Dietary supplementation of *Bacillus subtilis* modified microbiota metabolites in the intestine of weaned pigs

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Abstract

The objective of this experiment was to investigate the effects of dietary supplementation of a *Bacillus subtilis* probiotic on microbiota metabolites in the intestines of weaned pigs experimentally infected with an enterotoxigenic *Escherichia coli* (E. coli). Weaned pigs (n = 48, 6.17 ± 0.36 kg BW) were individually housed in disease containment rooms and randomly allotted to one of four dietary treatments: negative control (NC, control diet without *E. coli* challenge), positive control (PC, control diet with *E. coli* challenge), and supplementation of 50 mg/kg of carbadox or 500 mg/kg of a *Bacillus subtilis* probiotic. The experiment lasted 28 days with 7 days before and 21 days after the first *E. coli* inoculation. The F18 *E. coli* were given to pigs at 10^{10} CFU/3 mL dose for three consecutive days. All pigs were euthanized at the end of the experiment. Ileal and colon digesta were collected for metabolome analysis. The analysis was performed using gas chromatography coupled with time-of-flight mass spectrometry. Logarithmic normalization was performed on the peak intensities of identified metabolites and univariate analysis was performed to identify significant metabolites. Statistical significance was declared at $P < 0.05$ and the false discovery rate–adjusted P value (q value) < 0.20. Metabolites that were significantly impacted were uploaded to MetaboAnalyst (https://www.metaboanalyst.ca/) for pathway enrichment analysis. A total of 282 (141 identified and 121 unidentified) metabolites were detected in ileal mucosa. Forty-nine identified metabolites in ileal digesta differed significantly among experimental groups ($P < 0.05$; q < 0.20). The most impacted metabolic pathways were galactose metabolism, aspartate and glutamate metabolism, fructose and mannose degradation, pentose phosphate pathway, and urea cycle. A total of 196 (127 identified and 69 unidentified) metabolites were detected in colon digesta. Only 7 identified metabolites in colon digesta significantly differed among experimental groups ($P < 0.05$; q < 0.20) with majority of them involved in purine metabolism. In conclusion, supplementation of *Bacillus subtilis* could alter the ileal and colon metabolic profiles of pigs infected with *E. coli. Bacillus subtilis* sp. modified gut metabolites indicate improved intestinal integrity and enhanced carbohydrate, protein and lipid metabolism in *E. coli* infected pigs.

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Introduction

- Enterotoxigenic *E. coli* (ETEC) are the most common type of *E. coli* that cause diarrhea in post-weaning piglets.
- FDA regulations prohibited the use of in-feed antibiotics as growth promoter (FDA's GFI #213).
- Supplementation of *Bacillus* spp. probiotics reduces incidence of diarrhea and improves growth performance of post-weaning pigs (Bhandari et al., 2008; Pan et al., 2017).
- limited research on the action of mechanisms of dietary probiotics supplementation.

Figure 1. Post-weaning diarrhea caused by *E. coli* in US

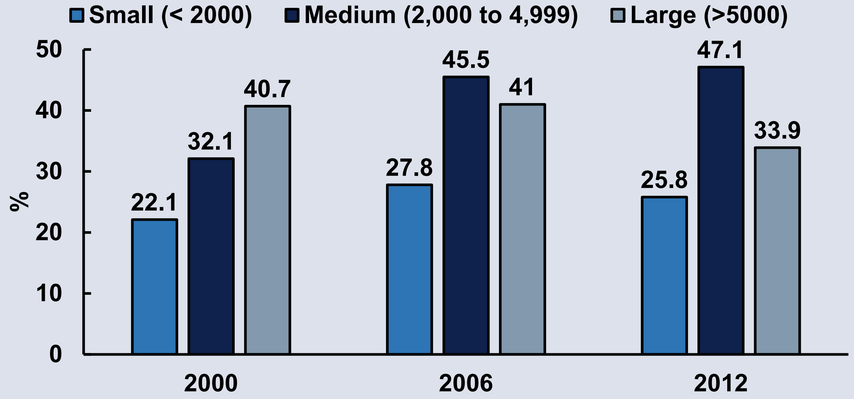
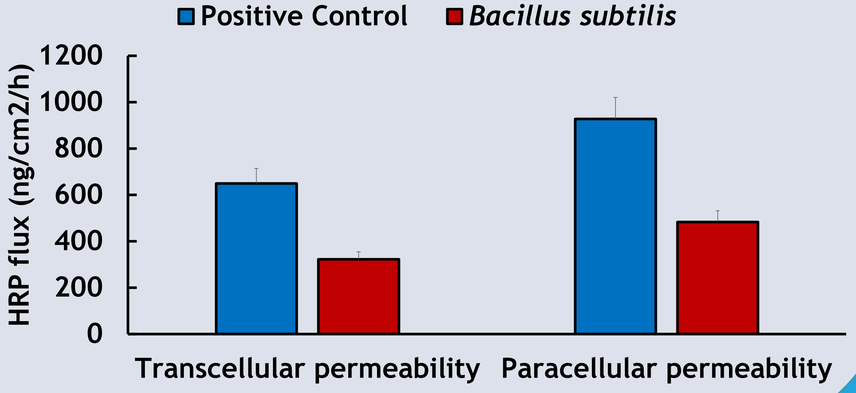


Figure 2. *Bacillus subtilis* reduced gut permeability



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Abstract

Introduction

Material and Methods

Results

Conclusions

Material and Methods

- **Animals and facility**
  - 48 weanling pigs (6.17 ± 0.36 kg)
  - Weaned at 21 d of age into 2 confinement nursery rooms
- **F18 *E. coli* challenge**
  - Enterotoxigenic F18 *E.coli* (LT, STb, SLT-2)
  - Oral inoculation, 10^{10} cfu/dose with 3 doses
- **Experimental design**
  - Randomized Complete Block Design
  - Blocking factors: body weight x gender
- **Dietary treatments:** 12 pigs/treatment
  - Negative control: basal diet without E. coli challenge (**A**)
  - Positive control: basal diet with E. coli challenge (**B**)
  - Antibiotic: Basal diet + 50 mg/kg Carbadox with *E. coli* challenge (**C**)
  - Probiotic: Basal diet + 500 mg/kg *Bacillus* sp. probiotic with *E. coli* challenge (**D**)
- Experimental period included a 7-d adaptation period and 21 days after the first *E. coli* inoculation.
- **Sample collection and analysis**
  - All pigs were euthanized on d 21 post-inoculation (d 28).
  - Ileal mucosa and colon digesta were collected.
  - Gas chromatograph time of flight mass spectrometer (GC-TOF/MS).
- **Statistical analysis**
  - Data were analyzed by ANOVA and p-values corrected for false discovery rate (q value).
  - Plots of principle components analysis, partial least squares-discriminant analysis, heatmap and enrichment pathways were generated using R by an online Metaboanalyst tool.

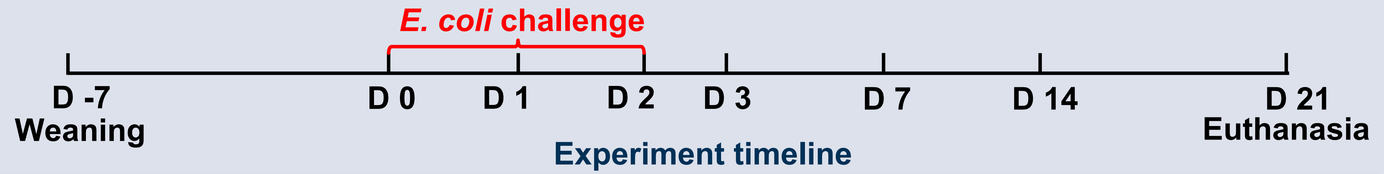

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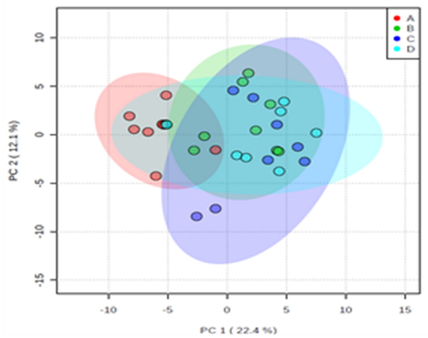
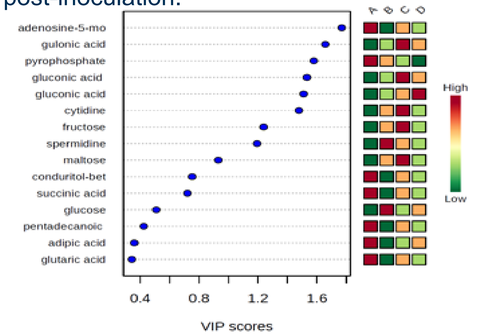
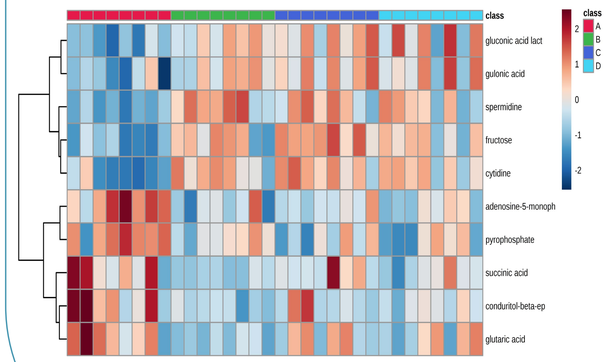
Figure 3. Changes in ileal mucosa metabolites in pigs among different treatment groups on d 21 post-inoculation.



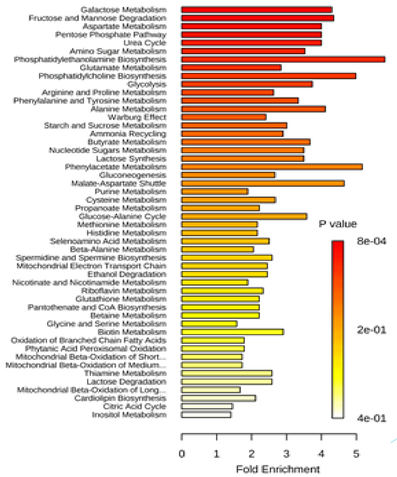
(A) Two-dimension score plots of principal component analysis and 95% confidence range were depicted as circled areas.



(C) Features identified by PLS-DA. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group.



(B) Heatmap and hierarchical clustering.



(D) Bar plot for metabolite sets enrichment.

Results

- **Ileal Mucosa Metabolites**
  - Distinct partition between negative and positive control groups.
  - Forty-nine identified metabolites significantly differed among treatment groups.
  - *E. coli* infection increased metabolites such as D-glucose, D-mannose, sorbitol, pyrophosphate, D-fructose, glucose-1-phosphate, and glucose-6-phosphate.
  - Galactose metabolism, fructose and mannose degradation, aspartate metabolism, pentose phosphate pathway, and urea cycle were significantly different among groups.

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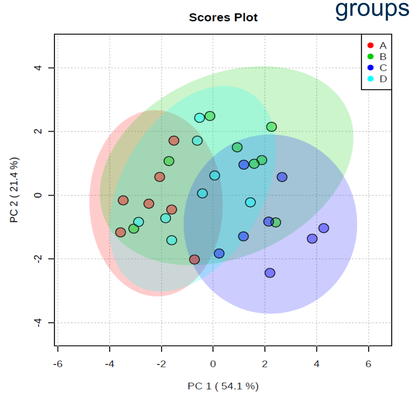
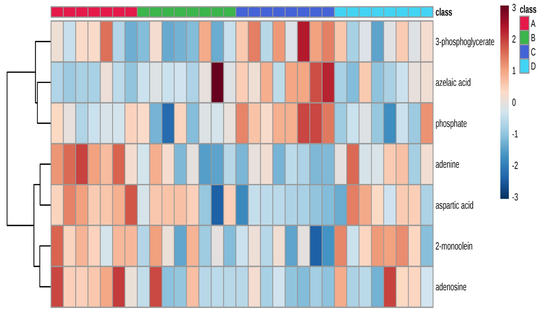
Figure 4. Changes in colon digesta metabolites in pigs among different treatment groups on d 21 post-inoculation.



(A) Two-dimension score plots of principal component analysis and 95% confidence range were depicted as circled areas.



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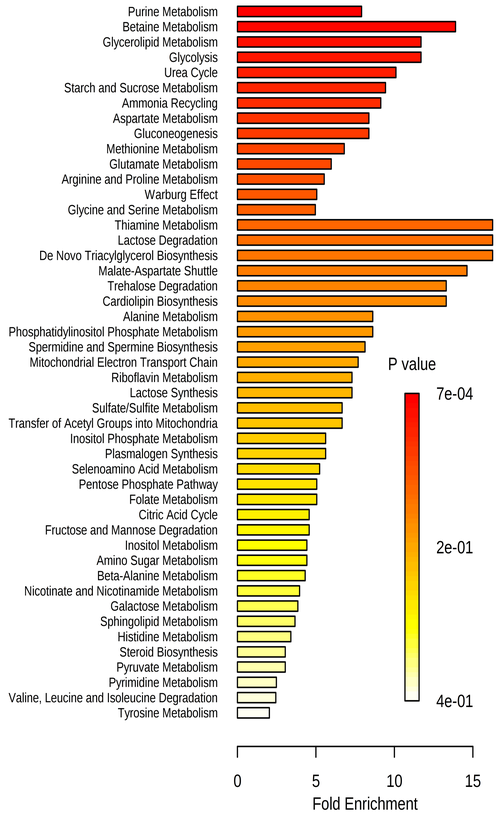

(C) Bar plot for metabolite sets enrichment.

Results

- **Colon Digesta Metabolites**
  - Distinct partition between negative and positive control groups.
  - There were 7 identified metabolites significantly differed among treatment groups, including adenine, adenosine, L-aspartic acid, phosphate, 2-monoolein, azelaic acid and 3-phosphoglycerate.
  - Purine metabolism was significantly different among groups.

Conclusions

- Disturbed metabolic pathways indicate inhibition in energy production in ETEC challenged pigs.
- *Bacillus subtilis* sp. modified gut metabolites improving intestinal integrity and enhancing carbohydrate, protein and lipid metabolism.