Small molecular weight metabolites regulating growth and immunity as postbiotic antibiotic alternatives

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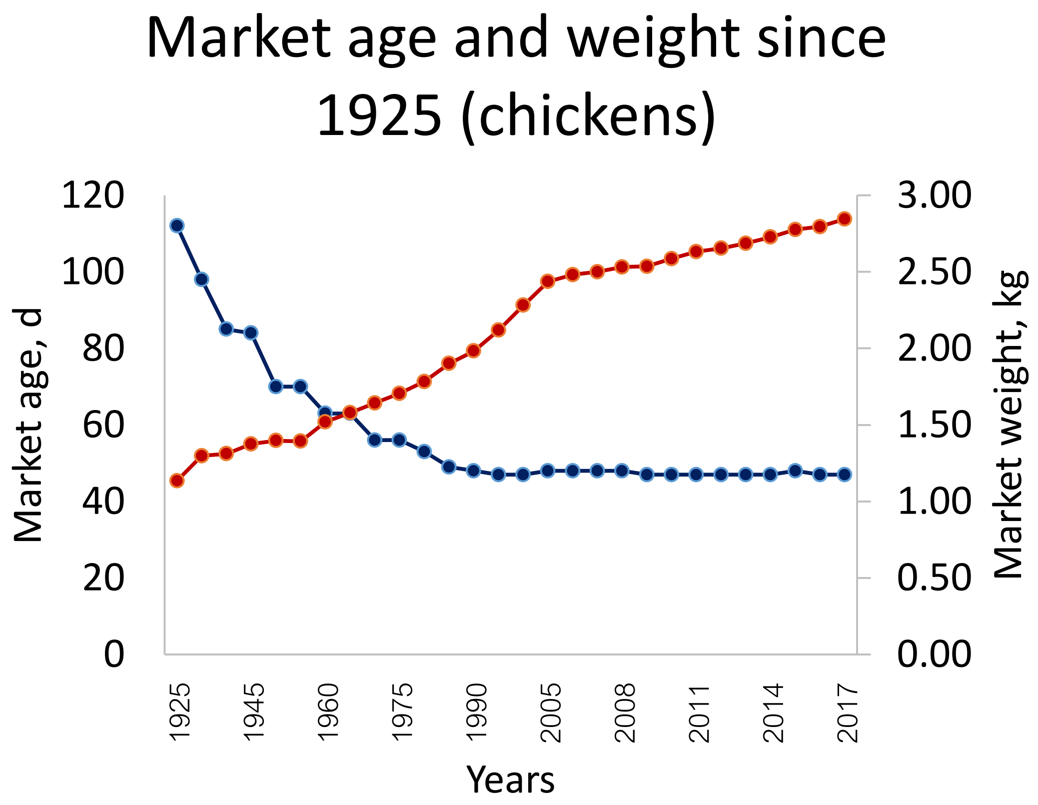
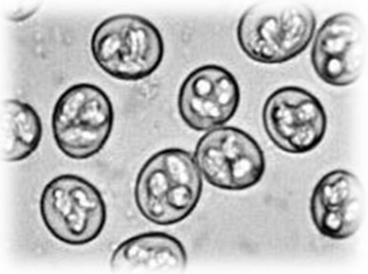
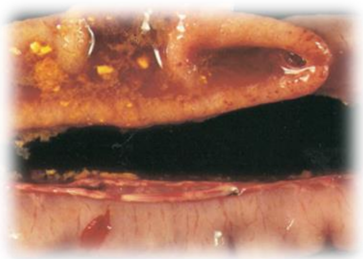
Introduction: antibiotic alternatives



- Avian coccidiosis with *Emeria* spp
- Necrotic enteritis
- With AGP withdrawal, there is an urgent need to develop antibiotic alternatives.

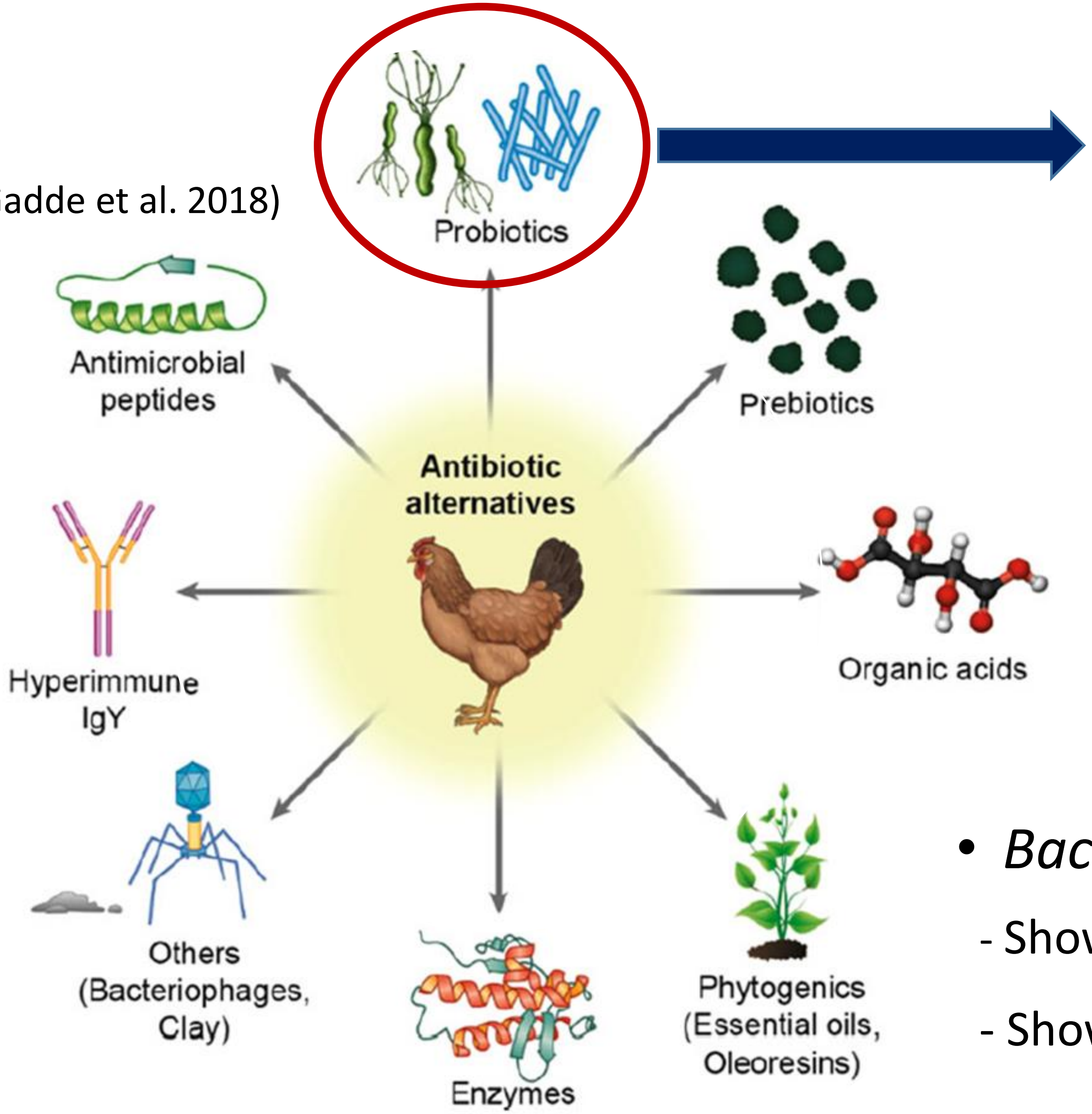


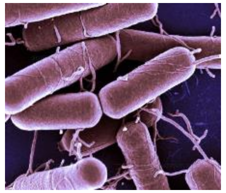


Gallucci and Matzinger, 2001; Shirley and Lillehoj, 2012

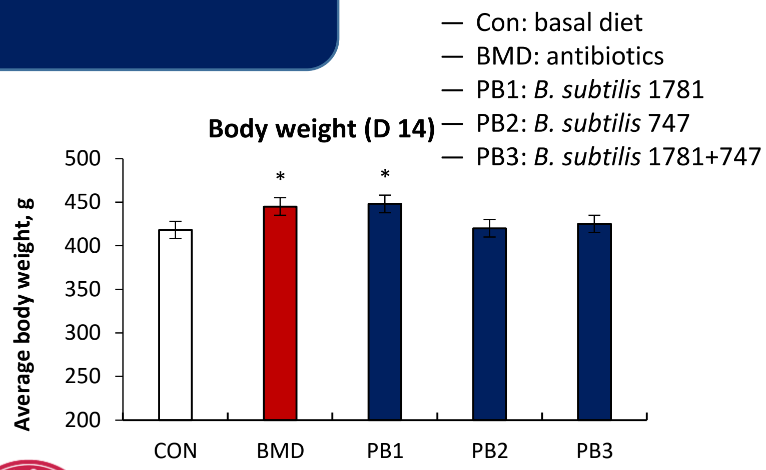
Introduction: antibiotic alternatives

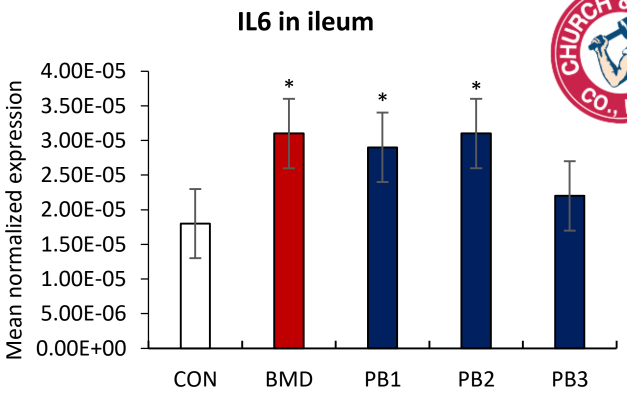
(Gadde et al. 2018)

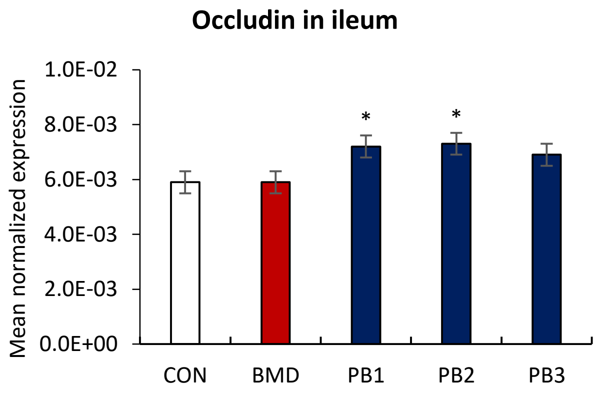




Bacillus subtilis
by Nano Creative/Science Source



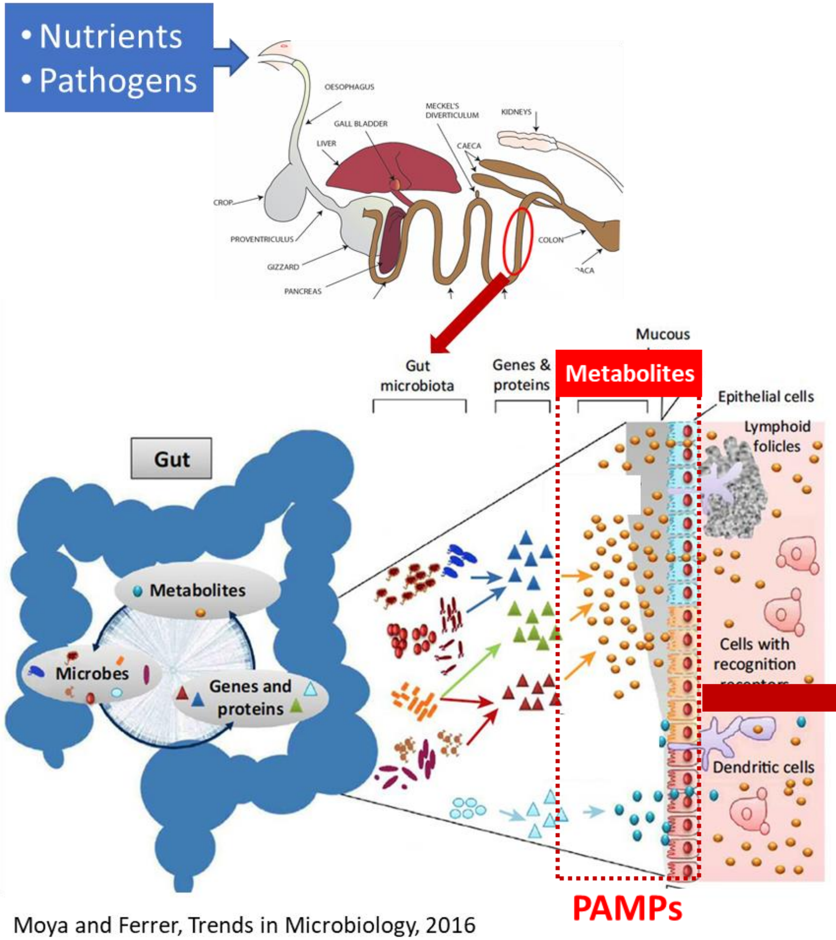




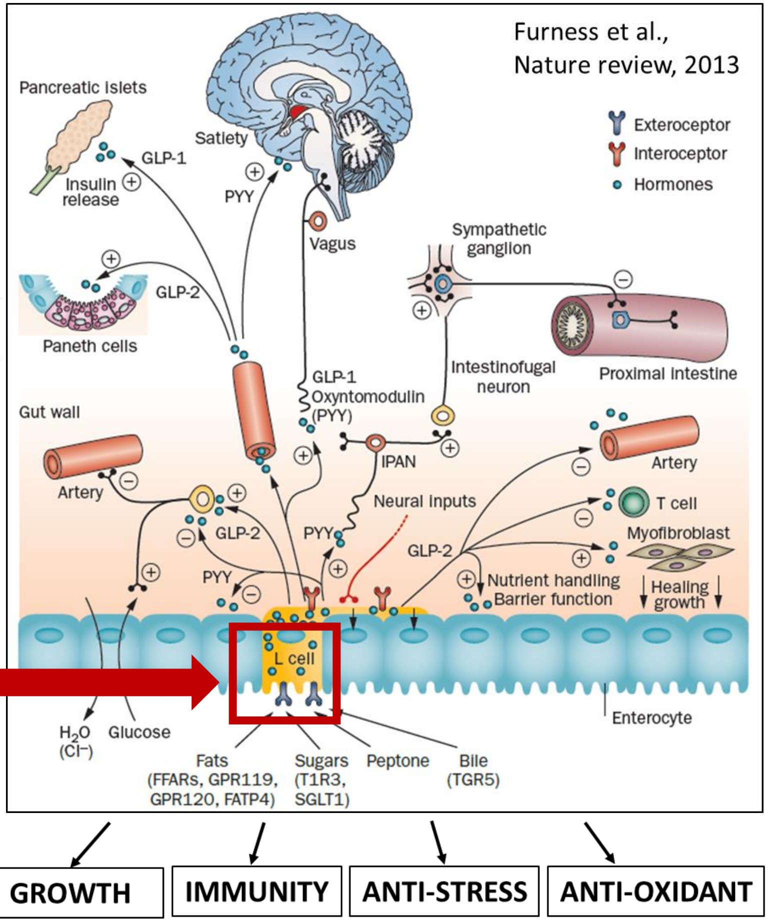
- *Bacillus subtilis* as probiotics in chicken feed
  - Shows growth-promoting effect (Gadde et al. 2017)
  - Shows a protective role against chicken pathogen (Park et al. 2019)

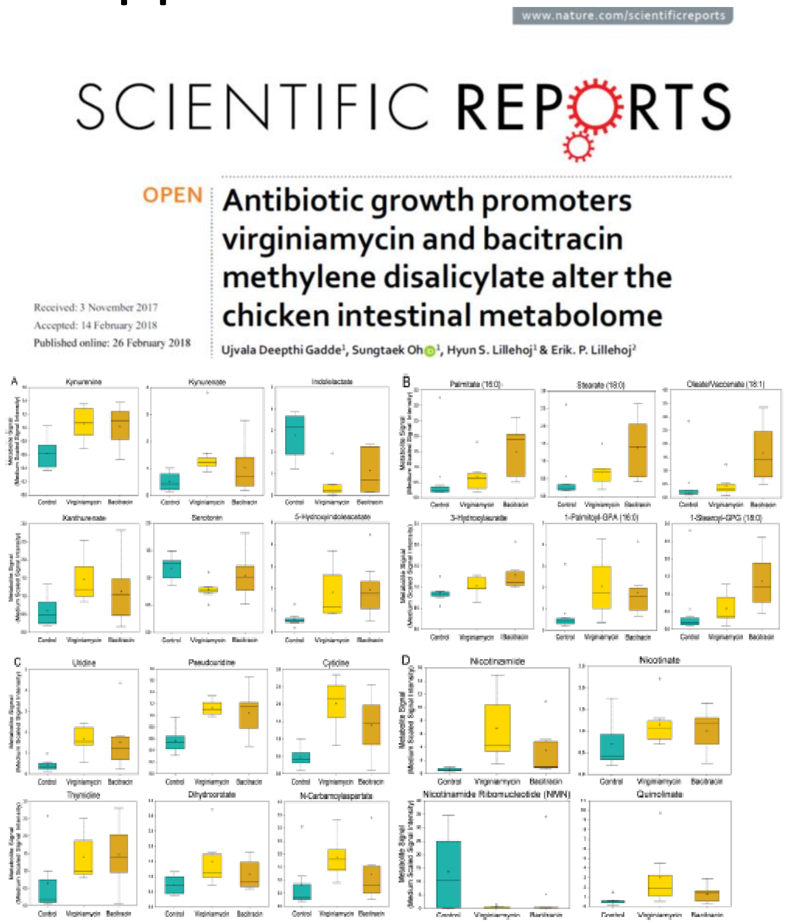
Introduction: gut microbiota and metabolites

- What is the mechanisms of dietary *Bacillus subtilis* supplementation?



Moya and Ferrer, Trends in Microbiology, 2016





"Postbiotics" novel materials promoting gut health as functional additive of diet

Objective of this study

- Therefore, the current study was undertaken to characterize the metabolic alterations in the chicken gut following dietary supplementation with *B. subtilis* DFMs with the goal of identifying potential chemical compounds that might be directly used to improve poultry growth performance without the use of AGPs.

Materials and Methods

84 male day-old Ross 708 broilers

ileal content from euthanized chciekn
(2 chickens/pen = total 8 chickens/treatment)

d 0 — d 14 — d 21

Normal feed (d 0 – d 14)

Experimental feed (d 14 – d 21)

d 14:

- Initial body weight
- Allocation to 3 treatments

d 21:

- Body weight
- Feed intake

Measurements:

- Metabolomic profiling of the ileal contents by mass spectrometry (Metabolon, Durham, NC)

Metabolic pathways

Treatments	Supplements	Dose	Chickens/cage	Replication
CON	Basal diet		7	4
B. subtilis 1781	CON+*B. subtilis* 1781	1.5×10^5 CFU/g feed	7	4
B. subtilis 747	CON+*B. subtilis* 747	1.5×10^5 CFU/g feed	7	4

- *B. subtilis* strains were obtained from Church & Dwight Co. Inc. (Waukesha, WI).

Materials and Methods

- Statistical Analysis
  - Growth performance

    - PROC MIXED in SAS (SAS Inst. Inc., Cary NC)

    - *P* values < 0.05

    - PDIFF option
  - Ileal biochemicals

    - Array Studio software (OmicSoft, Cary, NC)

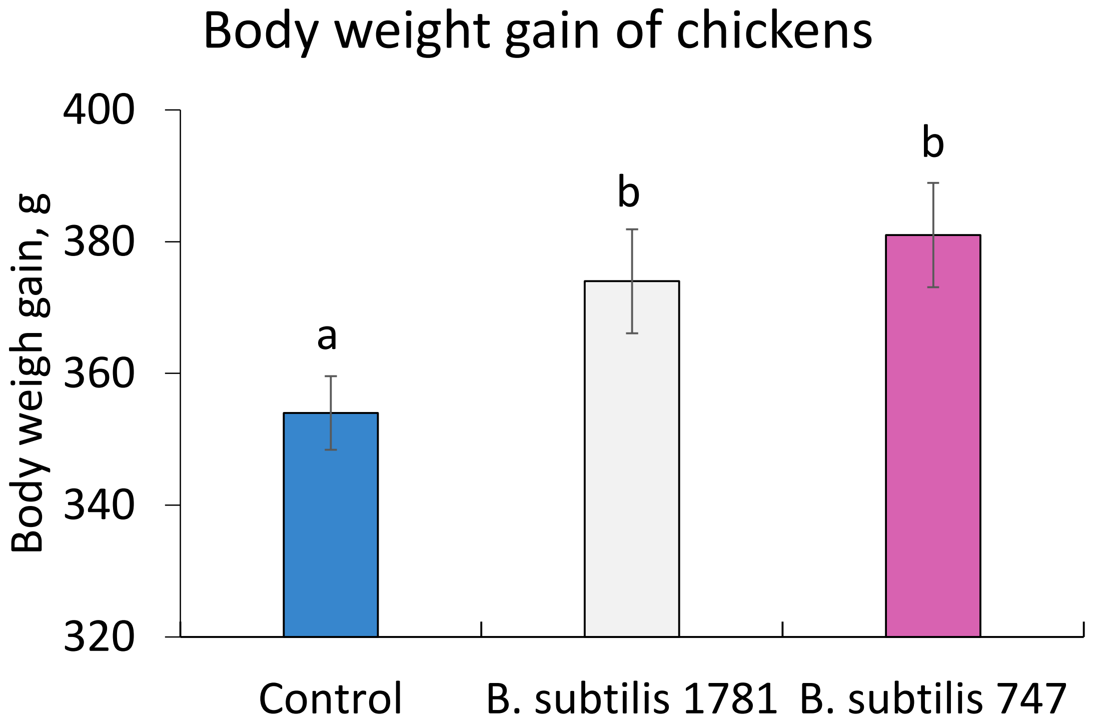
    - the programs R (R Foundation for Statistical Computing, Vienna, Austria)

    - JMP (SAS Institute)

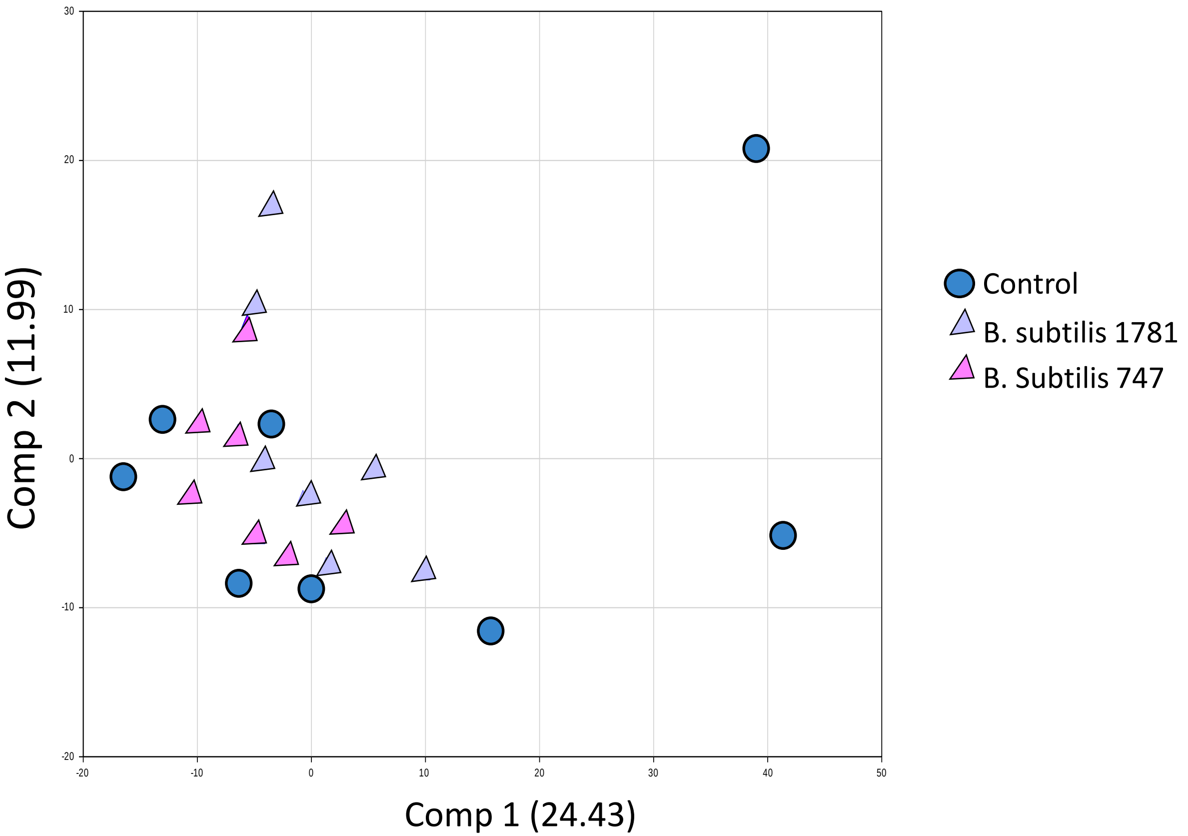
    - *P* values < 0.05

    - Random Forest Analysis (RFA) by computing the Mean Decrease Accuracy

Result: growth effect and biochemical distribution

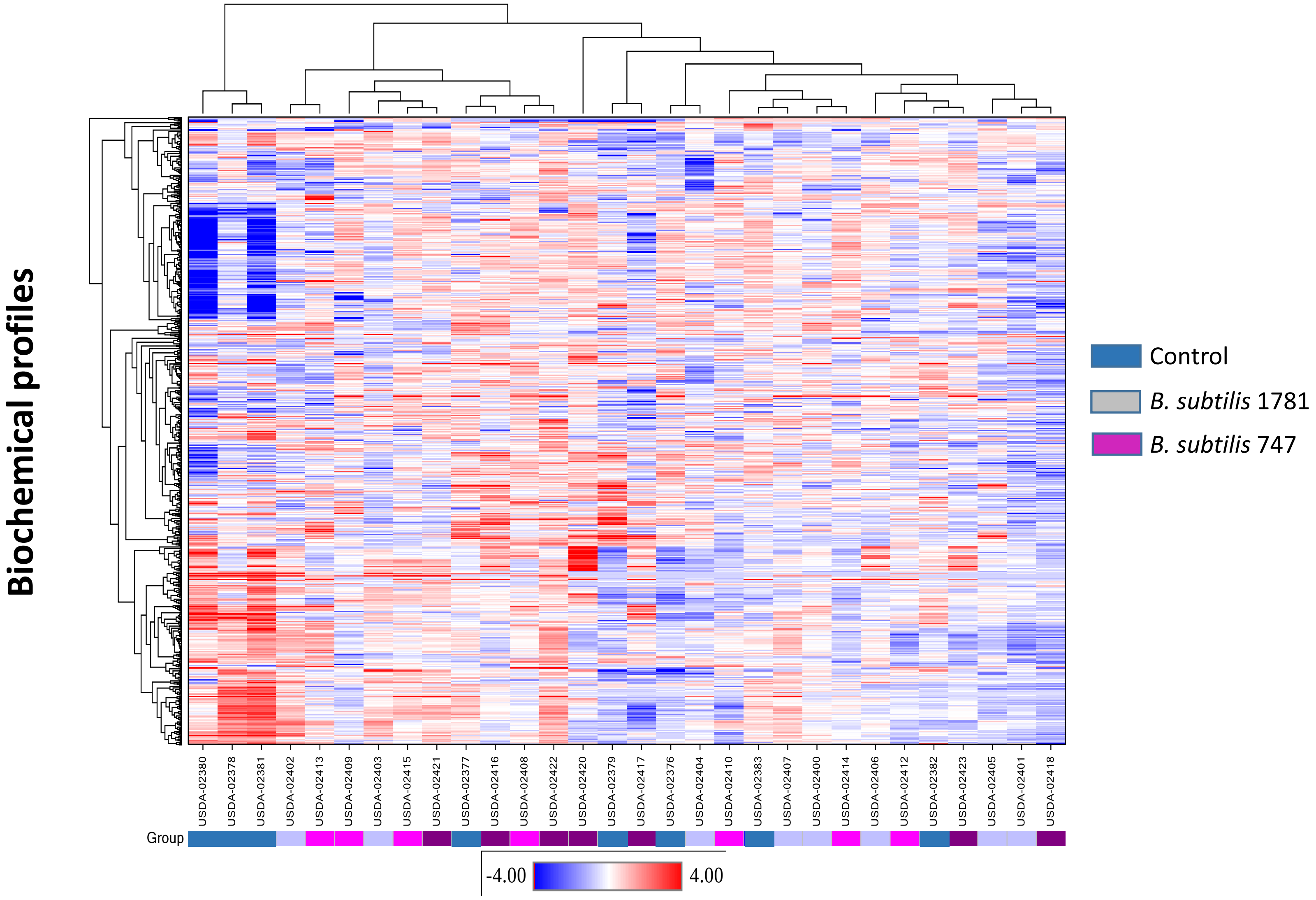


Body weight gain = final body weight – initial body weight



Principal component analysis of ileal biochemicals

Result: hierarchical clustering heatmap

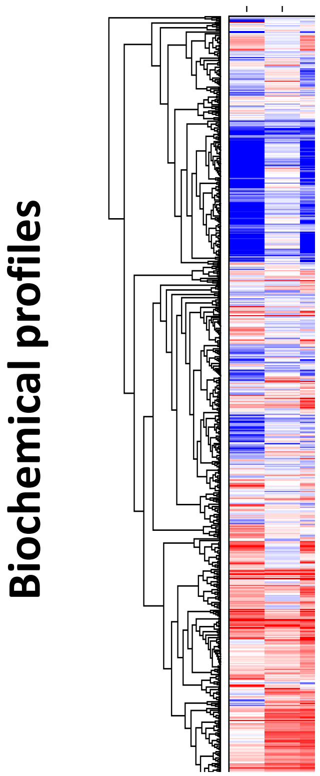


- Total 674 biochemicals

- Con vs *B. subtilis* 1781
  - Increased 209 (25; $P < 0.05$)
  - Decreased 461 (58; $P < 0.05$)

- Con vs *B. subtilis* 747
  - Increased 265 (12; $P < 0.05$)
  - Decreased 402 (38; $P < 0.05$)

Result: pathways in this study

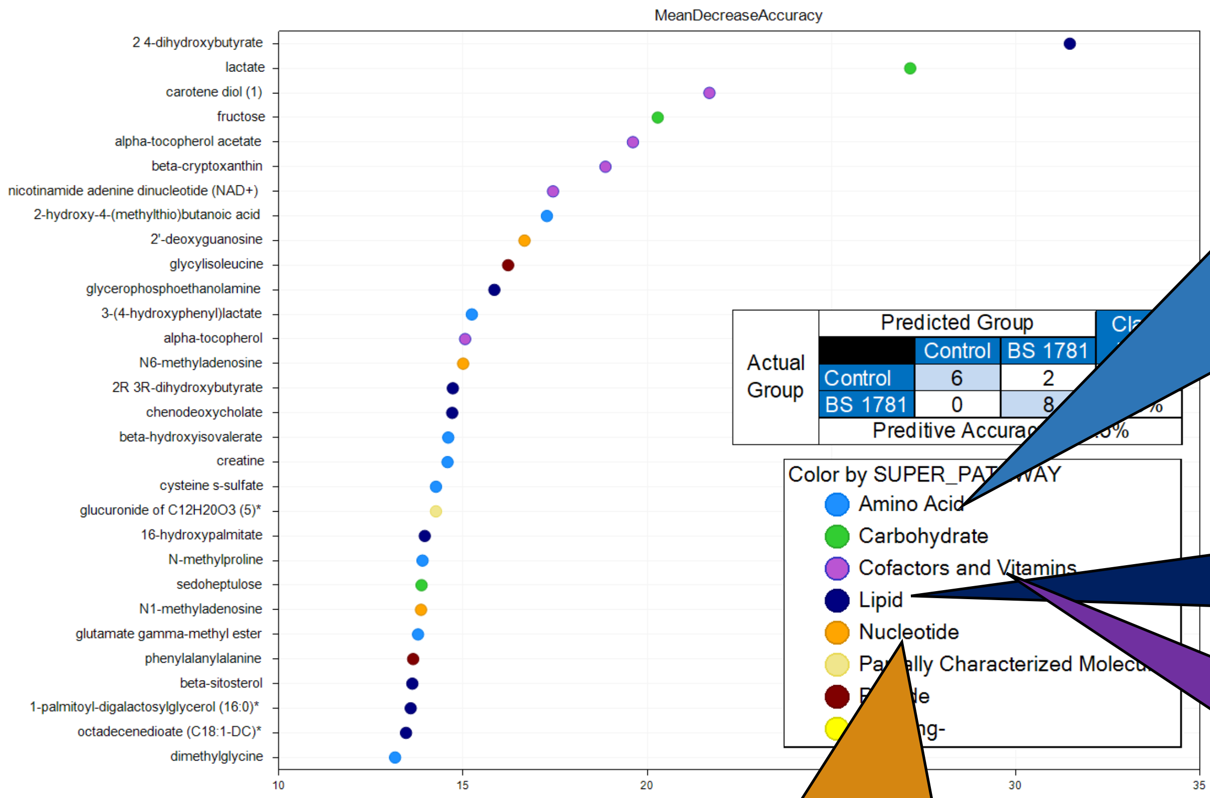


- 193 amino acid pathways
- 32 carbohydrate pathways
- 263 lipid pathways
- 42 cofactors and vitamins pathways
- 51 nucleotide pathways
- 12 energy pathways
- 81 unknown pathways

- 498 Human Metabolome Database (HMDB)
- 336 Kyoto Encyclopedia of Genes and Genomes (KEGG) codes

Result: random forest analysis and plot

CON vs *B. subtilis* 1781



- **8 amino acids (26.7%)**
  - 2-hydroxy-4-(methylthio)butanoic acid
  - 3-(4-hydroyphenyl)lactate
  - beta-hydroxycholate
  - creatine
  - cystein s-sulfate
  - N-methylproline
  - glutamate gamma-methyl ester
  - dimethylglycine

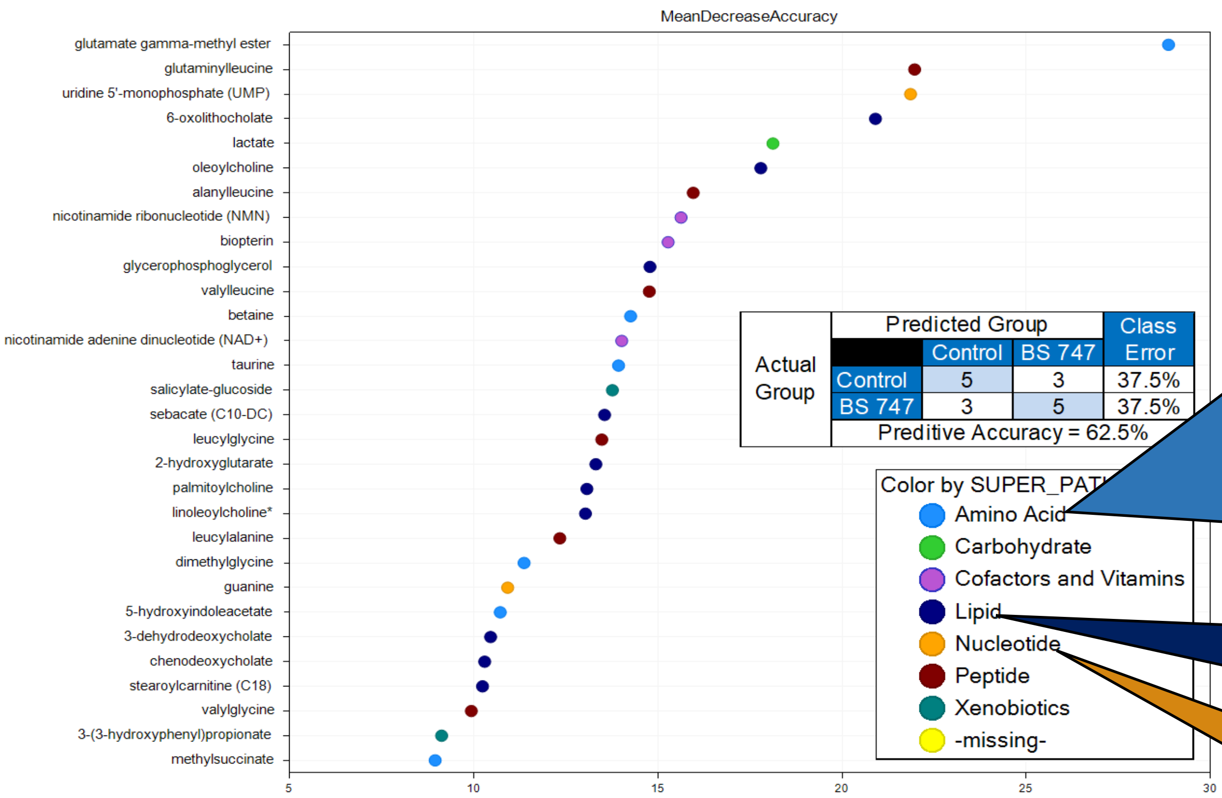
- **8 lipids (26.7%)**
  - 2,4-dihydroxybutyrate
  - glycerophosphoethanolamine
  - 2R 3R-dihydroxybutyrate
  - chenodeoxycholte
  - 16-hydroxypalmitate
  - beta-sitosterol
  - 1-palmitoyl-digalactosylglycerol (16:0)
  - octadecenedioate

- **5 vitamins and cofactors (16.7%)**
  - carotene diol
  - alpha-tocopherol acetate
  - beta-cryptoxanthin
  - nicotinamide adenine dinucleotide (NAD+)
  - alpha-tocopherol

- **3 nucleotides (10.0%)**
  - 2'deoxyguanosine
  - N6-methyladenosine
  - N1-methyladenosine

Result: random forest analysis and plot

CON vs *B. subtilis* 747



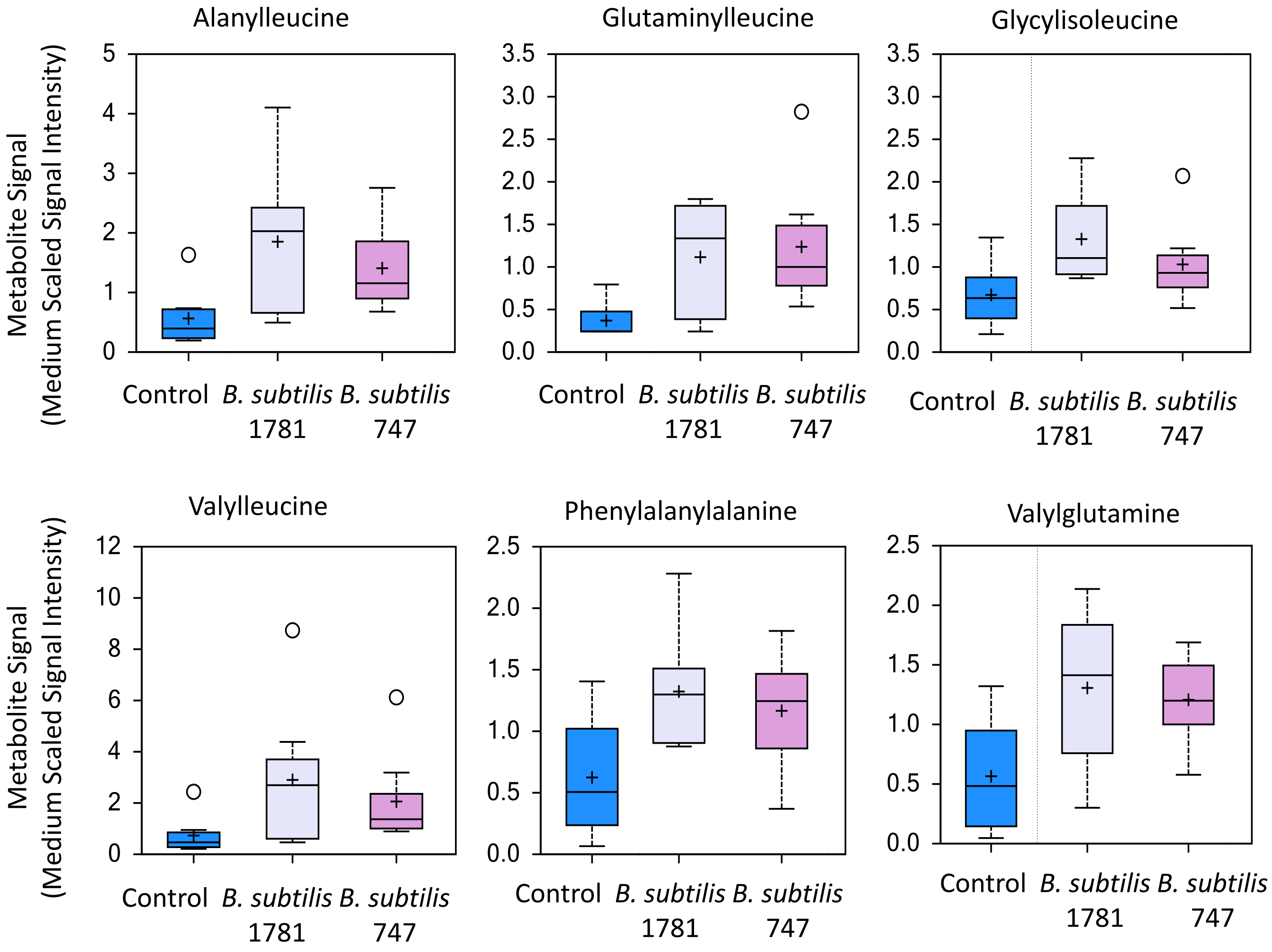
- **6 amino acids (20.0%)**
  - glutamate gamma-methyl ester
  - betaine
  - taurine
  - dimethylglycine
  - 5-hydroxyindoleacetate
  - methylsuccinate

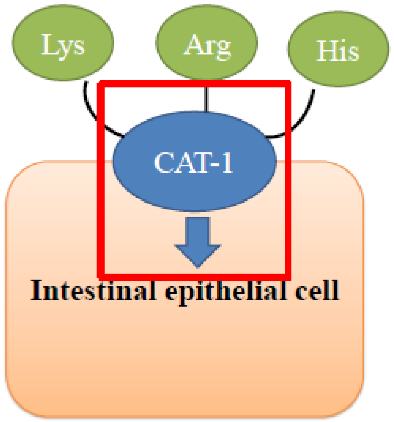
- **6 peptides (20.0%)**
  - glutaminylleucine
  - alanylleucine
  - valylleucine
  - leucylglycine
  - leucylalanine
  - valylglycine

- **10 lipids (33.0%)**
  - 6-oxolithocholate
  - oleoylcholine
  - glycerophosphoglycerol
  - sebacate (C10-DC)
  - 2-hydroxyglutarate
  - palmitoylcholine
  - linoleoylcholine
  - 3-dehydrodeoxycholate
  - chenodeoxycholate
  - stearoylcarnitine (C18)

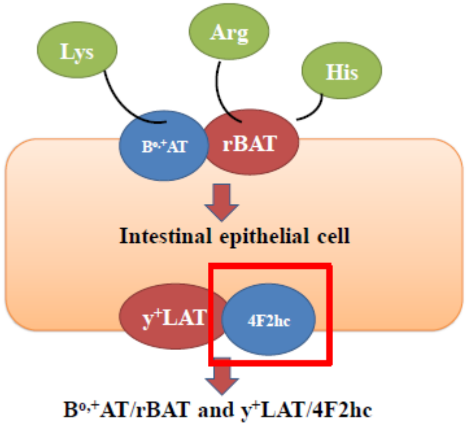
- **2 nucleotides (0.1 %)**
  - uridine 5’-monophosphate (UMP)
  - guanine

Result: amino acids (dipeptides)

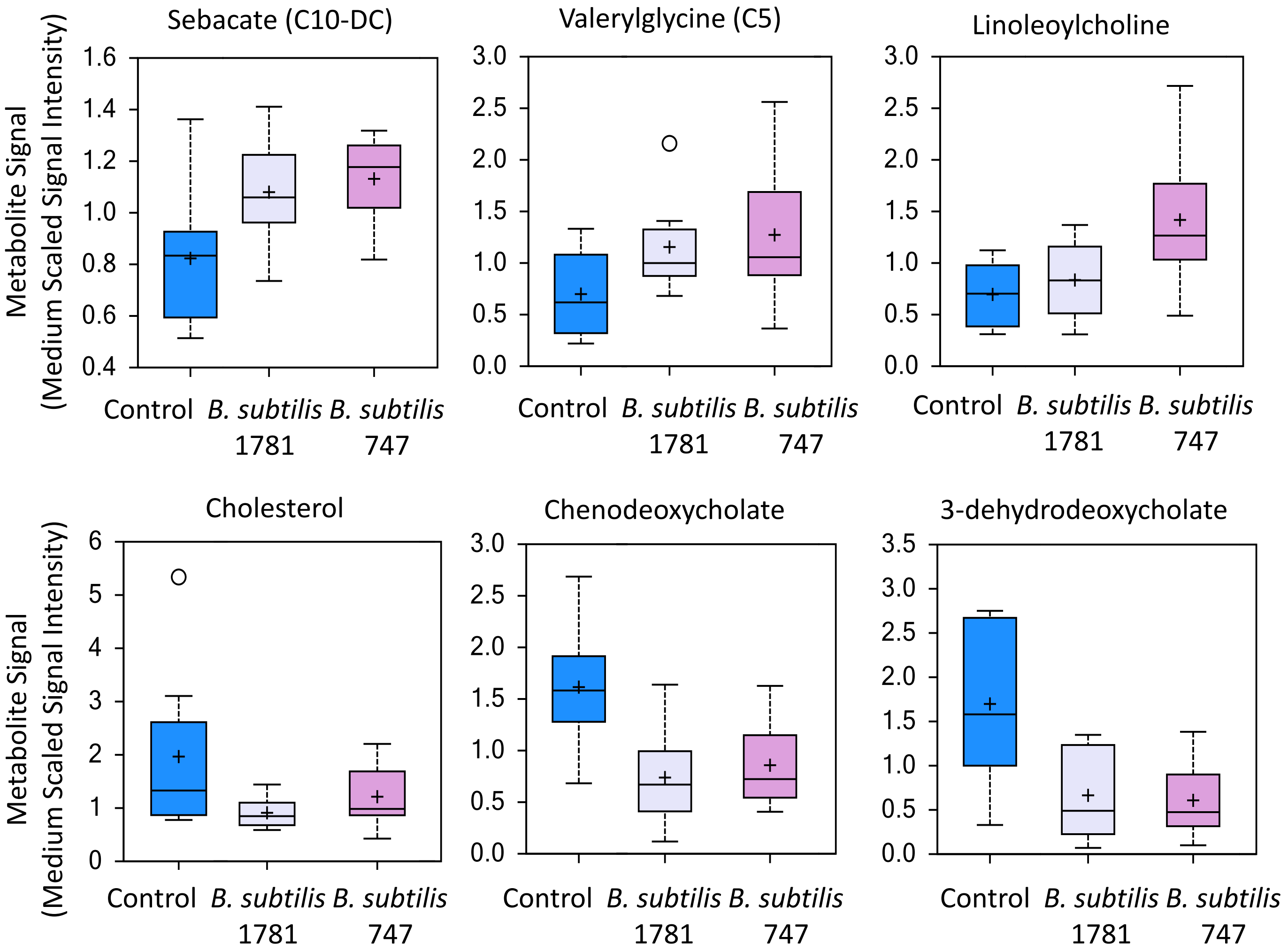


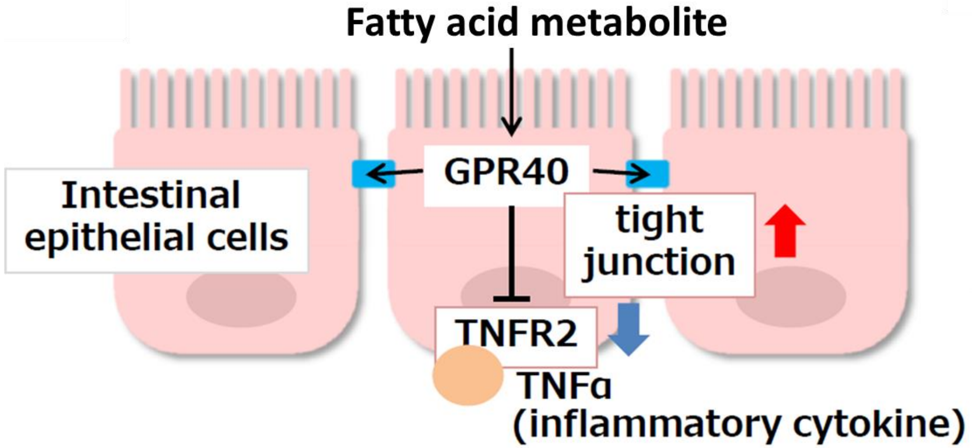


CAT-1



Result: lipids

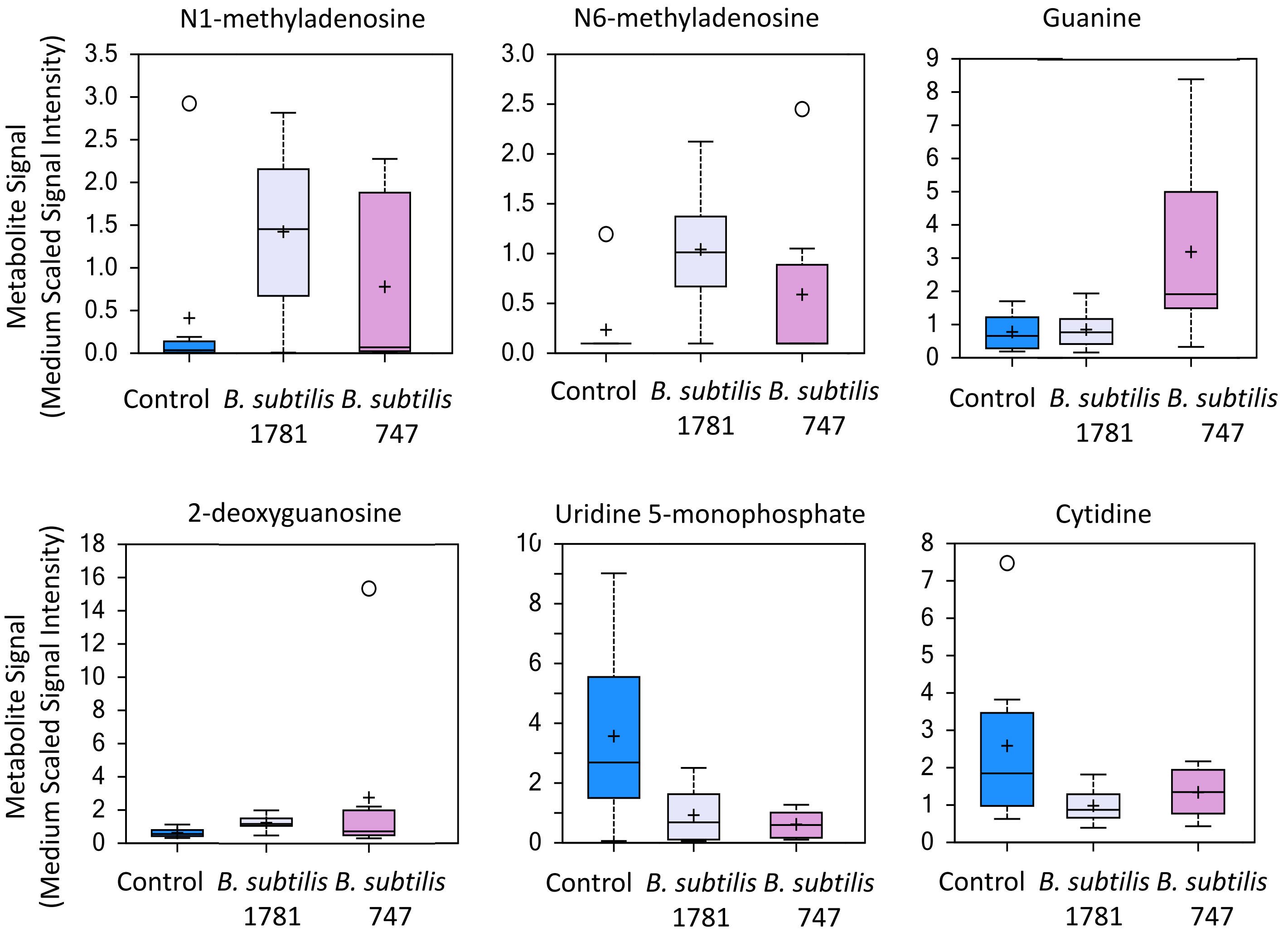


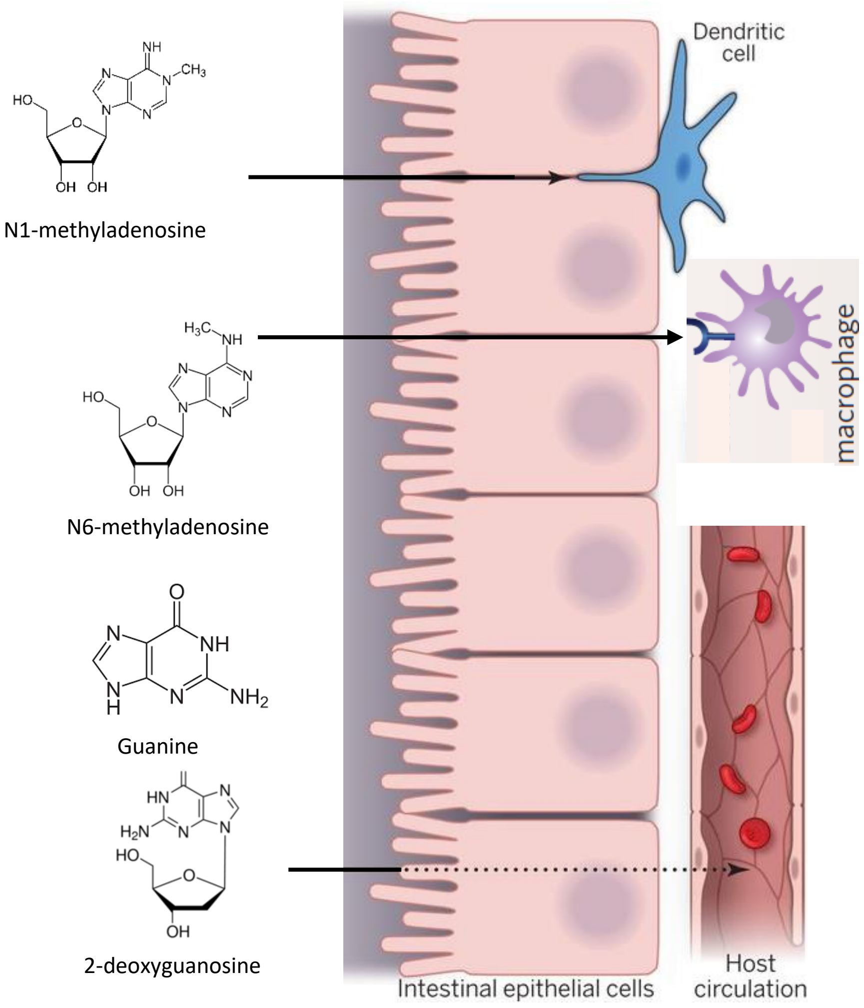


GPR : G protein-coupled receptor
TNFR : Tumor necrosis factor receptor

Miyamoto et al., 2015

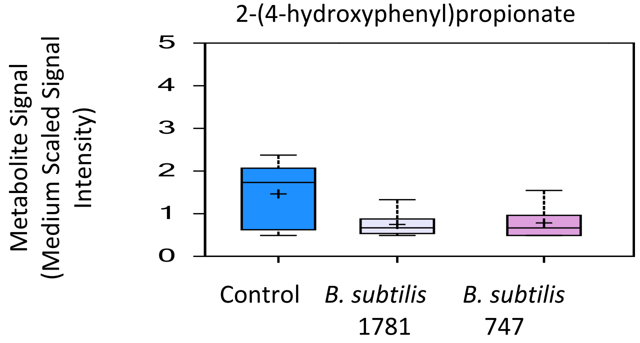
Result: nucleotides

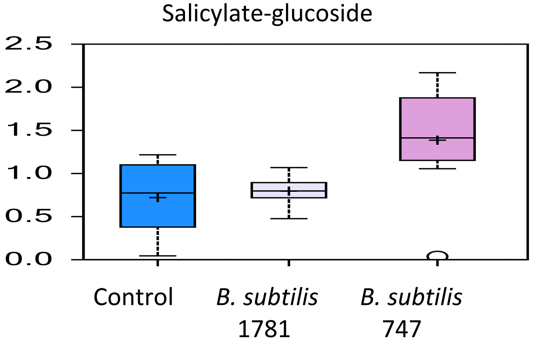


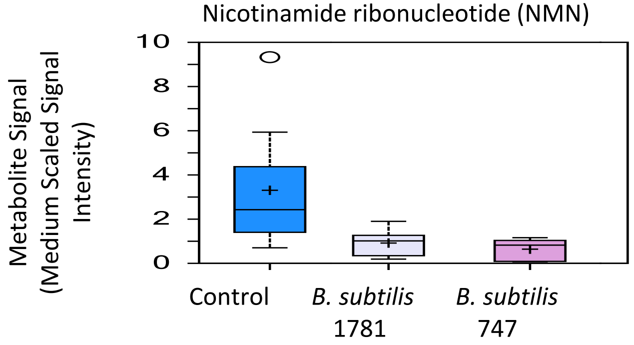


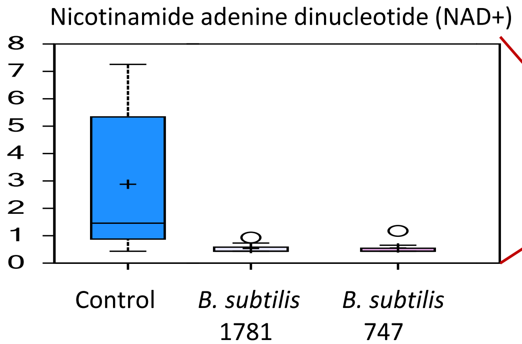
Donia and Fischbach, Science, 2015

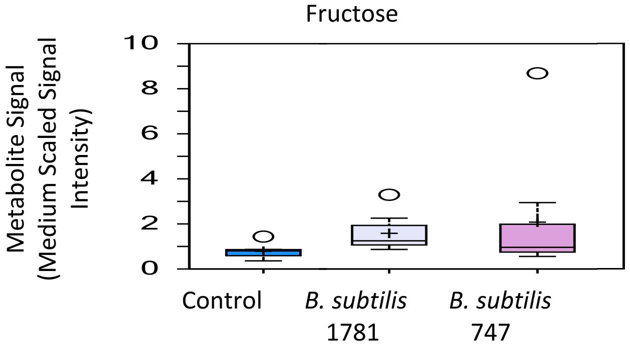
Result: others

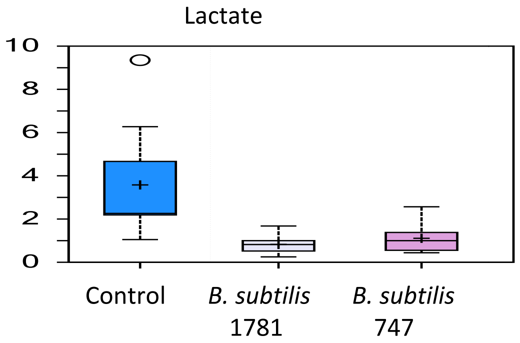


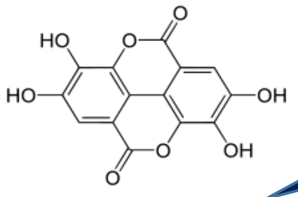






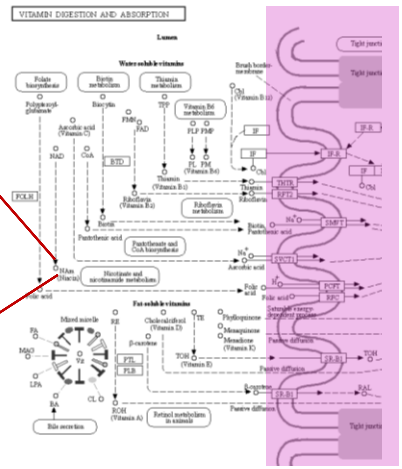




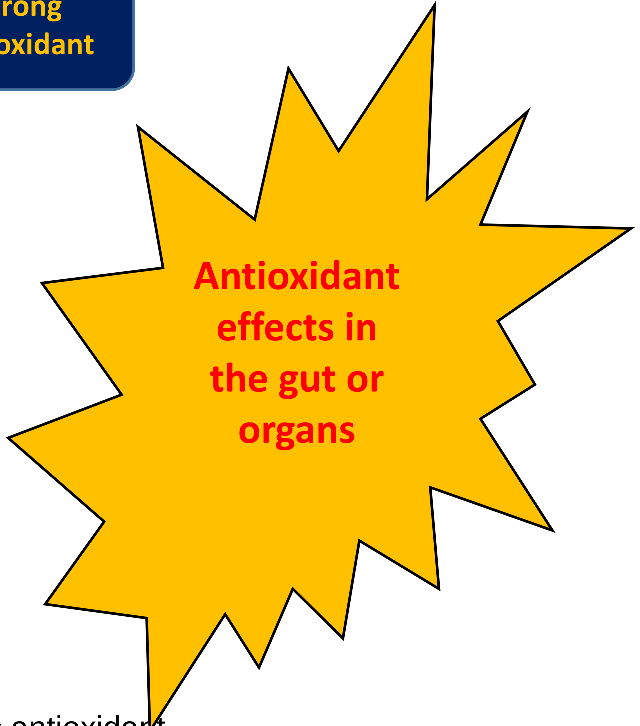


Polyphenol

Strong antioxidant



epithelial barrier



- Fructose feeding negatively affects antioxidant capacity in the blood of hypertensive rats but improves this capacity in the liver (Girard et al., 2006, Nutrition).
- Lactate ion may prevent lipid peroxidation by scavenging free radicals such as O^{2-} and -OH but not lipid radicals (Groussard et al., 2000, J Appl Physiol).

Conclusions

- Dietary supplementation with *B. subtilis* has profound effects on the levels of a wide variety of chemical metabolites in the chicken gut.

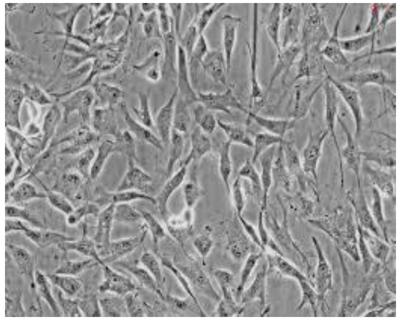
- These altered metabolite levels provide a biochemical signature unique to each *B. subtilis* supplementation group.

- Future studies are warranted to assess the growth promoting properties, if any, of the identified chemical compounds in lieu of antibiotics.

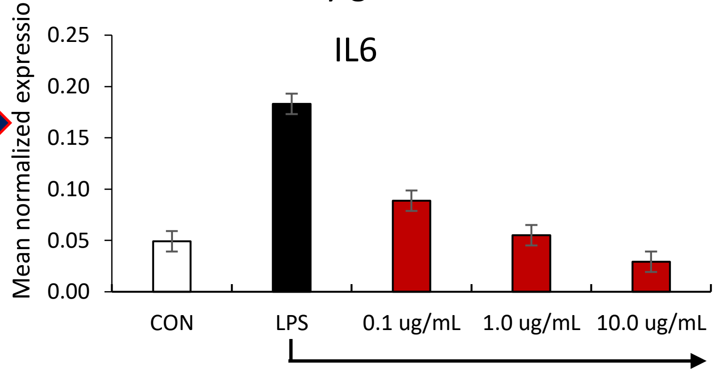
Further study: extra data

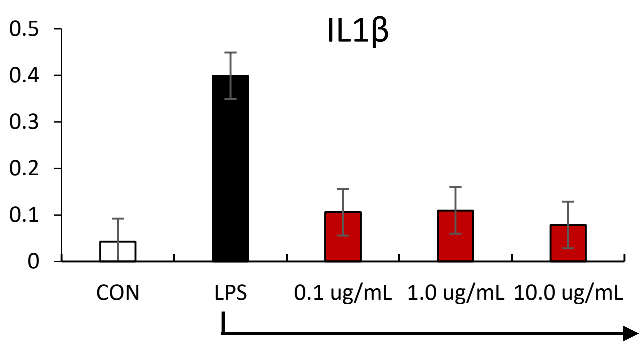
Proinflammatory responses

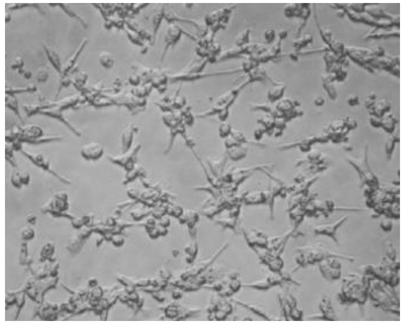
40 metabolites of Up-regulated common metabolites between *B. subtilis* 1781 and 747



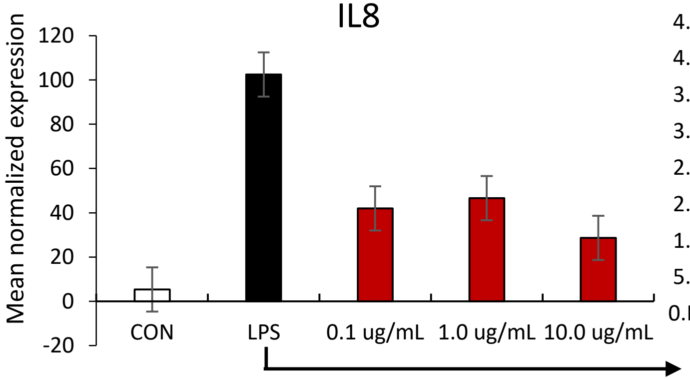
Chicken epithelial cells (8E11)

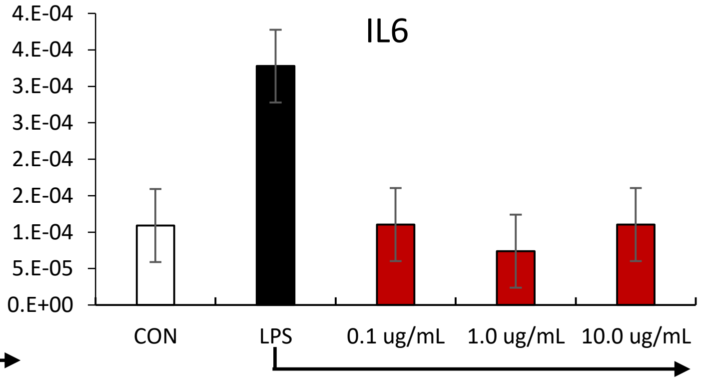






Chicken macrophage cells (HD11)





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