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Cross-kingdom Interactions in the Porcine Gut: Implications in Health and Performance

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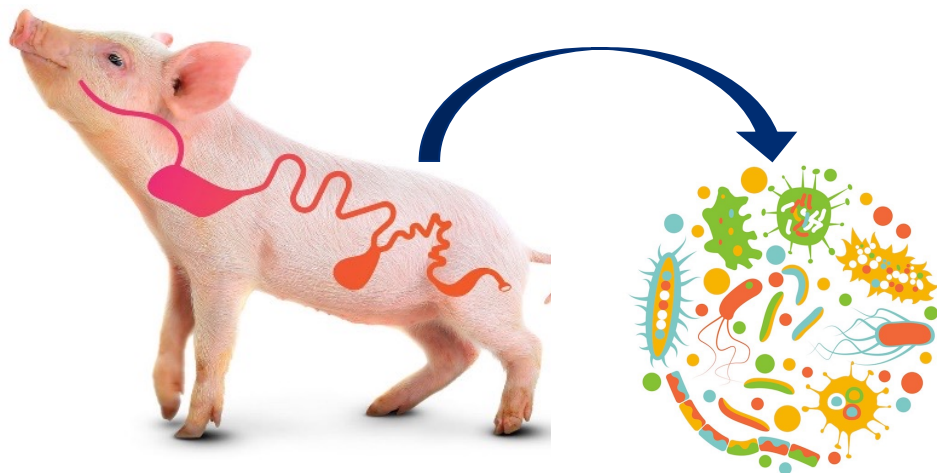
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Alternative Growth Promotion in Pigs



- Food production needs to increase by 70% by 2050
- One Health
- Gut as a research target



Weaning transition

- Weaning is a period of stress
 - Changes in environment
 - Predisposition to disease

- Antibiotics in-feed
 - Prevent weaning-associated diseases
 - Promote growth



Alternatives to Antibiotics

- In-feed antibiotics traditionally utilized in agricultural animals
 - prevent opportunistic diseases
 - promote weight gain
- Recent antibiotic ban necessitates novel strategies to enhance animal growth performance and resistance and resilience to disease





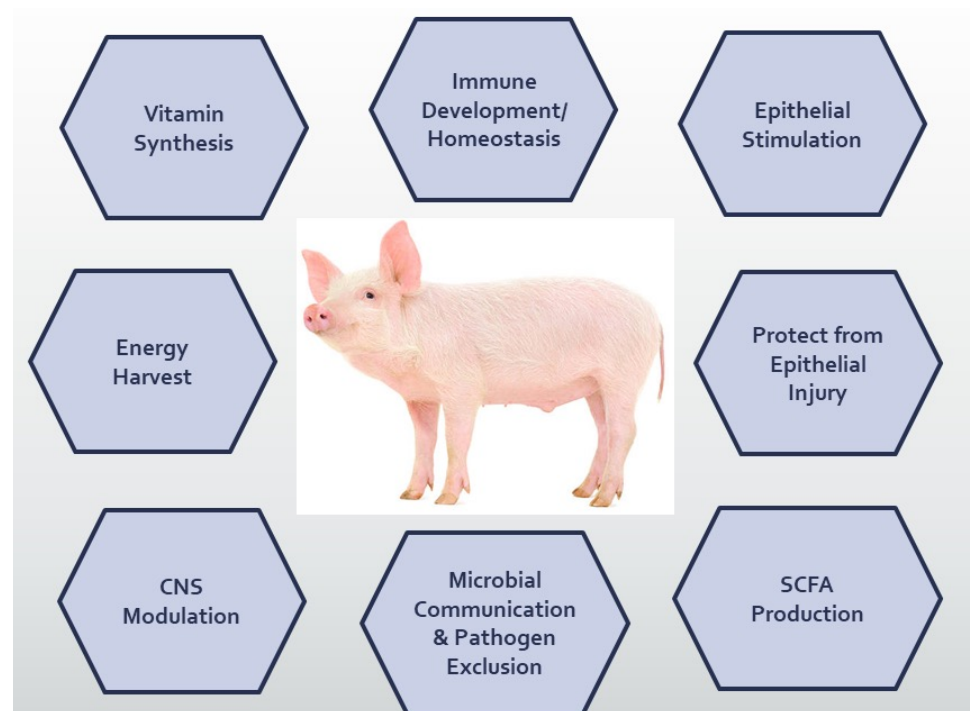
Role of Gut Microbes in Piglet Health and Disease Resilience

■ *In vivo*

- Bacteriome & Mycobiome
- Temporal development
- Microbial network interactions
- Dietary interventions

■ *In vitro*

- IPEC-J2 porcine jejunal cell line
- Biofilm competition assays
- Comparative genomics





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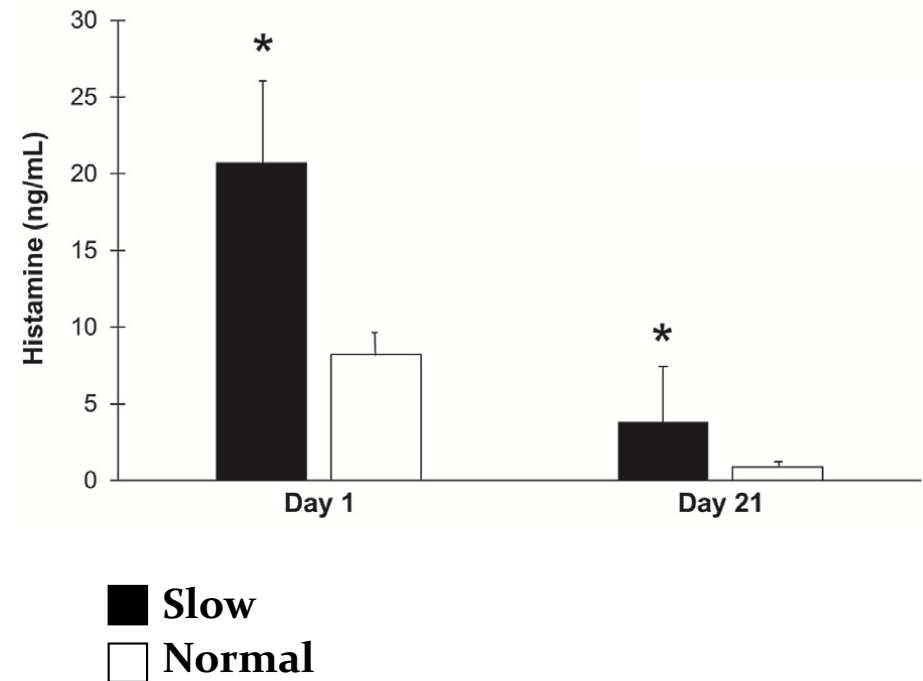
Why the Mycobiome?



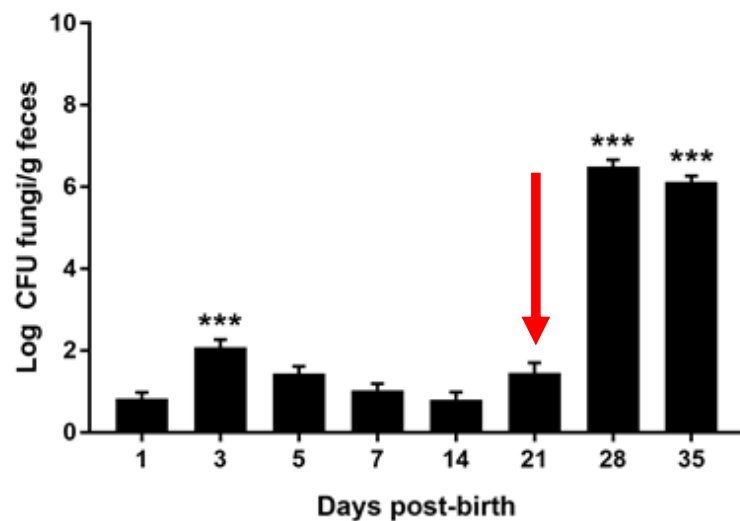


Histamine and Growth Performance

- Elevated histamine levels at birth were associated with reduced piglet growth
- Hypothesized that fungi may be promoting mast cells to release histamine
 - Allergic disease reducing animal performance?
 - Failure to thrive?

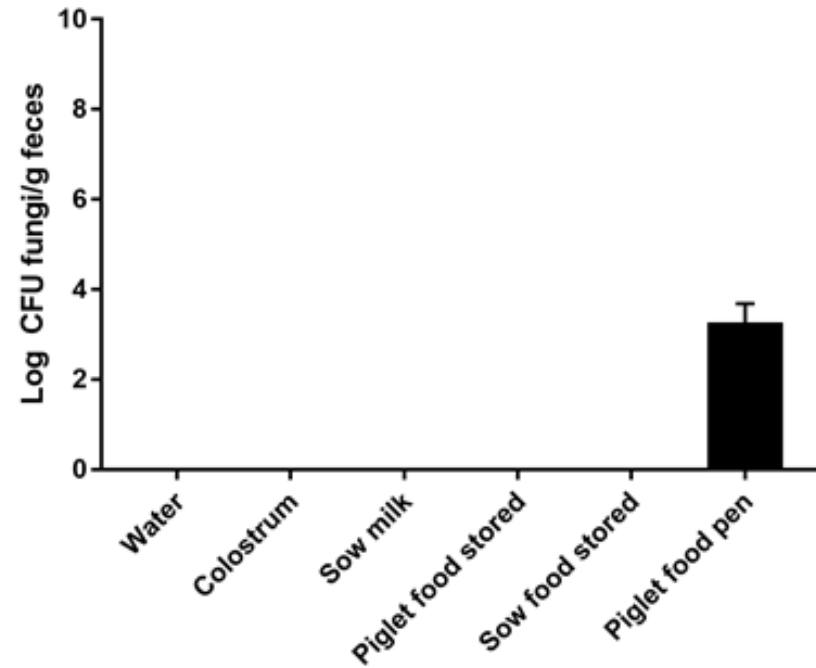
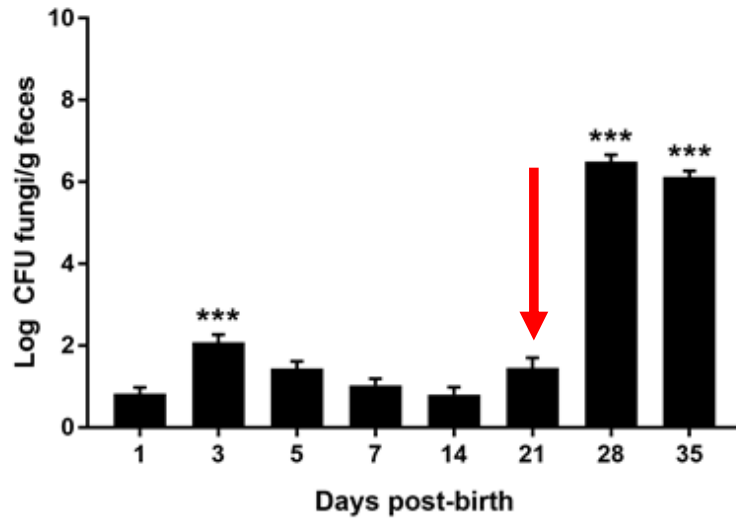


Cultural Fecal Fungal Levels Increase After Weaning

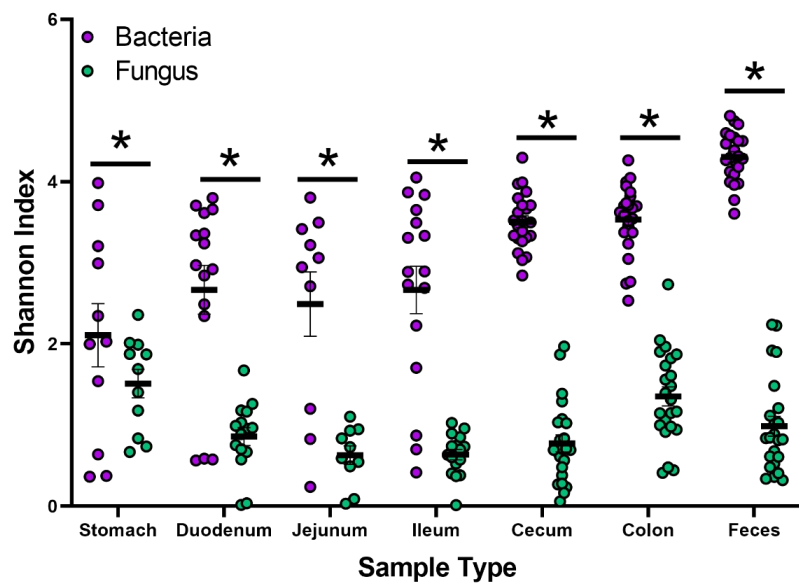




Cultural Fecal Fungal Levels Increase After Weaning



α -diversity of the bacteriome and mycobiome

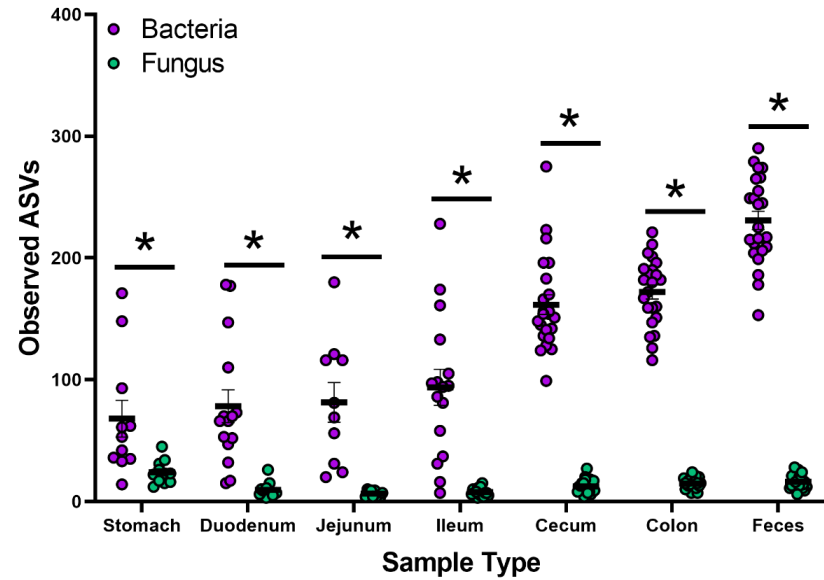
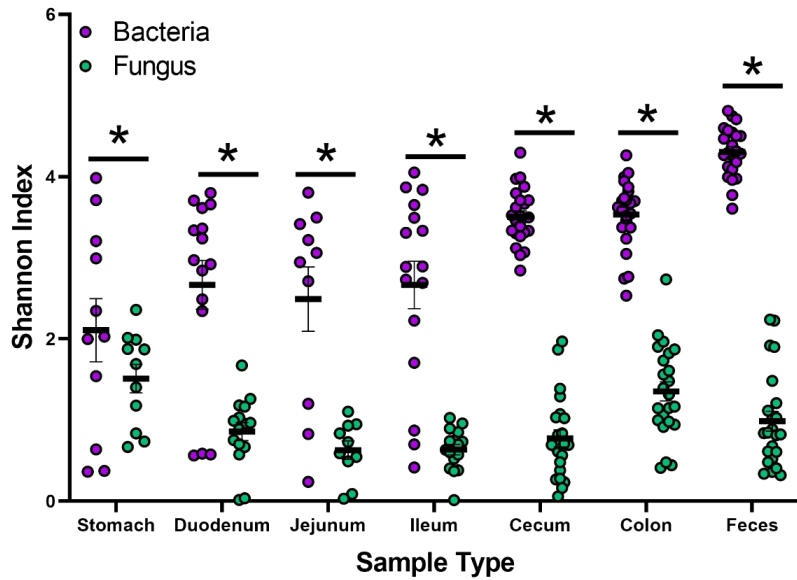


* $p < 0.001$





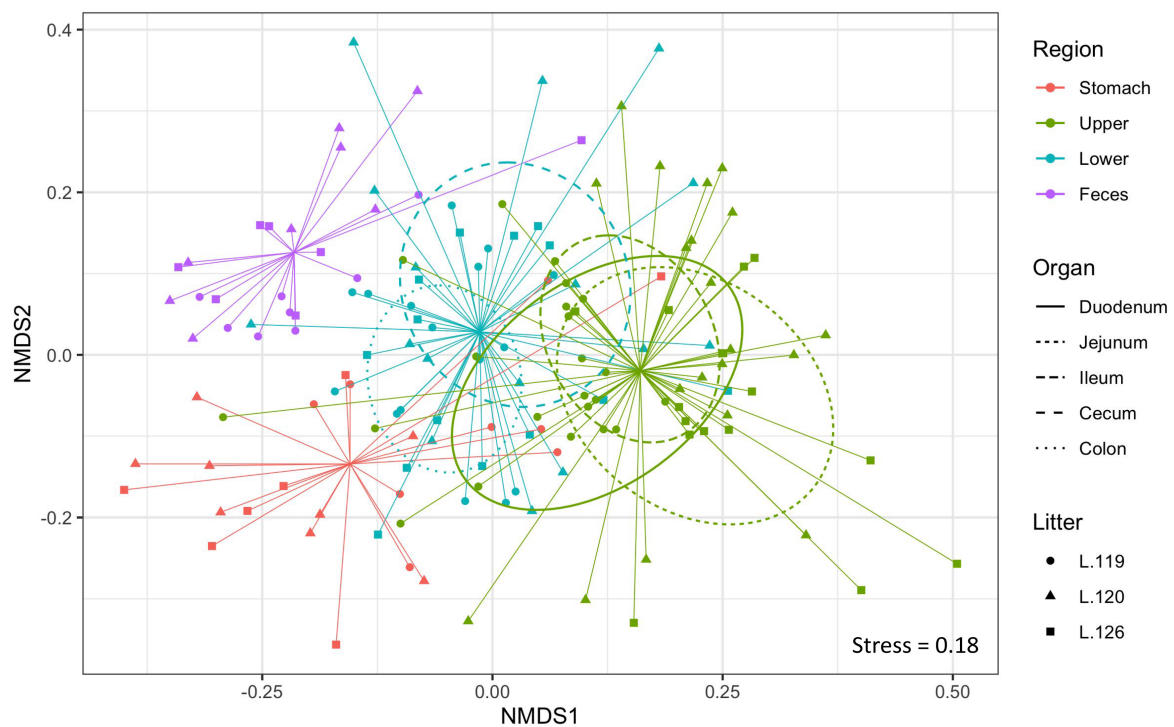
α -diversity of the bacteriome and mycobiome



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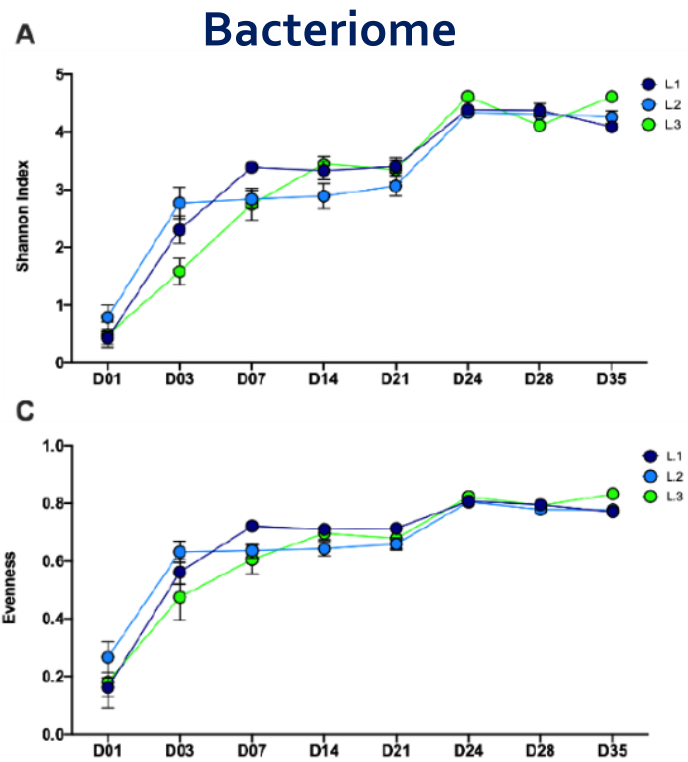


Organ variation of the mycobiome



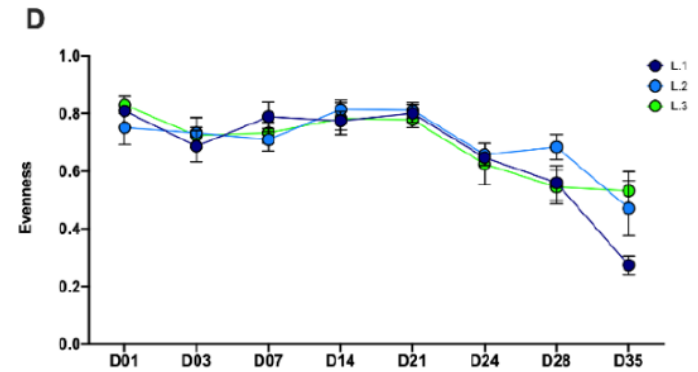
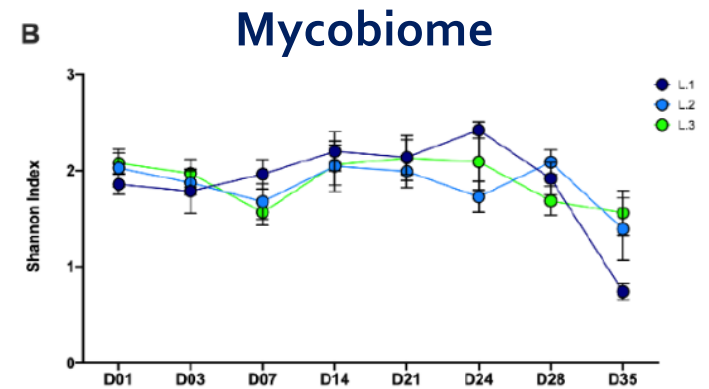
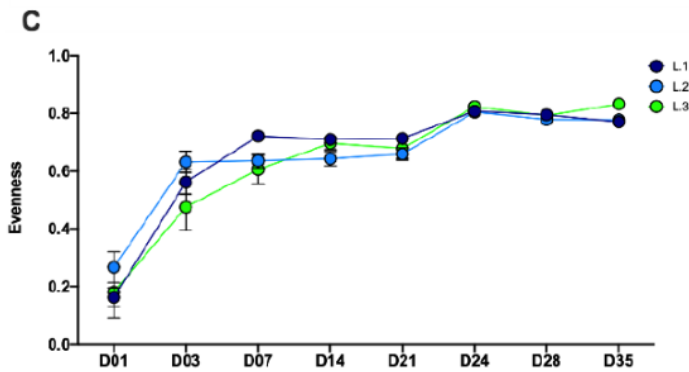
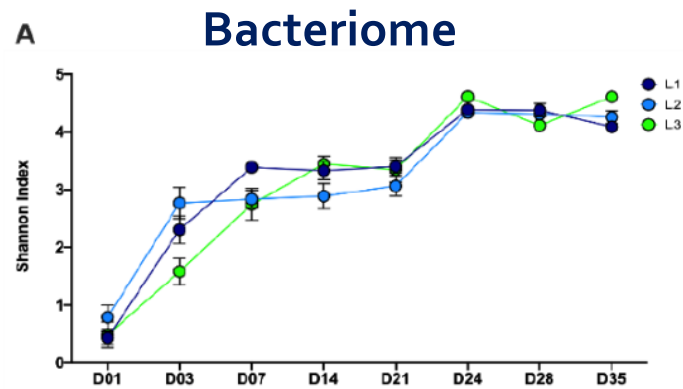


Temporal development of α diversity



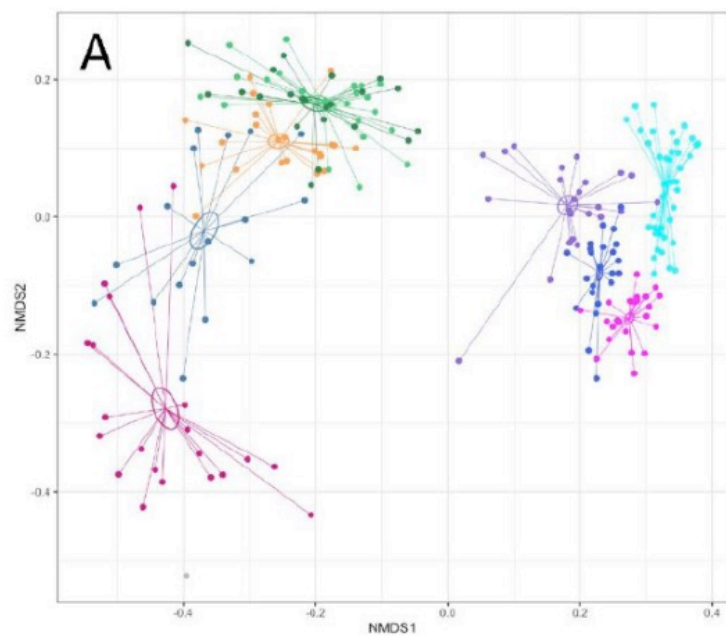


Temporal development of α diversity

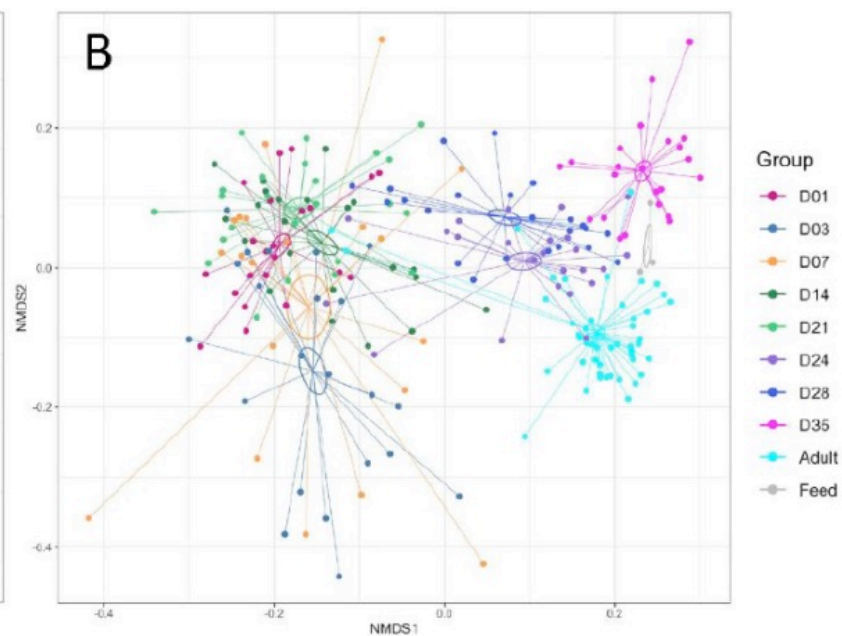


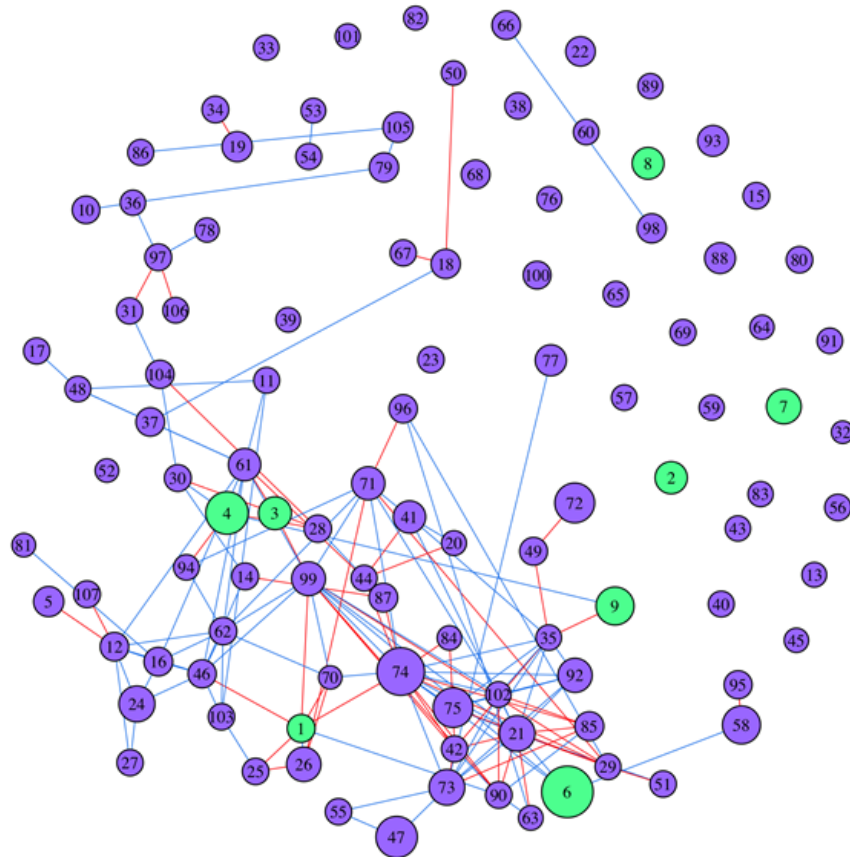
β diversity shows distinct patterns of development

Bacteriome



Mycobiome





Inferred interactions

- Bacteriome – mycobiome
 - genera level
- SparCC correlation network
- Node color = kingdom
- Node size proportional to mean centered-log ratio abundance

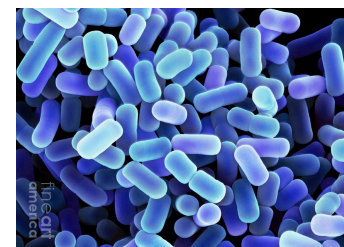




K. slooffiae

**positive
interactions**

Alloprevotella
Prevotella.2
Prevotella.9
Lactobacillus
Subdoligranulum

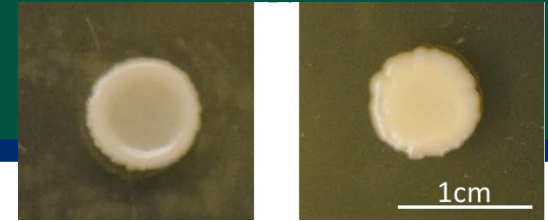


Aspergillus spp.

**negative
interactions**

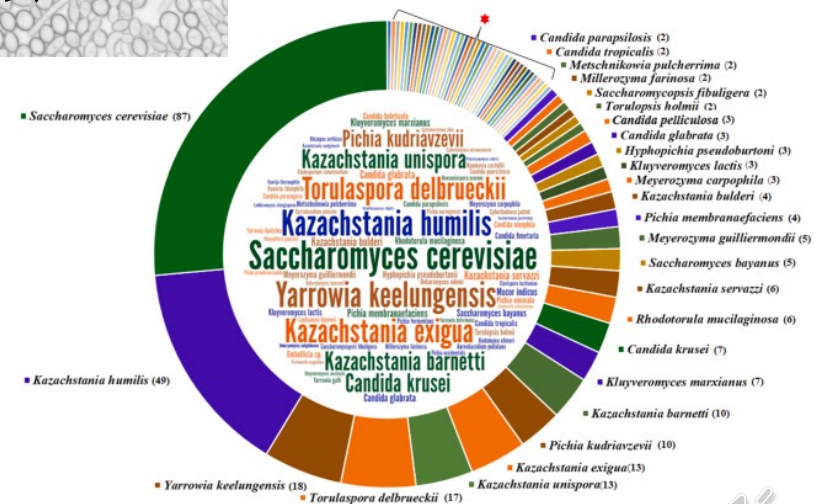
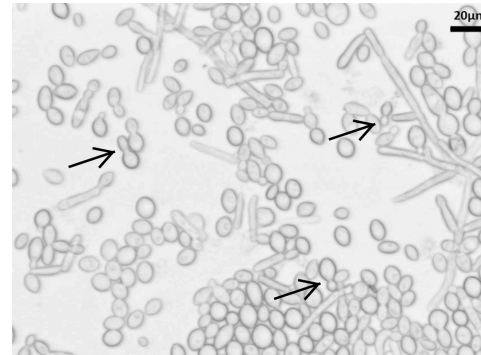
Alloprevotella
Prevotella.2
Prevotella.9
Subdoligranulum





Kazachstania slooffiae

- *Kazachstania telluris* complex
 - Related to *Candida* species
- Most dominant post-weaning fungi
- Cells are high in nitrogen and amino acids



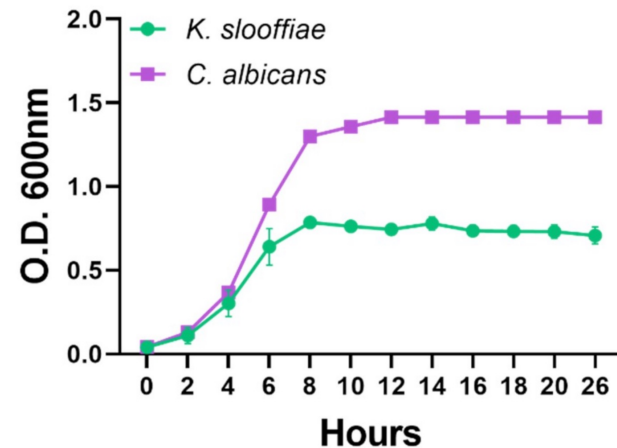


Kazachstania slooffiae

- Antimicrobial resistance not seen
- In vitro* growth curve lags behind pathogenic *C. albicans* CHN1
- Reduced pathogenicity?
 - Genome annotation

Antimicrobial	Susceptible [Y/N]	Antimicrobial Concentration	Drug class
Caspofungin	Y	0.094 ug/mL	Echinocandins
Itraconazole	Y	0.25 ug/mL	Azoles
Voriconazole	Y	0.023 ug/mL	Azoles
Fluconazole	Y	20 ug/mL	Azoles
Amphotericin B	Y	250 mg/mL	Polyenes
Flucytosine	Y	1.0 ug/mL	Pyrimidine analogue
Cefoperazone	N	100 mg/mL	3 rd generation cephalosporin
Ampicillin	N	100 mg/mL	Beta lactam

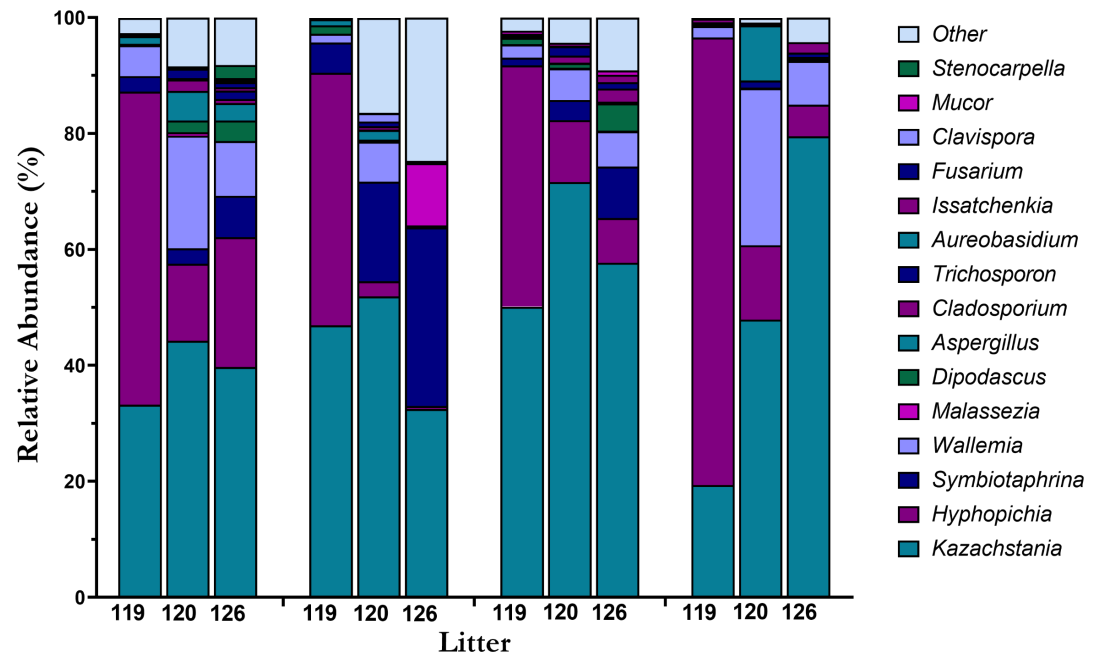
Growth Curve





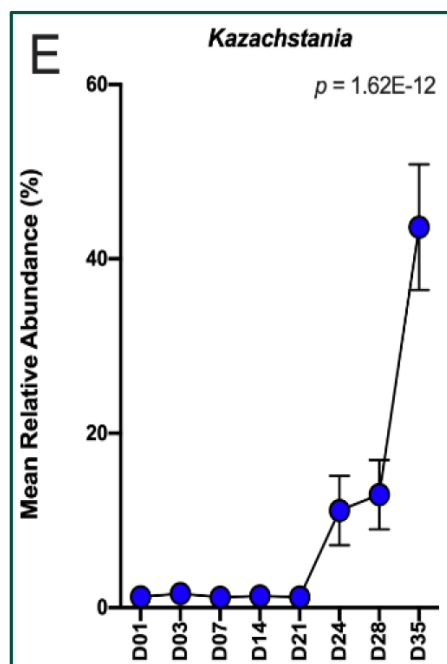
Piglet mycobiome

- *Kazachstania* is the most dominant post-weaning fungi in piglet gut and feces



Fungal colonization

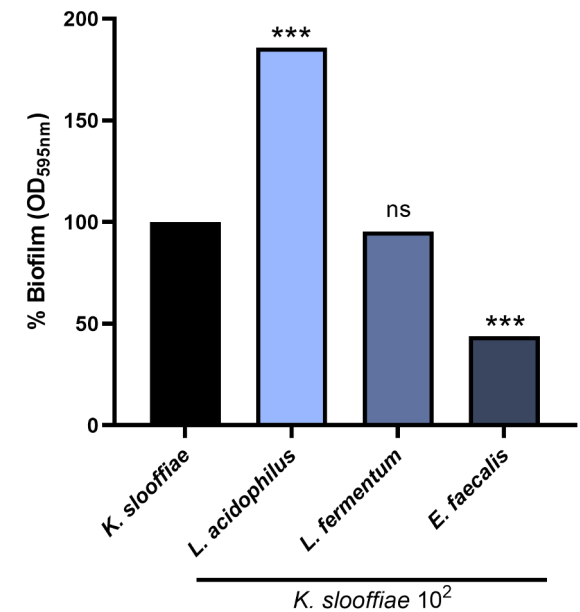
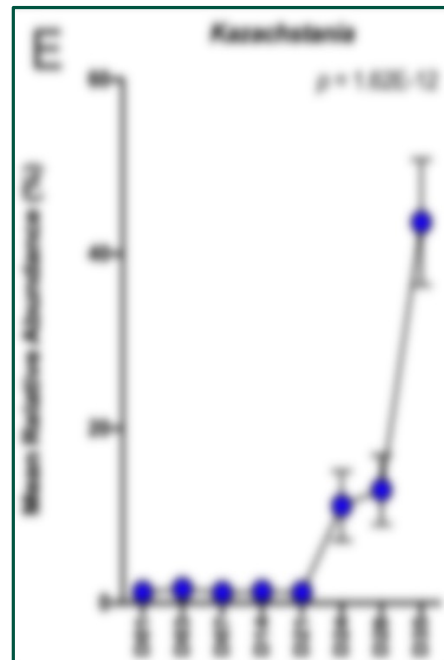
- Colonization increases over time with substantial change at weaning





Fungal colonization

- Colonization increases over time with substantial change at weaning
- In vitro*, *K. slooffiae* creates biofilms
 - Complexity and density reduced or enhanced by bacterial supernatants

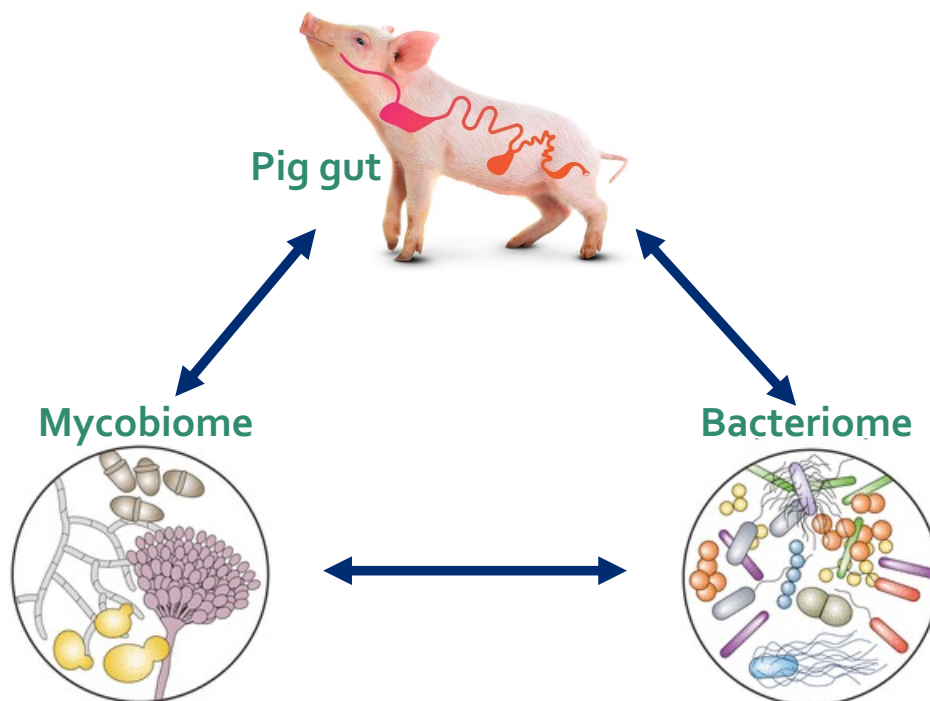




Antagonism and cooperation seen between fungi and bacteria

MYCOBIOME

Rare biosphere
 α -diversity high to low
Easily manipulated

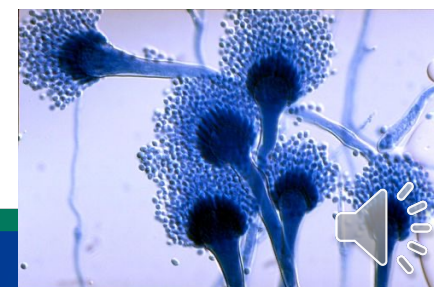


BACTERIOME

Succession pattern
 α -diversity low to high
Alterations can be transient

Fungal Conclusions and Implications

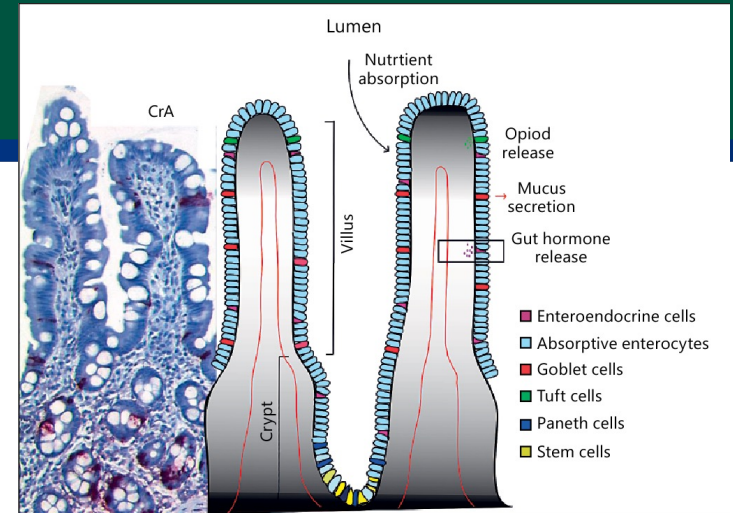
- Fungal importance in pig health
 - Different temporal patterns (bacteria vs. fungi)
 - GI environmental niche effects
- Mycobiome is more malleable than bacteriome
 - Can we enhance piglet growth through supplementation with specific fungi?
 - Can we prevent common GI infections in pigs (i.e. scours) through fungal intervention?
- *K. slooffiae* is the most dominant post-weaning fungus





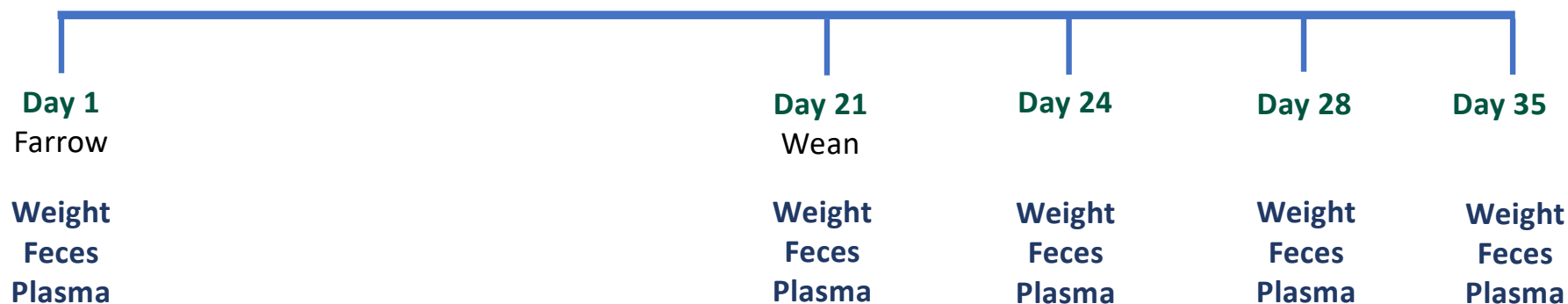
Enteroendocrine peptides and growth

- Enteroendocrine cells
 - Gut and pancreas
- Microbiome has demonstrated relationship with feed efficiency
- Growth rate/feed efficiency is linked to interactions between the microbiome and the enteroendocrine system
 - short chain fatty acids
 - biogenic amines
 - neuroendocrine secretions





Experimental Design



n=48

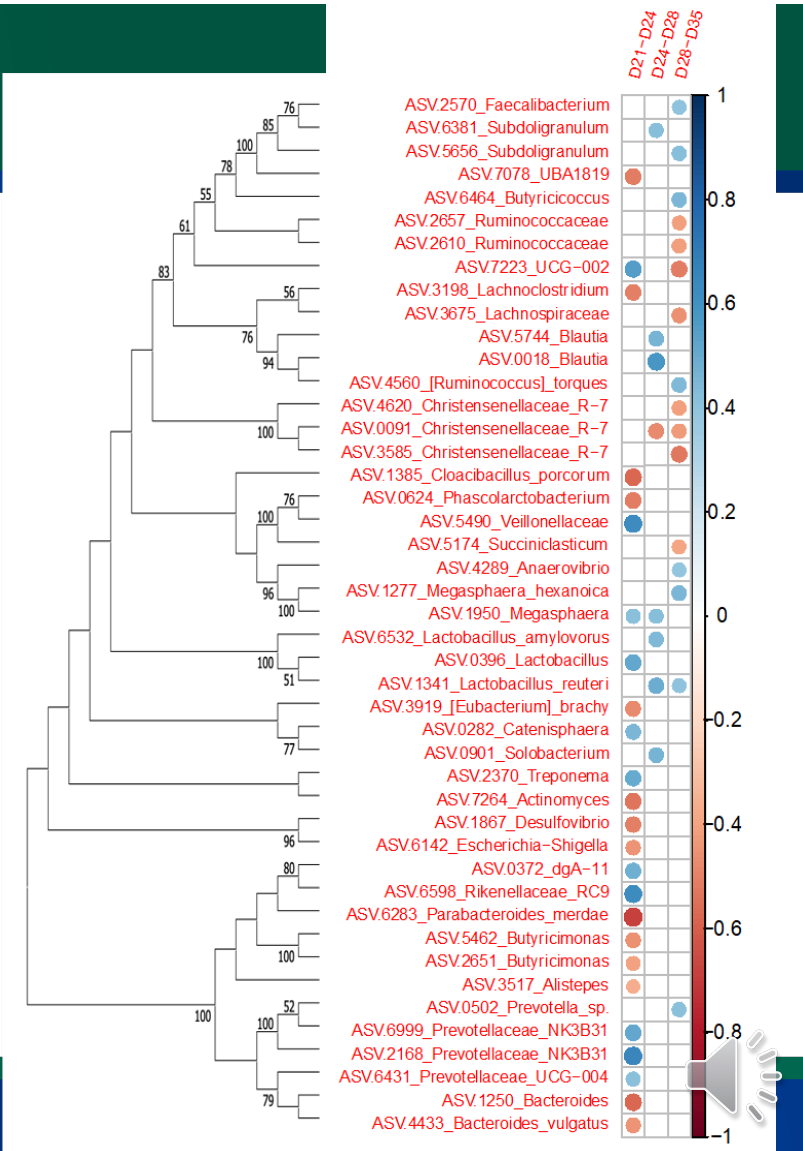
Plasma: ELISAs for hormones and peptides
Feces: 16S Illumina MiSeq
Weight: Growth rate determination

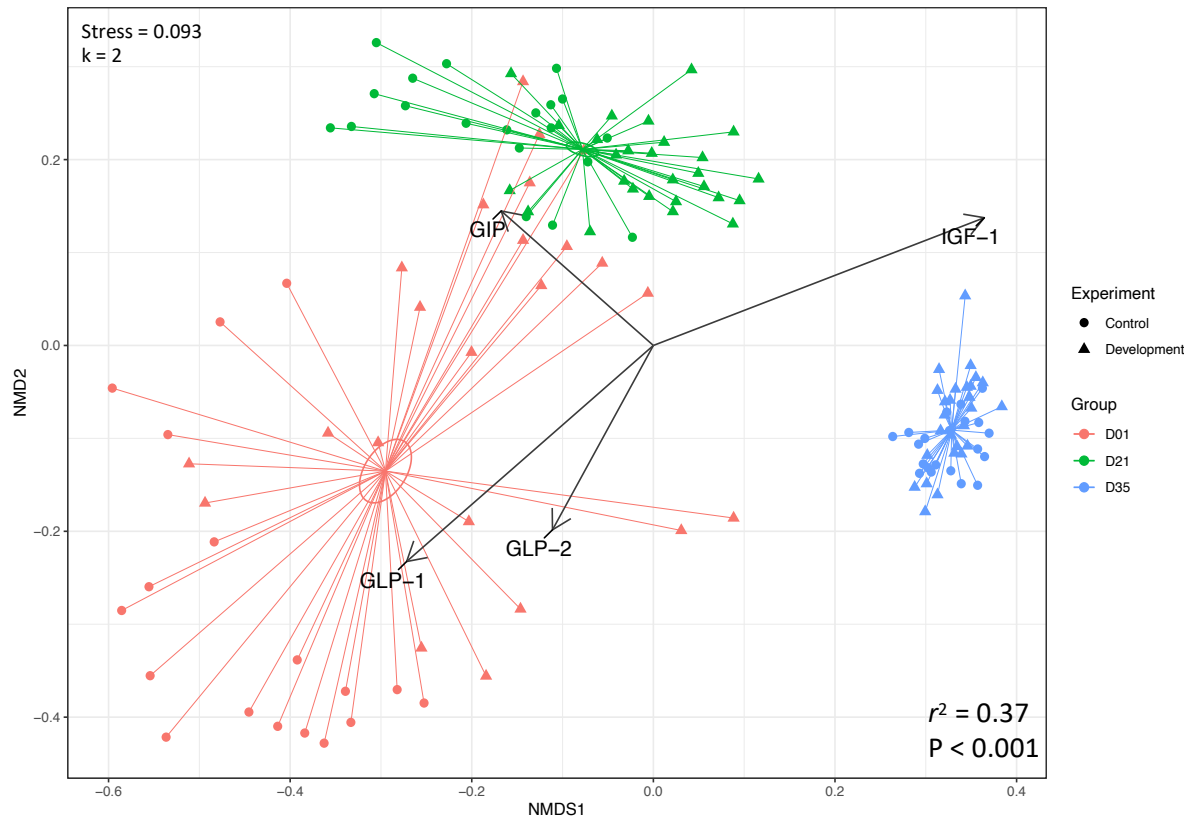




Correlations between weight gain and ASV

- D21-24 has highest # of significant correlations (n=25)
- D24-28 (n=8)
- D28-35 (n=16)
- *Prevotellaceae* NK3B31 had greatest correlation with weight gain at early age ($p < 0.001$)
- Role of diet change to complex polysaccharides utilized by *Prevotellaceae*?





Peptides and age

- IGF-1 – Insulin-like growth factor 1
- GLP-1 – Glucagon-like peptide 1
- GLP-2 – Glucagon-like peptide 2
- GIP – Glucose-dependent insulinotropic polypeptide



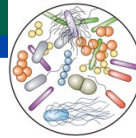


**Can we harness these interactions
to promote disease resistance
and/or growth promotion in the
absence of antibiotics?**

Peptides and age

- IGF-1 – Insulin-like growth factor 1
- GLP-1 – Glucagon-like peptide 1
- GLP-2 – Glucagon-like peptide 2
- GIP – Glucose-dependent insulinotropic polypeptide





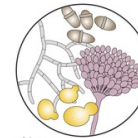
Determine interactions across kingdoms (bacterial, fungal, porcine) at the microbe-lumen interface of the gut.



Identify alternatives to antibiotics (ATA) through bioinformatics-based approaches through machine learning (microbiome, metagenome, metatranscriptome).



Examine the effect of candidate ATA on the innate immune response at the gut mucosa.



Identify the role of *Kazachstania slooffiae*, a porcine fungal commensal, in pig performance.



Identify mechanisms of action of antibiotics or ATA in promoting growth through machine learning.



Evaluate potential probiotic candidates, such as *Clostridium scindens*, on preweaning growth and health.

Overall research focus:

Determine the mechanisms behind antibiotic-induced animal growth and identify potential alternative growth promotants in swine during the weaning transition





Acknowledgements

Lab Members

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- Nora Carrillo

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- Lea Rempel



THE MICHIGAN
MICROBIOME
PROJECT



UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE
INSTITUTE FOR GENOME SCIENCES

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USDA National Programs AMR/ATA Grant

Current Post Doctoral Opportunities

- Immunology
 - Machine Learning
- katie.summers@usda.gov

