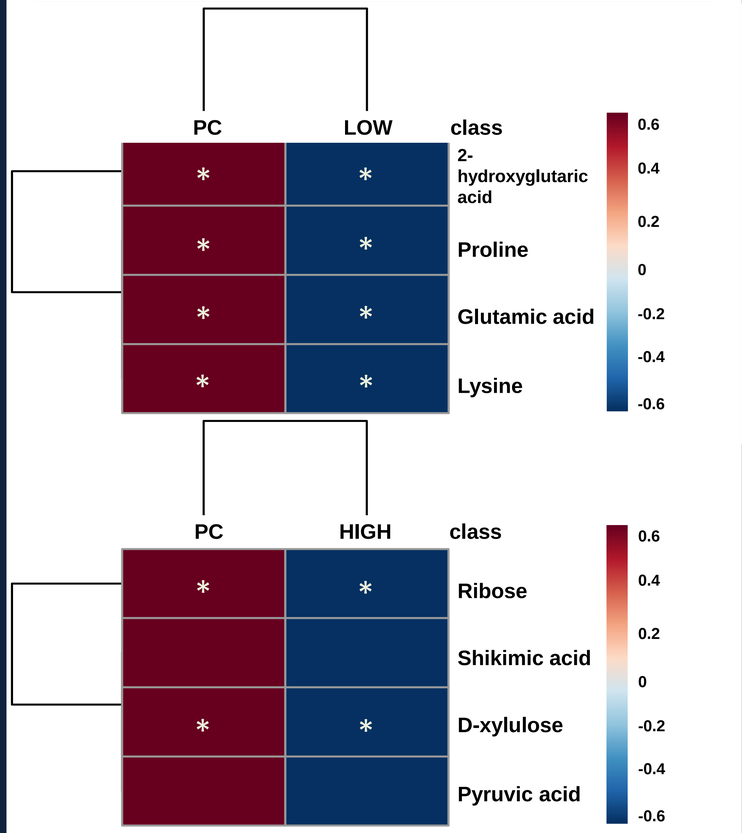
Results

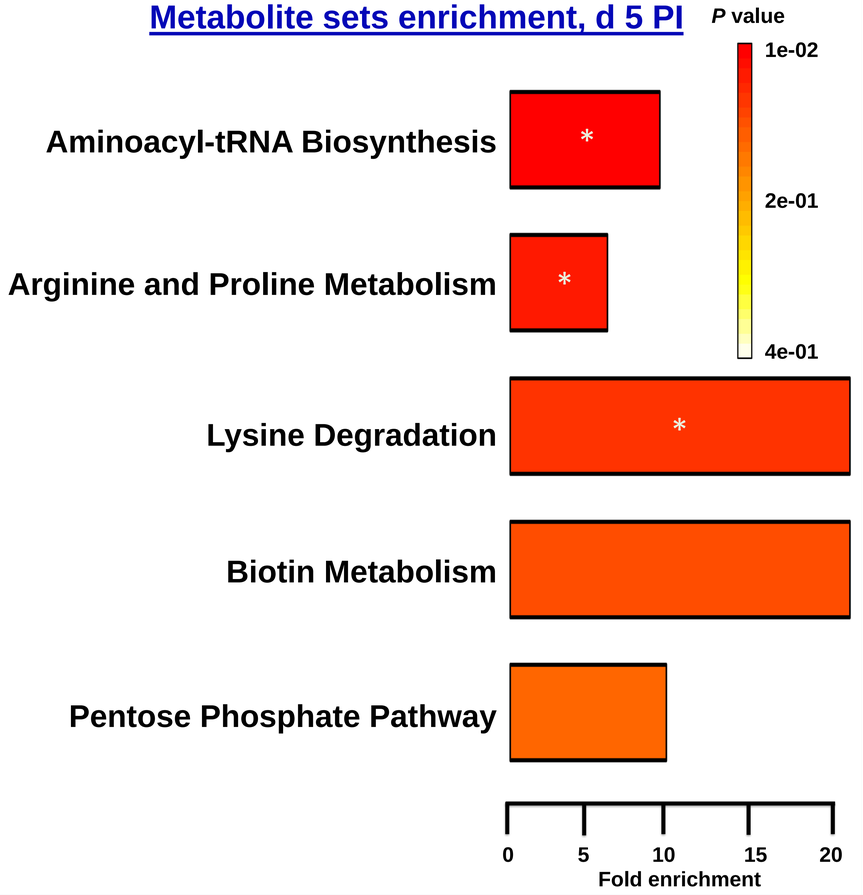
Synchronized 3D plots: Partial least squares – discriminant analysis (PLSDA), d 5 PI



Heatmap and hierarchical clustering, d 5 PI



Metabolite sets enrichment, d 5 PI



Abstract | Introduction | Objective & Methods | Results | Results & Conclusions

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Results

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.

Conclusions

- 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*.

References

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