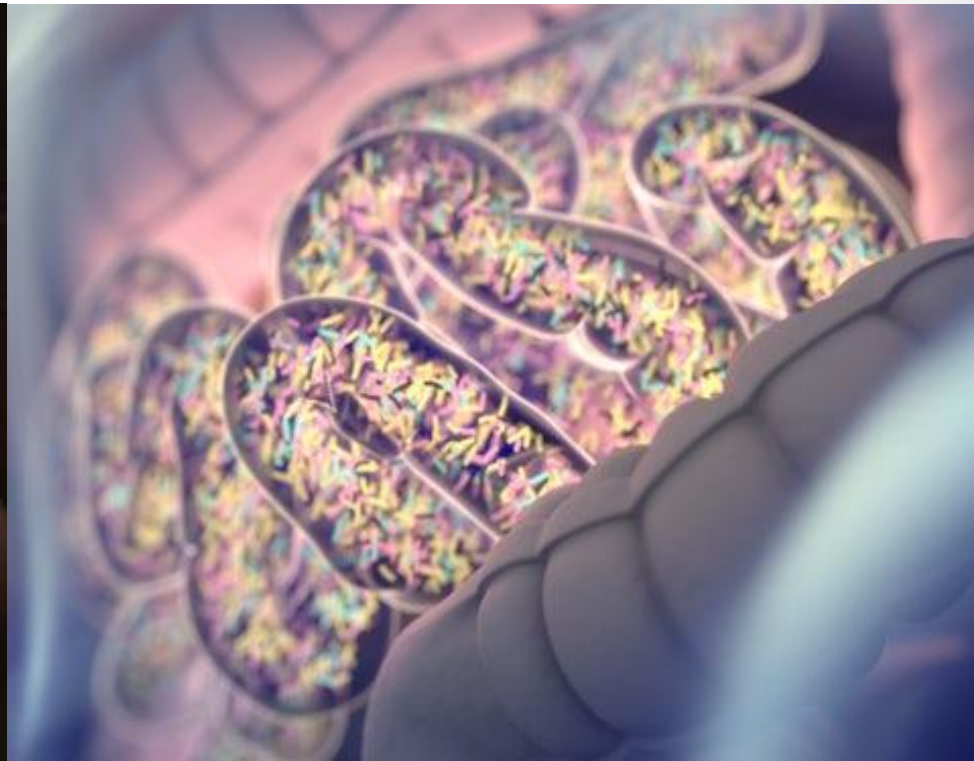


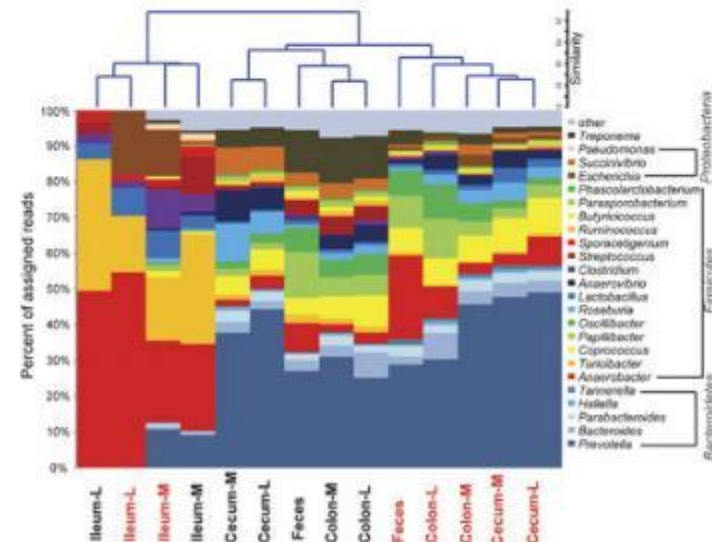
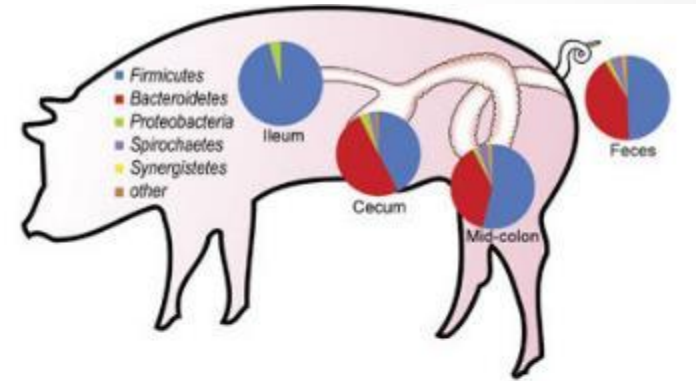
# Modulation of the gut microbiota: A tool to improve swine health and performance

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Food Biotechnology Laboratory, BIOTEC



# The presence of microbial community in pig gut

- Community structure varies from location to location
- > 1,000 bacterial species identified
- Major phyla: *Firmicutes*, *Bacteroidetes*, *Protiobacteria*, *Spirochaetes*



# The swine gut microbiota: a meta analysis

- **>25 million** quality-filtered 16S rRNA gene sequences from **939** swine GI samples
- Sequences from **3** continents and **10** countries (half from U. S.)
- Pig age range: pre-wean to slaughter



# “Core” microbiota

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- >99% of **fecal** samples contained the following bacterial genera:
  - *Prevotella* (an uncultured member was found in 75% of samples!)
  - *Clostridium*
  - *Alloprevotella*
  - *Ruminococcus*
  - the RC9 gut group (member of the *Rikenellaceae* family, related to *Prevotella*)

# Roles of the gut microbiota in swine health

- Revealed by the study in germ-free vs conventional pigs



# The Role in intestinal development

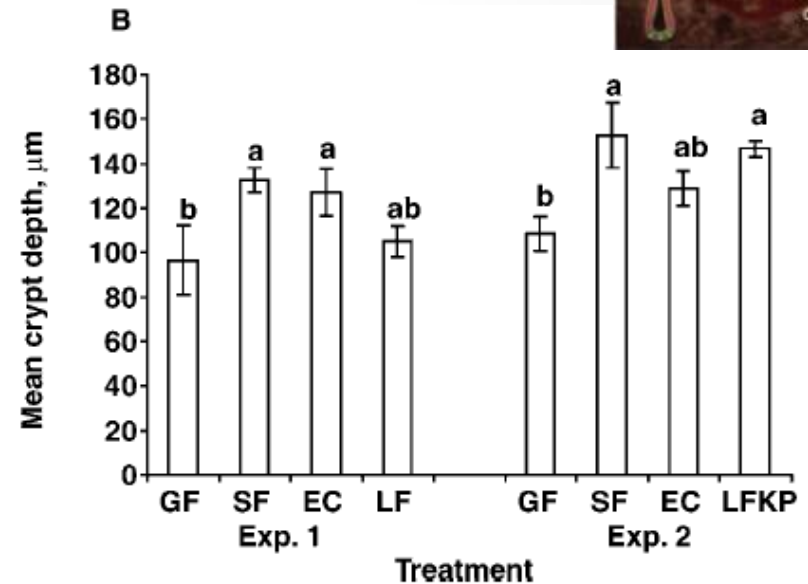
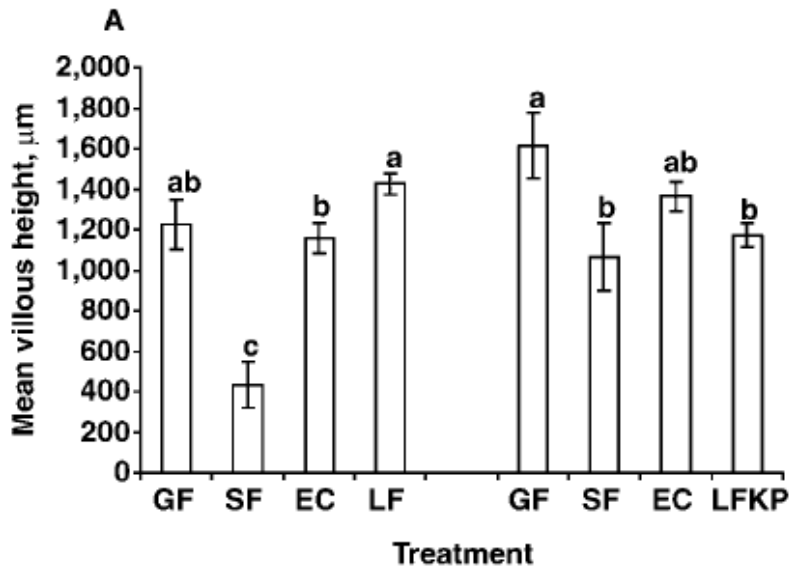
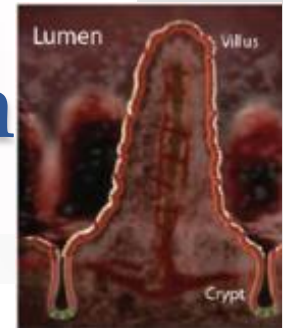


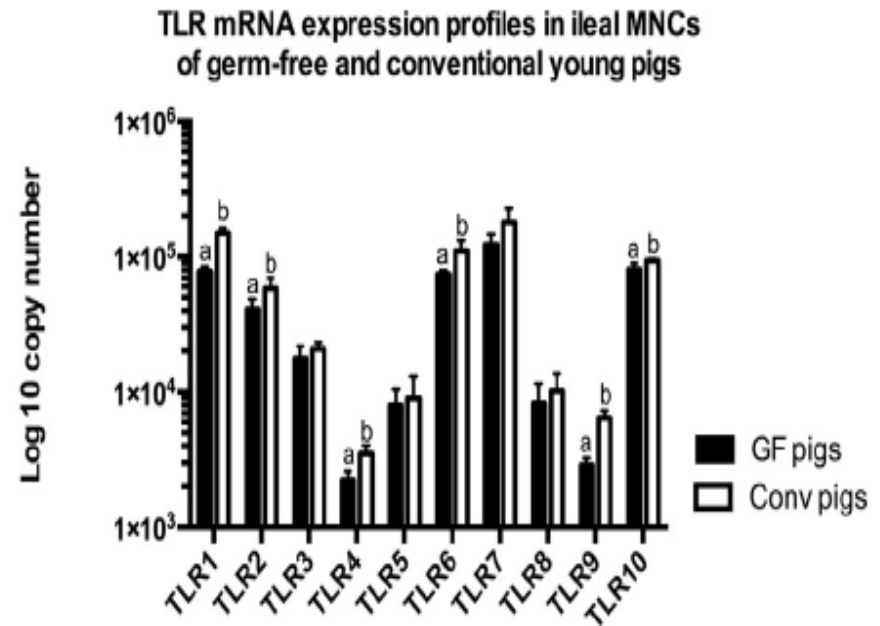
Figure 1. Mean villous height (A) and crypt depth (B) at the 75% location of the small intestine as measured from the pyloric sphincter for Exp. 1 and 2 in germ-free (GF), *Lactobacillus fermentum* (LF) or *Escherichia coli* (EC) monoassociated, LF and *Klebsiella pneumoniae* (LFKP) diassociated, and conventionalized (SF) pigs. Vertical bars represent SE. a,b Means within the same experiment with a different letter are different ( $P < 0.05$ ).

Willing BP, Van Kessel AG. 2007. Enterocyte proliferation and apoptosis in the caudal small intestine is influenced by the composition of colonizing commensal bacteria in the neonatal gnotobiotic pig. *J Anim Sci* 85:3256-66.

# The role in immune activation

Compared with a germfree pig, the intestine of a conventional pig displays

- markedly up-regulated expression of pro-inflammatory cytokines
- increased infiltration of immune cells
- increased organization of lymphoid follicles and Peyer's patches



Shao L. et. al., 2016. *Vet Immunol Immunopathol* 171:7-16.

Shirkey TW *et al.*, 2006. *Experimental Biology and Medicine* 231:1333-1345.

# The role in short-chain fatty acid production

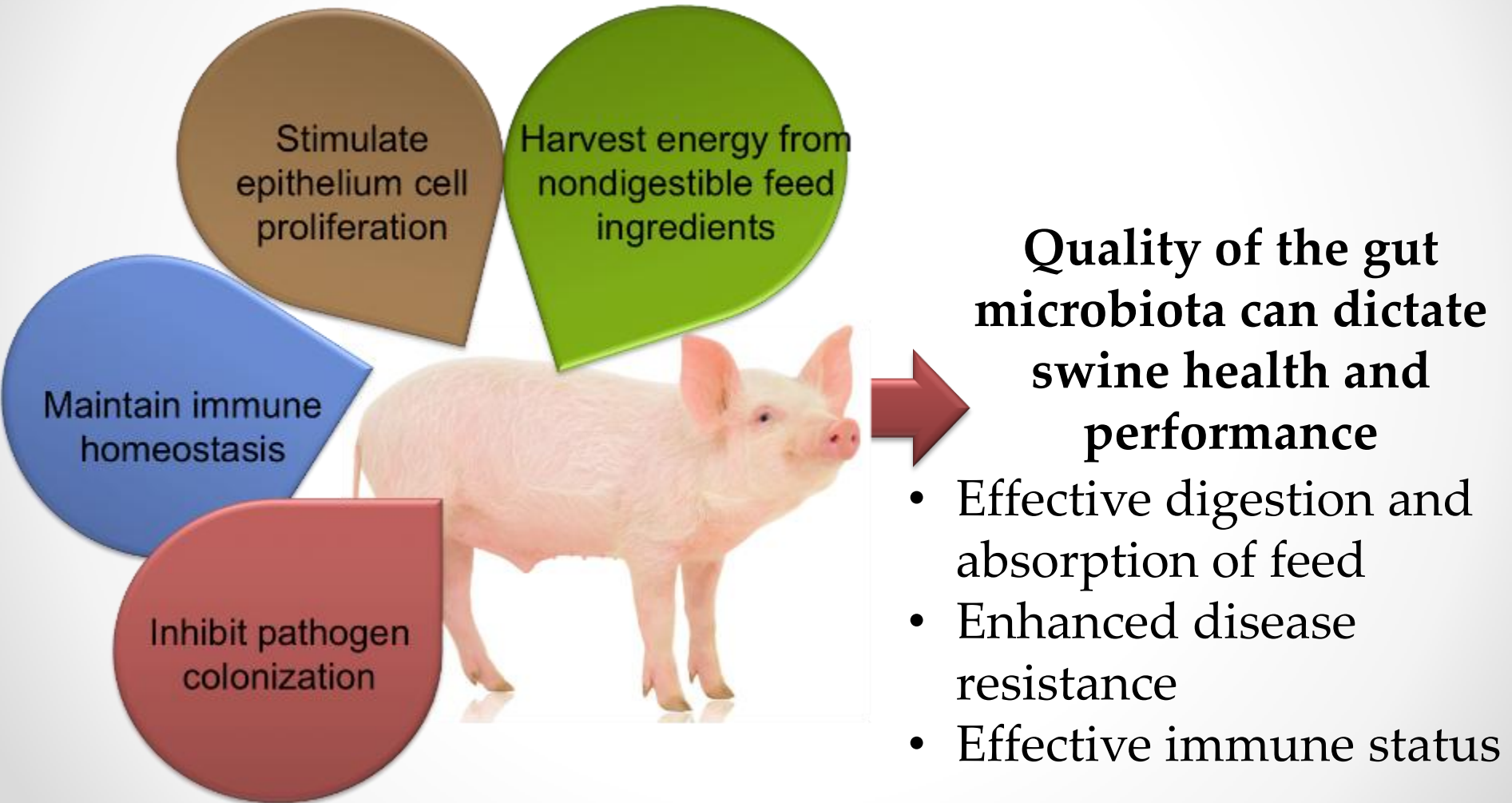
## The effect of SCFA on intestinal health of pigs

Fatty acids	Effects	Animals
SCFA	<ul style="list-style-type: none"> <li>↓Intestinal atrophy, ↑structural indices of GI adaptation, ↑enterocyte proliferation, ↓enterocyte apoptosis</li> <li>↓Diarrhoea incidence, ↑serum IgG concentration and jejunal IgA<sup>+</sup> cell count</li> <li>↑ Intestinal morphology and disaccharidase activity</li> <li>↓Intestinal injury by ↓ apoptosis, ↑ tight-junction formation, ↑EGFR signaling</li> <li>↑Recovering of intestinal wound healing</li> <li>↑Intestinal morphology, ↓total viable counts of proximal colon <i>Clostridium</i> and <i>Escherichia coli</i>, ↓serum TNF-<math>\alpha</math> and IL-6 levels, and intestinal DNA-binding activity of NF-<math>\kappa</math>B</li> <li>↓Gastric emptying and intestinal mucosa weight</li> <li>↑Host defense peptide gene expression</li> </ul>	<ul style="list-style-type: none"> <li>TPN-fed neonatal pigs</li> <li>Weaned piglets</li> <li>Newly weaned piglets</li> <li>Pig model of acetic acid-induced colitis</li> <li>Porcine IPEC J2</li> <li>Weaned piglets</li> <li>Piglets before or after weaning</li> <li>IPEC-J2</li> </ul>

Liu Y. 2015. Fatty acids, inflammation and intestinal health in pigs. J Anim Sci Biotechnol 6:41.

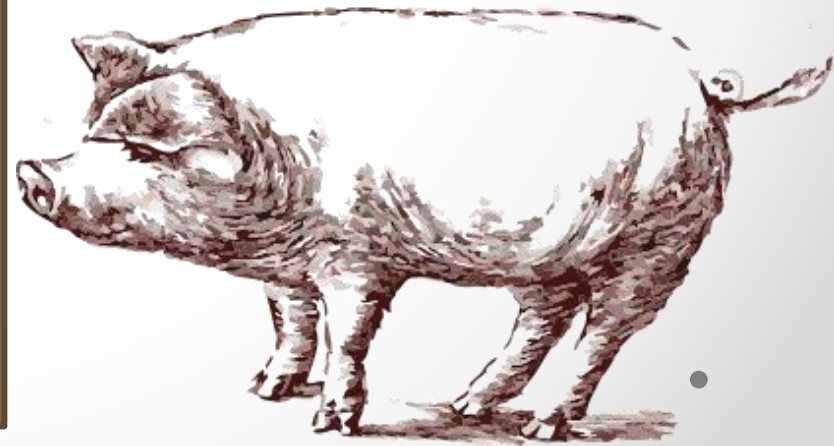


# Roles of the gut microbiota in swine health and performance



# How to modulate the gut microbiota in the directions that enhance swine health and performance

- Effective digestion and absorption of feed
- Enhanced disease resistance
- Effective immune status



Direction 1:  
Identify and enrich microbial  
composition that promotes  
growth



# A link between gut microbiota composition and growth traits

Large White, 28-day old piglets  
(No significant difference in BW)



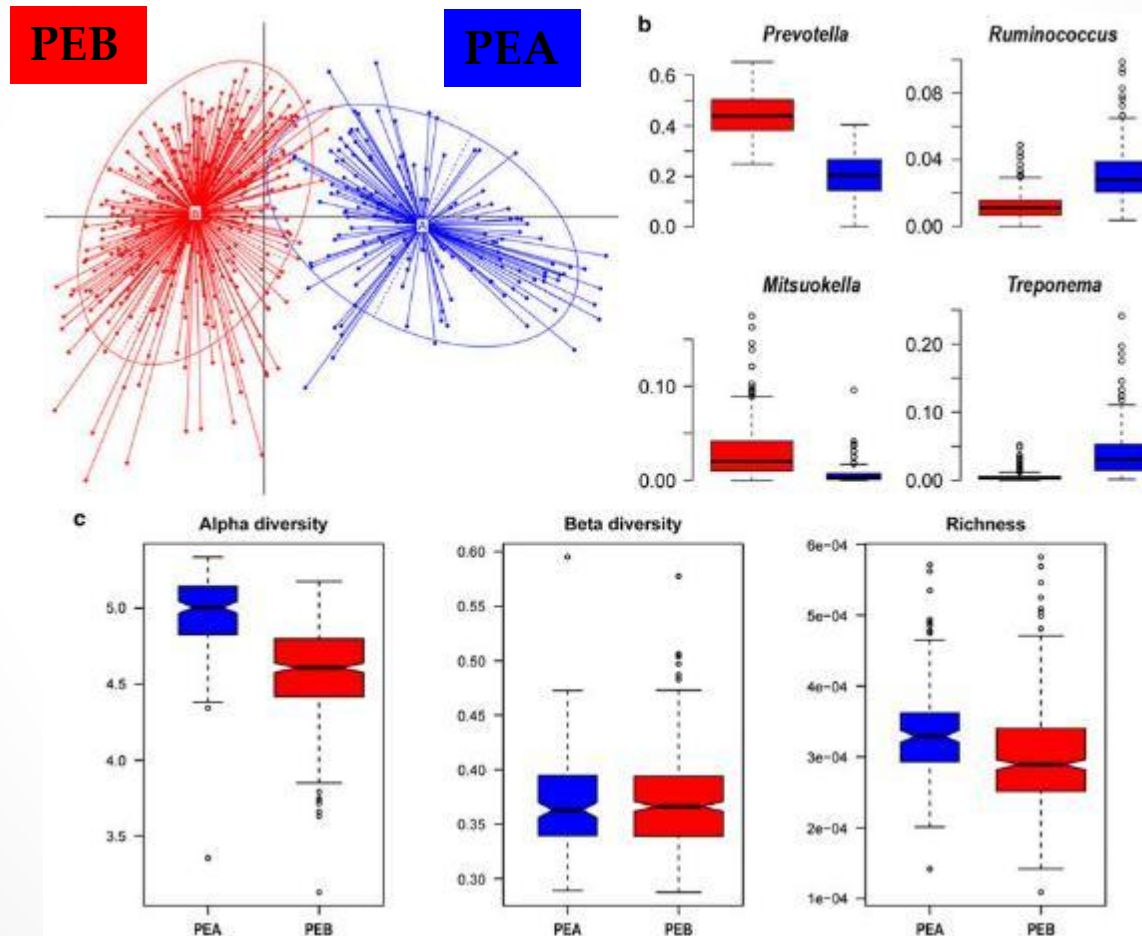
Randomly assigned into different pens  
(Controlled management, environment, housing factors)



- Fed with cereal-based diets
- Reared without antibiotics

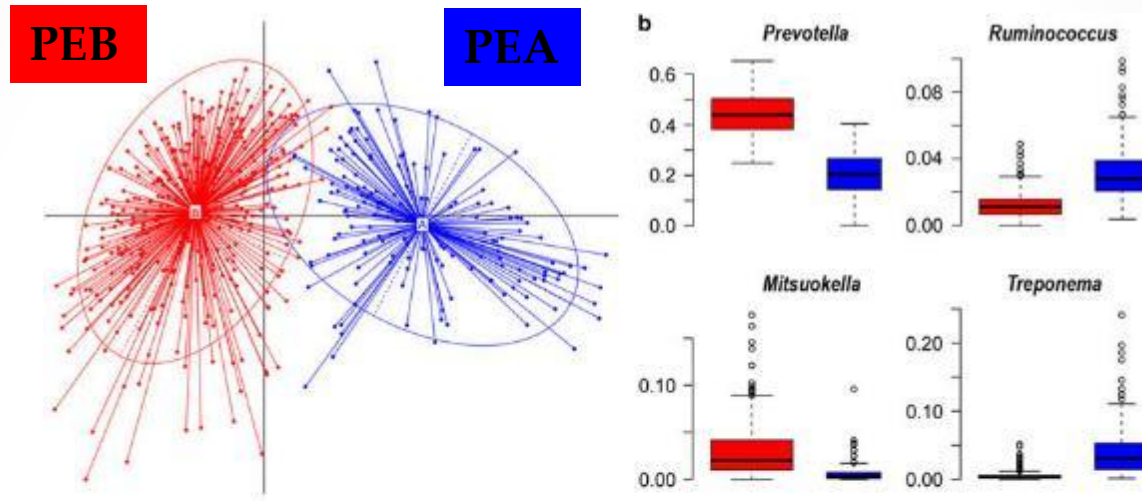
Analyzed fecal microbiota of **518** healthy pigs  
at **60** days of age using **16S rRNA gene pyrosequencing**

# Enterotype-like clustering of 518 piglets' microbiota, based on genus abundance



Ramayo-Caldas *et al.*, 2016. Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. ISME J 10:2973-2977.

# Enterotype-like clustering and association with host performance



Enterotypes	BW (Kg)	ADG (g/day)
<b>PEA</b>	25.8 ± 0.2	438.3 ± 6.7
<b>PEB</b>	26.7 ± 0.1**	456.3 ± 4.4*

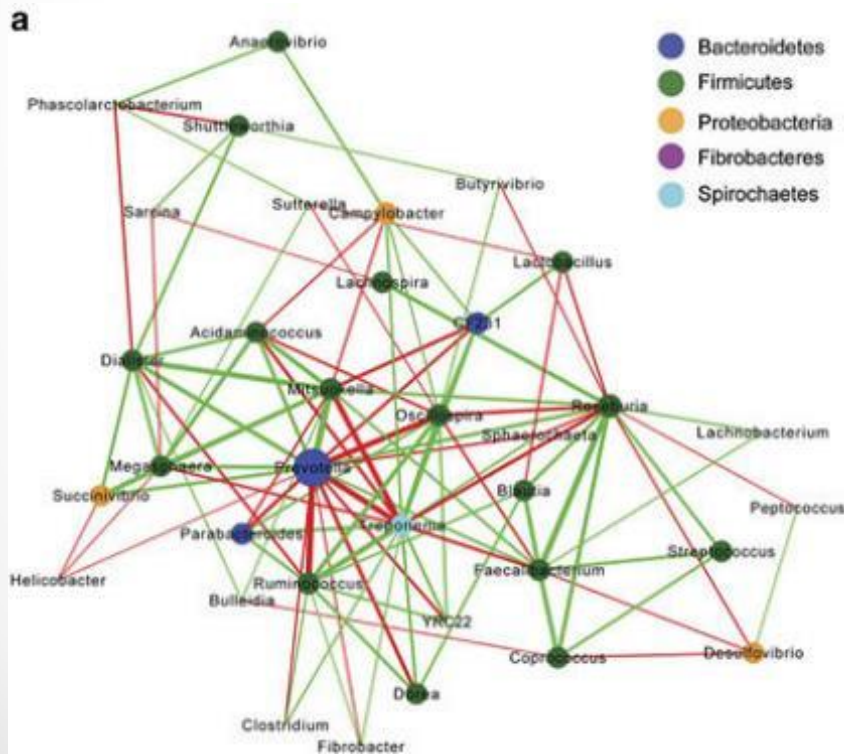
\* p-value < 0.05    \*\* p-value < 0.01

Ramayo-Caldas *et al.*, 2016. Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. ISME J 10:2973-2977.

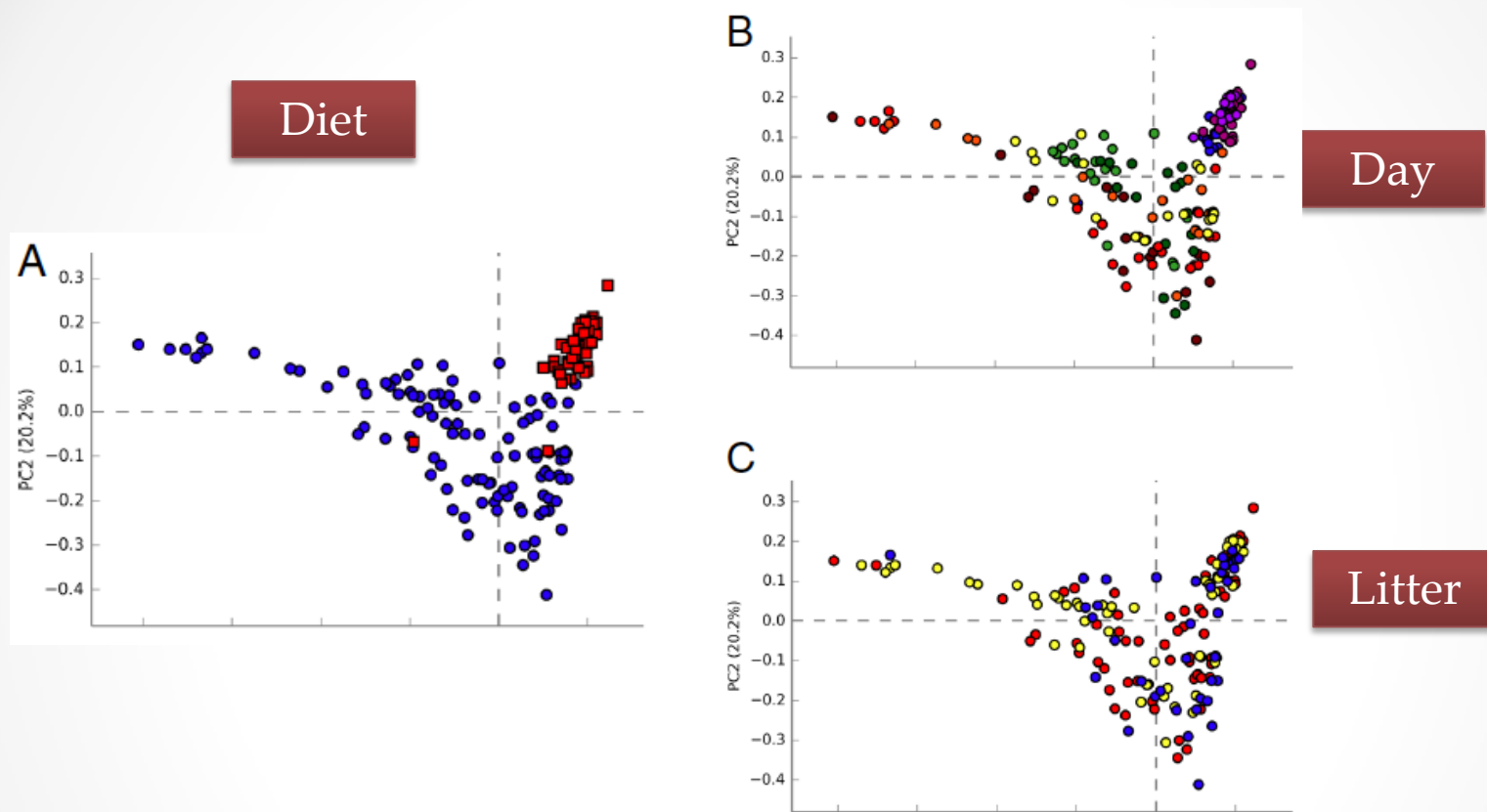
# From enterotypes to translational research for swine farming

Enrich *Prevotella* cluster in the swine gut microbiome

- Probiotic *Prevotella* + relating species
- Prebiotics



# Diet shapes the gut microbiota of pigs



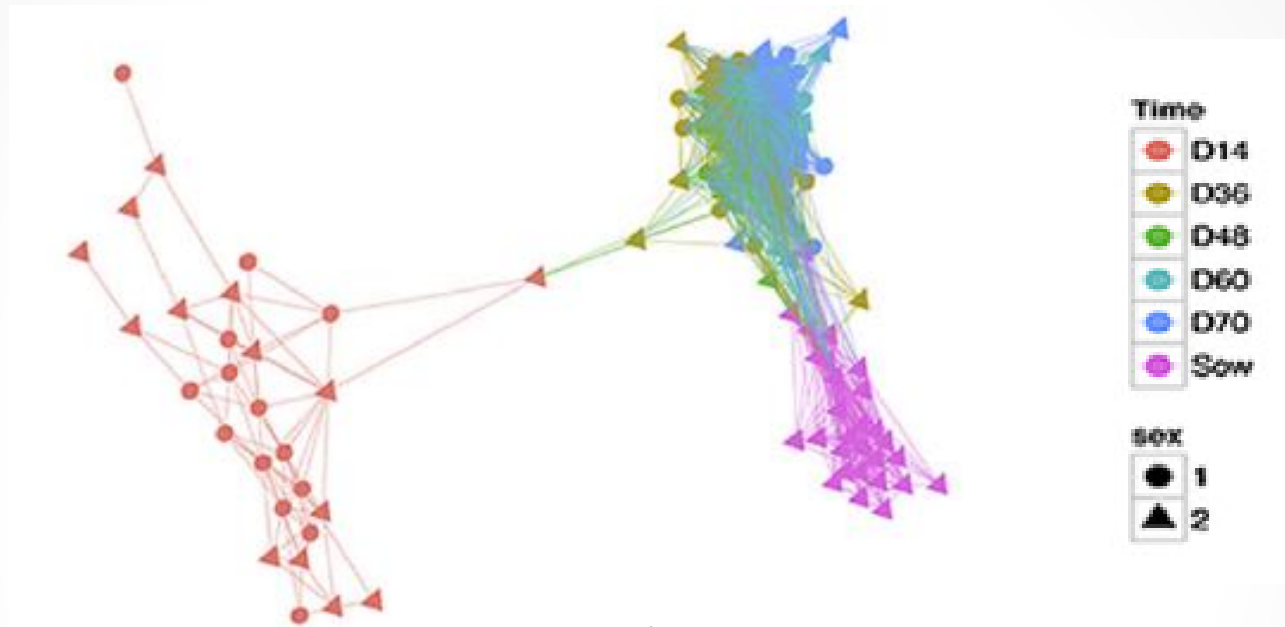
Principal component analysis plots of pig fecal microbiota colored by diet (**a** nursing, blue; weaned, red), day (**b** day 1, dark red; day 3, red; day 5, orange; day 7, yellow; day 14, green; day 21, dark green; day 28, blue; day 35, dark blue; day 42, purple), or litter (**c** litter 1, red; litter 2, yellow; litter 3, blue)

Frese SA *et al.*, 2015. Diet shapes the gut microbiome of pigs during nursing and weaning. *Microbiome* 3:28.



Direction 2:  
Stimulate the protective gut  
microbiota during weaning

# Diversity relationship of fecal microbiota (genus level) in piglets at different ages



## Representative genera:

### Suckling piglets

*Bacteroides*,  
*Oscillibacter*,  
*Escherichia/Shigella*,  
*Lactobacillus*  
*Unclassified Ruminococcaceae*

### After weaning

*Acetivibrio*,  
*Dialister*,  
*Oribacterium*,  
*Succinivibrio*  
*Prevotella*

Mach N. *et. al.*, 2015. Early-life establishment of the swine gut microbiome and impact on host phenotypes. *Environ Microbiol Rep* 7:554-69.

# Disruption of the gut microbiota during weaning



Weaning stresses



Disrupted the gut microbial succession

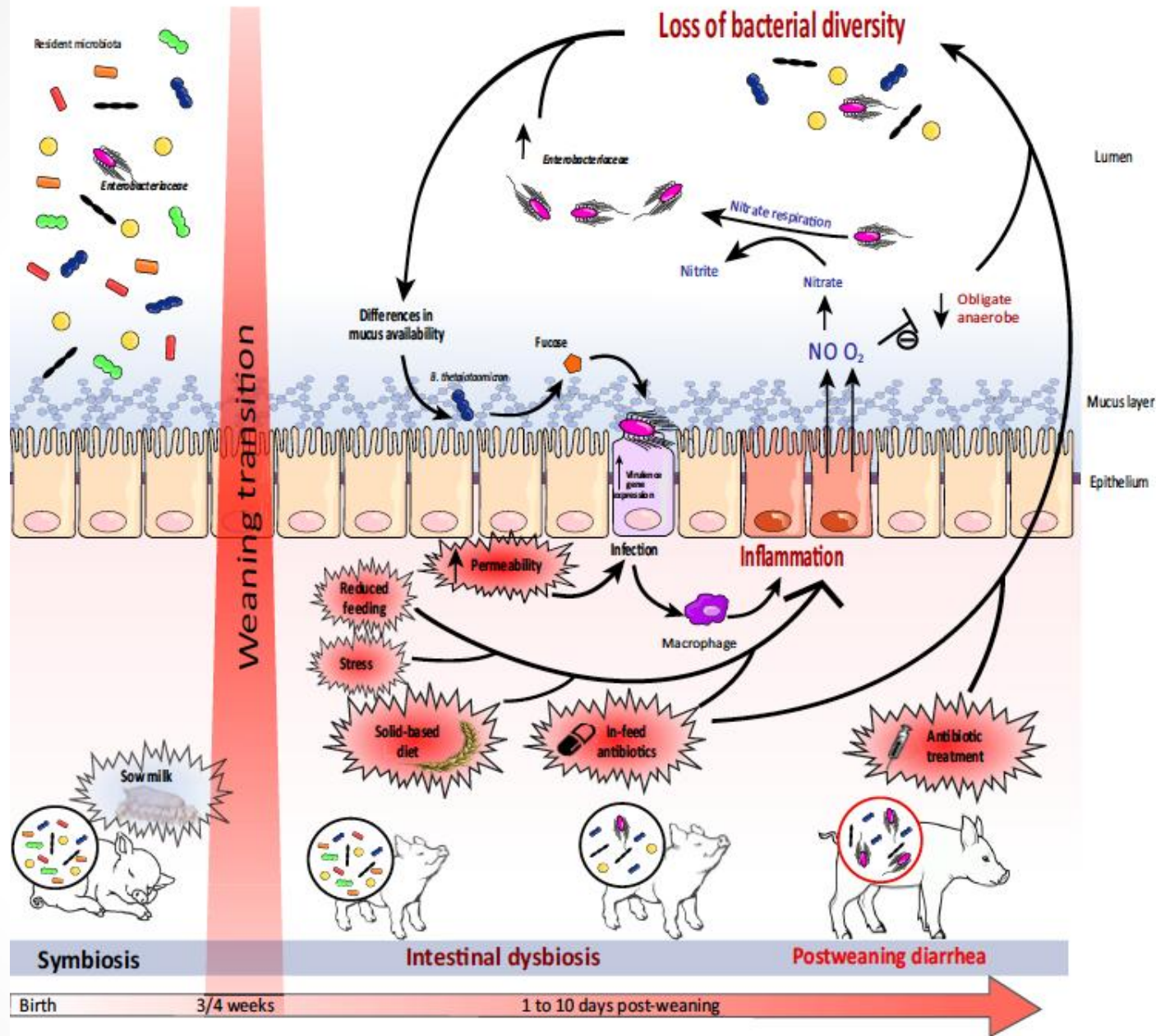


Outgrowth of pathobionts



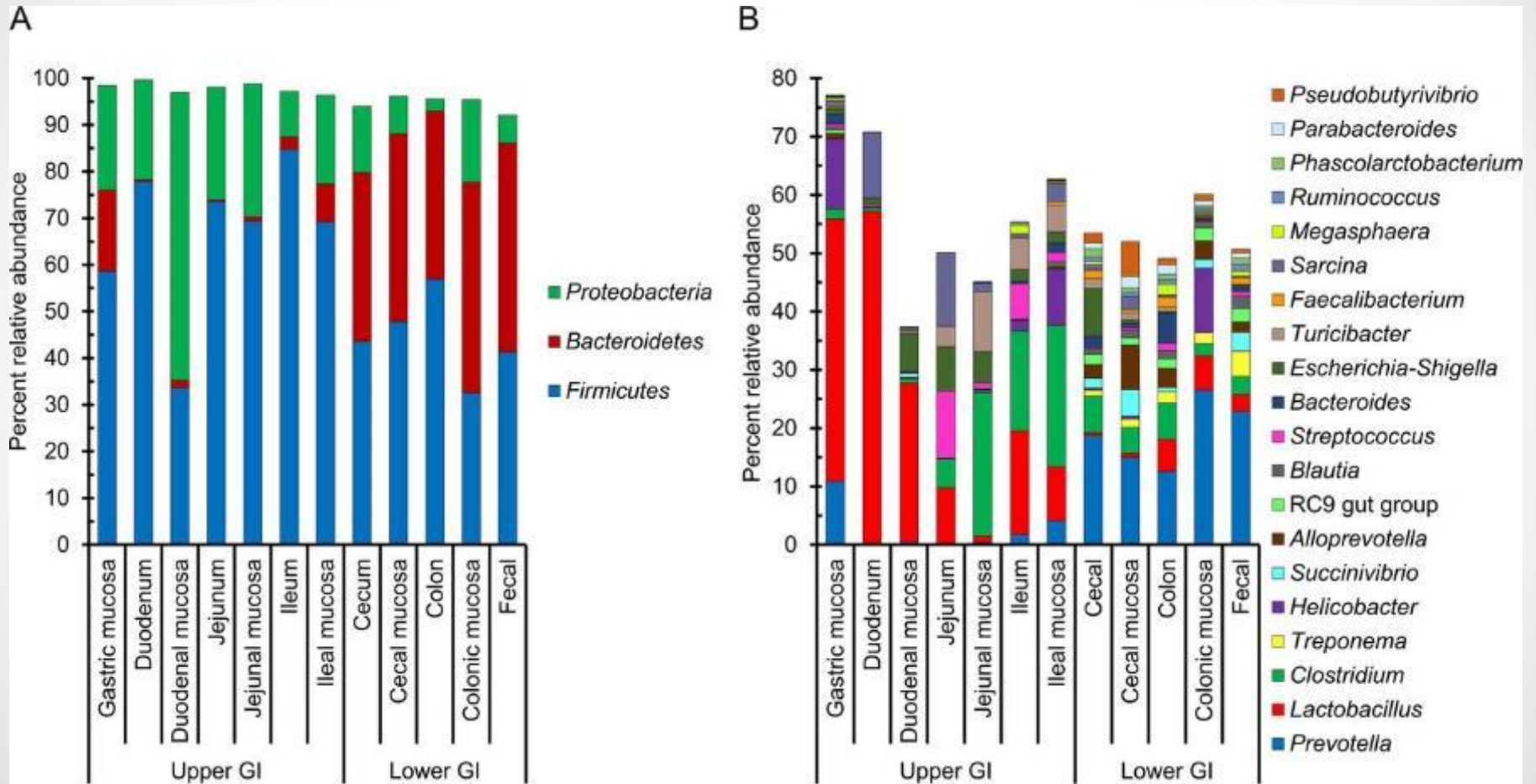
Diseases

# Impact of weaning on piglet gut microbiota and pathogen expansion



Gresse R *et al.*, 2017. Gut Microbiota Dysbiosis in Postweaning Piglets: Understanding the Keys to Health. Trends Microbiol 25:851-873.

**Percent relative abundances of the three most abundant phyla (A) and 20 most abundant genera (B) by gastrointestinal tract sample type.**



# Diversity of ileal lumen microbiota in neonatal, weaned, and unweaned piglets

**Table 2.** Real-time PCR results for *Lactobacillus sobrius*, *L. reuteri*, *L. acidophilus* and total lactobacilli (LAB) in porcine ileal samples.

Day-treatment	<i>L. sobrius</i>	<i>L. reuteri</i>	<i>L. acidophilus</i>	LAB
2	$4.72 \pm 3.9 \times 10^7$	$1.34 \pm 0.5 \times 10^7$	$4.84 \pm 0.1 \times 10^4$	$7 \pm 0.1 \times 10^7$
5	$9.63 \pm 1.2 \times 10^8$	$2.13 \pm 2.17 \times 10^7$	$7.61 \pm 0.9 \times 10^4$	$9.7 \pm 0.5 \times 10^8$
12	$1.01 \pm 1.05 \times 10^8$	$3.08 \pm 3.97 \times 10^7$	$7.22 \pm 1.1 \times 10^4$	$1.4 \pm 0.9 \times 10^8$
19	$6.85 \pm 0.1 \times 10^8$	$1.48 \pm 1.3 \times 10^7$	$1.04 \pm 0.1 \times 10^4$	$7.1 \pm 2.6 \times 10^8$
23-UW	$4.72 \pm 3.9 \times 10^7$	$1.34 \pm 0.5 \times 10^7$	$4.84 \pm 0.1 \times 10^4$	$7.1 \pm 1.3 \times 10^7$
23-W	$< 10^{3*}$	$< 10^{3*}$	$< 10^{3*}$	$4.4 \pm 0.5 \times 10^{5*}$
27-UW	$2.46 \pm 0.9 \times 10^8$	$1.07 \pm 0.8 \times 10^7$	$4.91 \pm 0.1 \times 10^4$	$8.2 \pm 0.8 \times 10^8$
27-W	$< 10^{3*}$	$< 10^{3*}$	$< 10^{3*}$	$5.1 \pm 0.1 \times 10^{6*}$
32-UW	$1.18 \pm 0.9 \times 10^7$	$6.13 \pm 1.3 \times 10^7$	$< 10^3$	$6.1 \pm 0.5 \times 10^7$
32-W	$< 10^{3*}$	$< 10^{3*}$	$< 10^{3*}$	$2.1 \pm 0.1 \times 10^{6*}$

\*Significant differences from the respective values compared at  $P < 0.05$ .

Two samples were examined for the samples of 2-, 5- and 12-day-old piglets. Four samples were quantified for all 23-, 27- and 32-day-old piglets. UW, unweaned piglets; W, weaned piglets. Counts are expressed as mean  $\pm$  SD cells  $g^{-1}$  wet weight.

Konstantinov SR *et al.*, 2006. Post-natal development of the porcine microbiota composition and activities. *Environ Microbiol* 8:1191-9.

# Disruption of the gut microbiota during weaning



Antibiotics



Weaning stresses



Disrupted the gut microbial succession



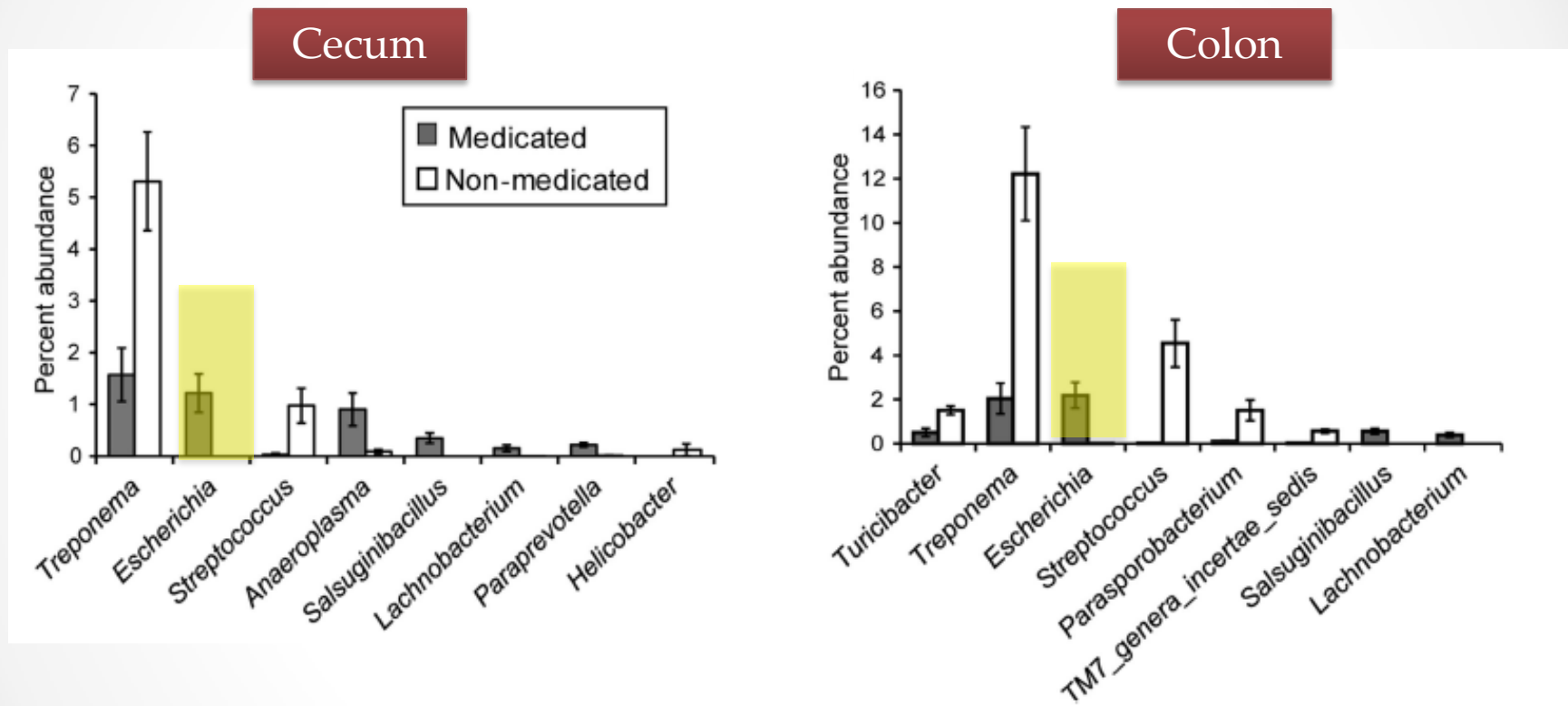
Outgrowth of pathobionts



Diseases



# Microbiota disturbance: Impact of in-feed antibiotics ASP250 (chlortetracycline, sulfamethazine and penicillin)

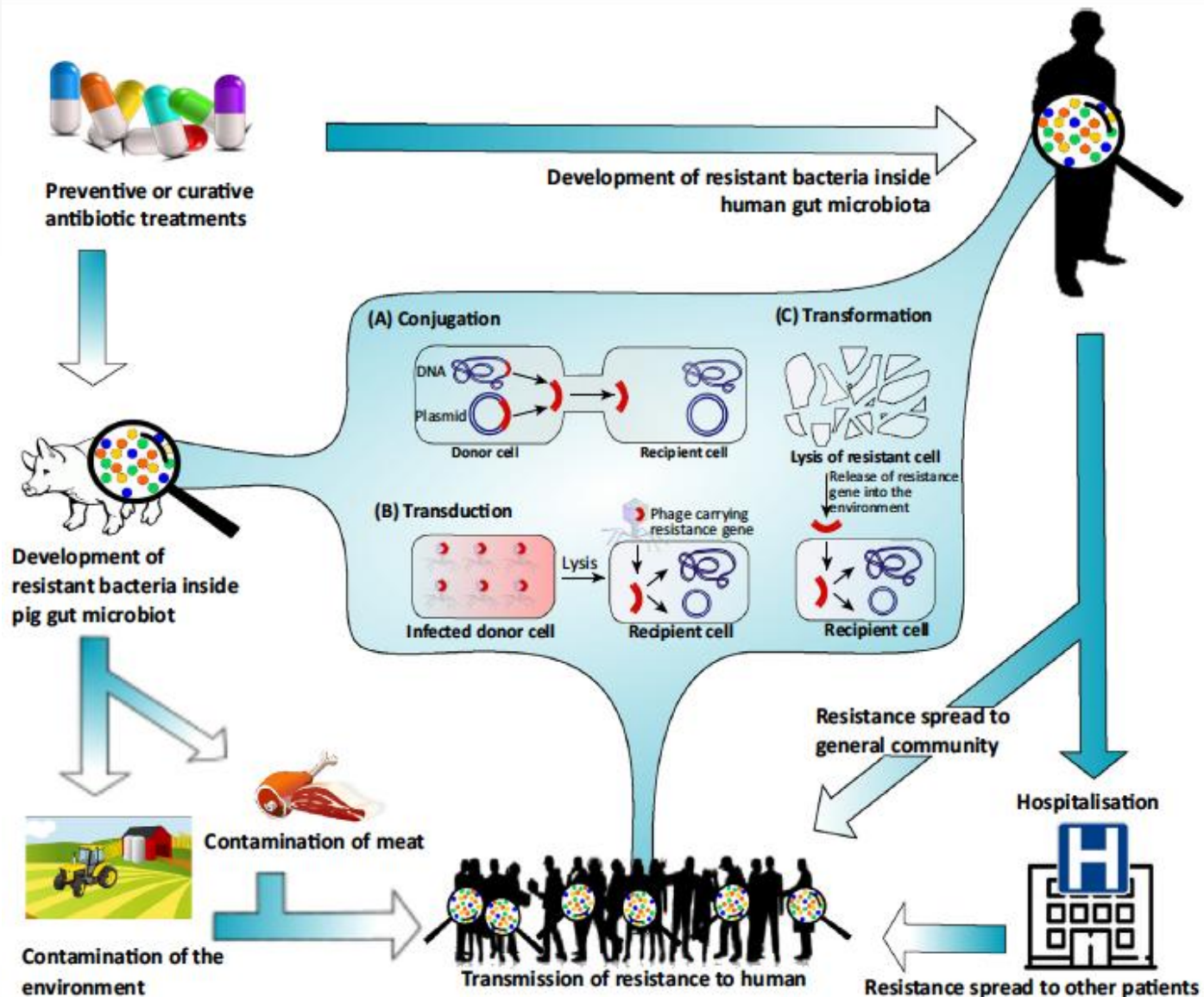


Bacterial genera that are differentially present due to antibiotic treatment, based on taxonomic inference of bacteria (16S rRNA sequences) from (a) cecal and (b) colonic intestinal samples ( $P < 0.01$ ).

Looft T *et al.*, 2014. Bacteria, phages and pigs: the effects of in-feed antibiotics on the microbiome at different gut locations. ISME J 8:1566-76.



# Mechanisms of antibiotic resistance dissemination

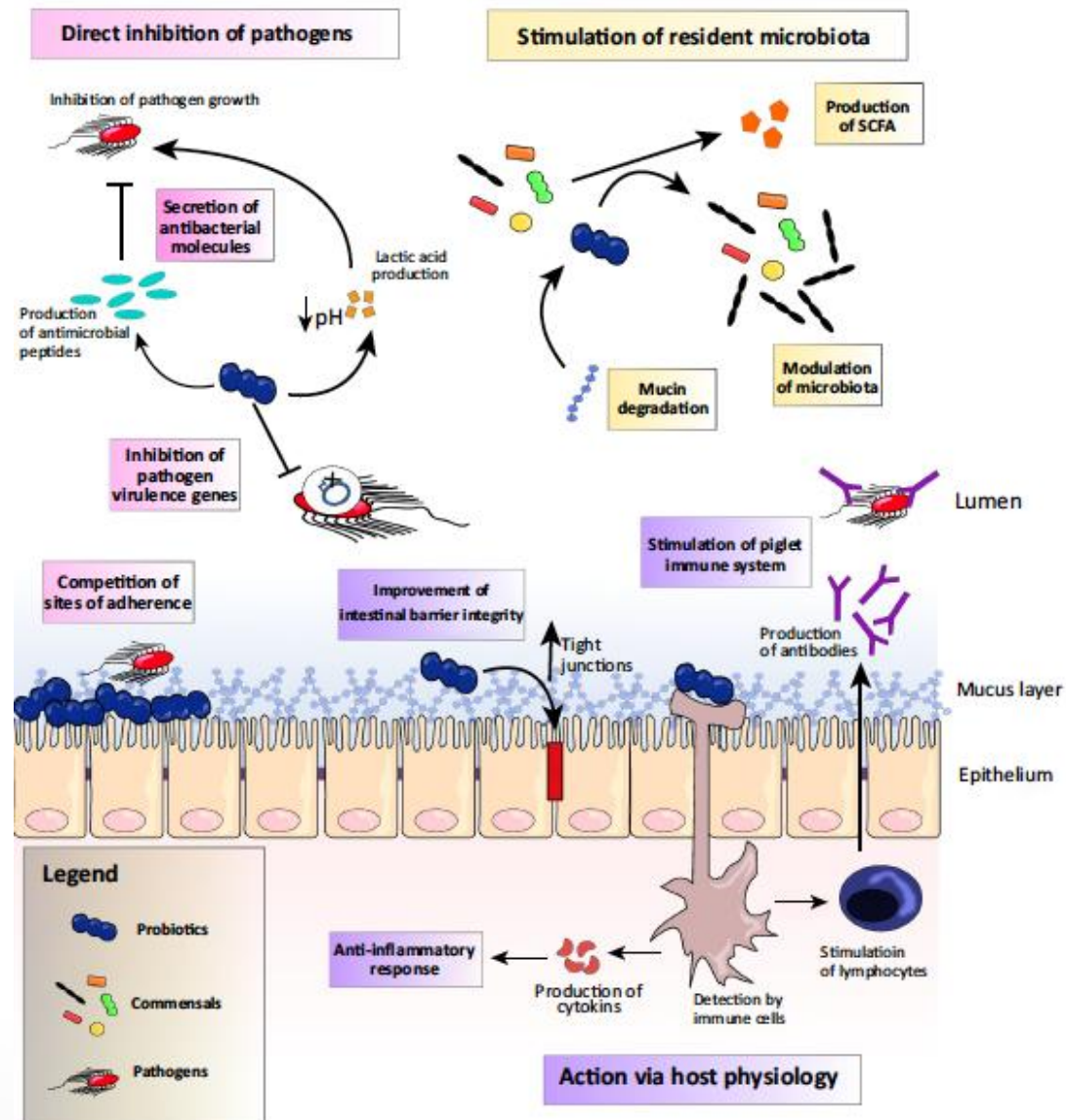


Gresse R *et al.*, 2017. Gut Microbiota Dysbiosis in Postweaning Piglets: Understanding the Keys to Health. Trends Microbiol 25:851-873.

# A way to improve protective effect of the gut microbiota

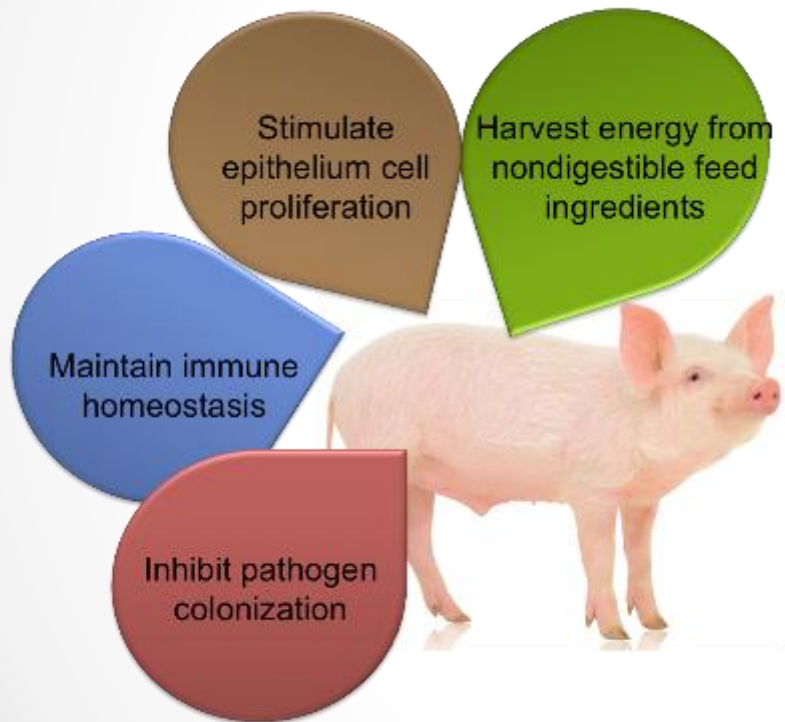
Add protective members into the disturbed intestinal microbial community

- Probiotic lactobacilli



Gresse R *et al.*, 2017. Trends Microbiol 25:851-873.

# Summary



- The gut microbiota plays crucial roles in swine health and performance
- Finding the key players in such microbial community
- Optimizing their population

# Happy pigs make happy pork



# Antibiotic resistance from the farm to the table

## ANTIBIOTIC RESISTANCE

from the farm to the table

### RESISTANCE

Animals can carry harmful **bacteria** in their intestines

When **antibiotics** are given to animals... Antibiotics kill most bacteria But resistant bacteria can survive and multiply

### SPREAD

Resistant bacteria can spread to...

- animal products
- produce through contaminated water or soil
- prepared food through contaminated surfaces
- the environment when animals poop

### EXPOSURE

People can get sick with resistant infections from...

- contaminated food
- contaminated environment

Learn 4 steps to prevent food poisoning at [www.foodsafety.gov](http://www.foodsafety.gov)

### IMPACT

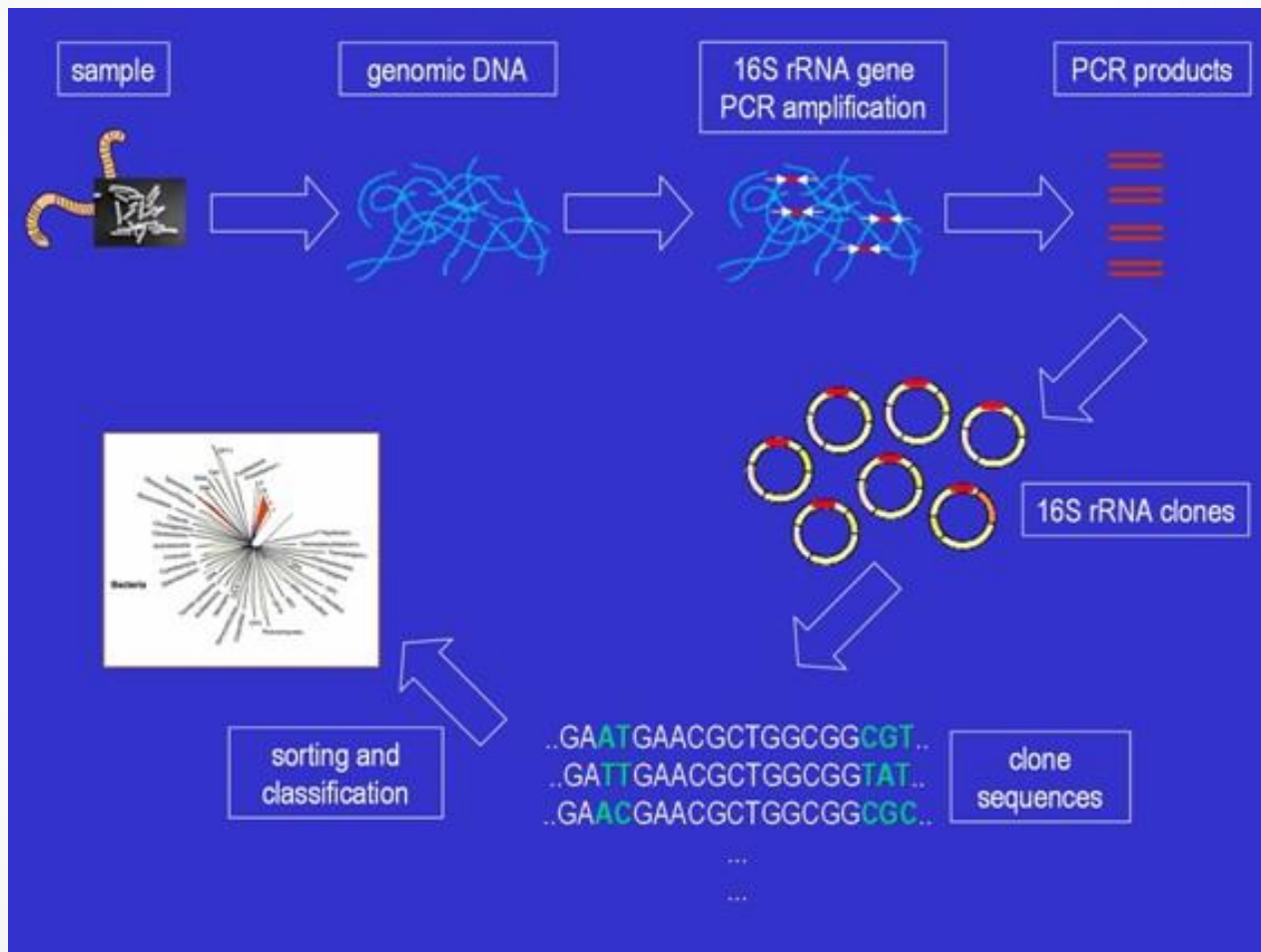
Some resistant infections cause...

- mild illness
- severe illness and may lead to death

About **1 in 5** resistant infections are caused by germs from food and animals.

Source: *Antibiotic Resistant Threats in the United States, 2013*

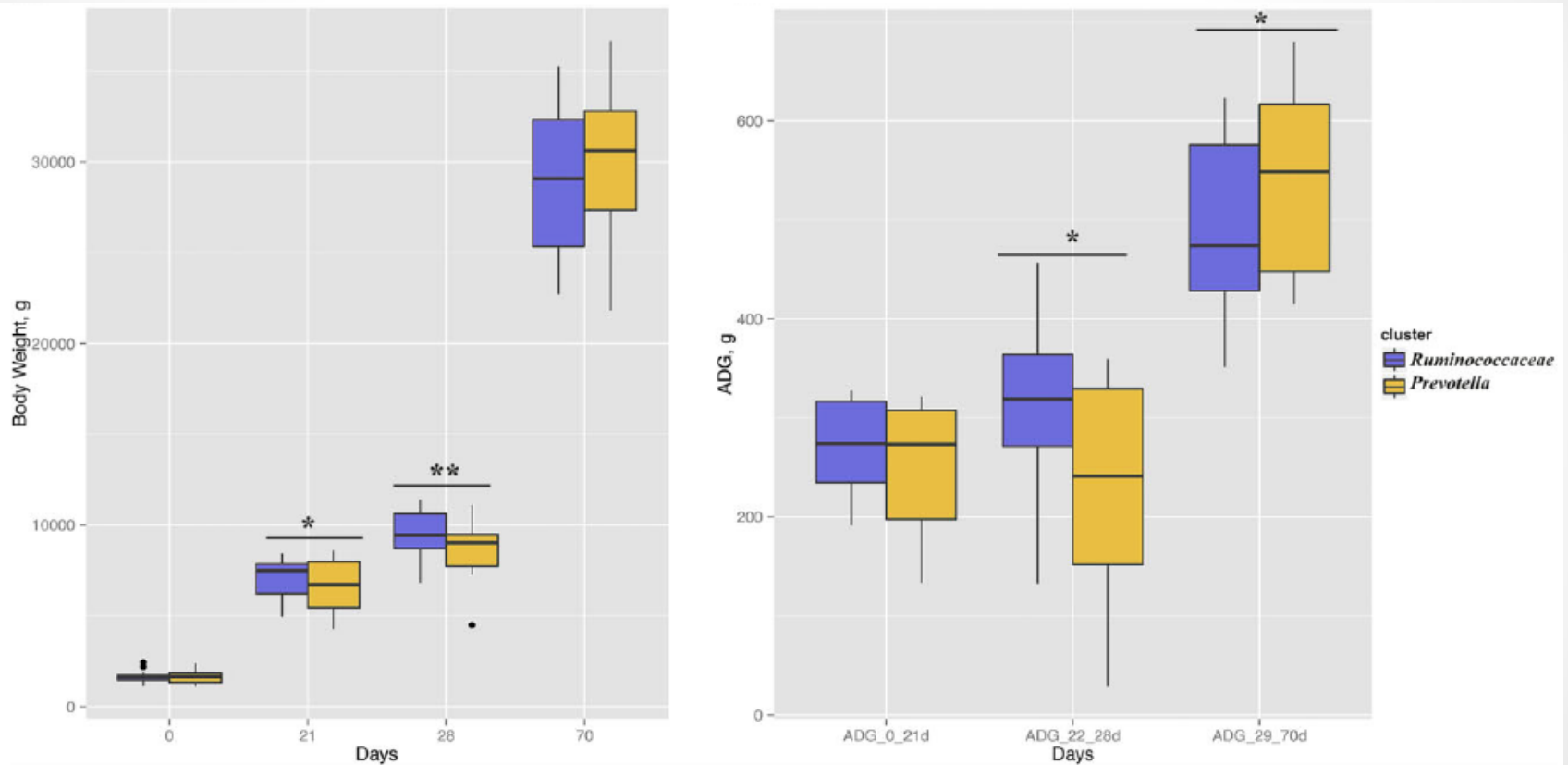
Learn more about antibiotic resistance and food safety at [www.cdc.gov/foodsafety/antibiotic-resistance.html](http://www.cdc.gov/foodsafety/antibiotic-resistance.html)  
Learn more about protecting you and your family from resistant infections at [www.cdc.gov/drugresistance/protecting\\_yourself\\_family.html](http://www.cdc.gov/drugresistance/protecting_yourself_family.html)



# Early life gut microbiota predestines pig phenotypes

- Microbial colonization of the piglet gut begins immediately following birth. Initial colonization by *E. coli* and *Streptococcus* spp. creates an anaerobic environment for subsequent colonizers, *Bacteroides*, *Bifidobacterium*, *Clostridium*, and *Lactobacillus*
- A known beneficial microbe, *Lactobacillus*, predominates the small intestine of piglets until weaning and is a major player in disease prevention

Konstantinov SR et al., 2006. Post-natal development of the porcine microbiota composition and activities. *Environ Microbiol* 8:1191-9.



**Fig. 3.** Evaluation of growth performance distribution according to the two enterotype-like clusters in piglets at day 36. A. The box plot graph represents the BW distribution across ages between the two enterotype-like clusters found at day 36; (B) The box plot graph represents the ADG distribution across ages between the two enterotype-like clusters at day 36. In all plots, individuals are represented

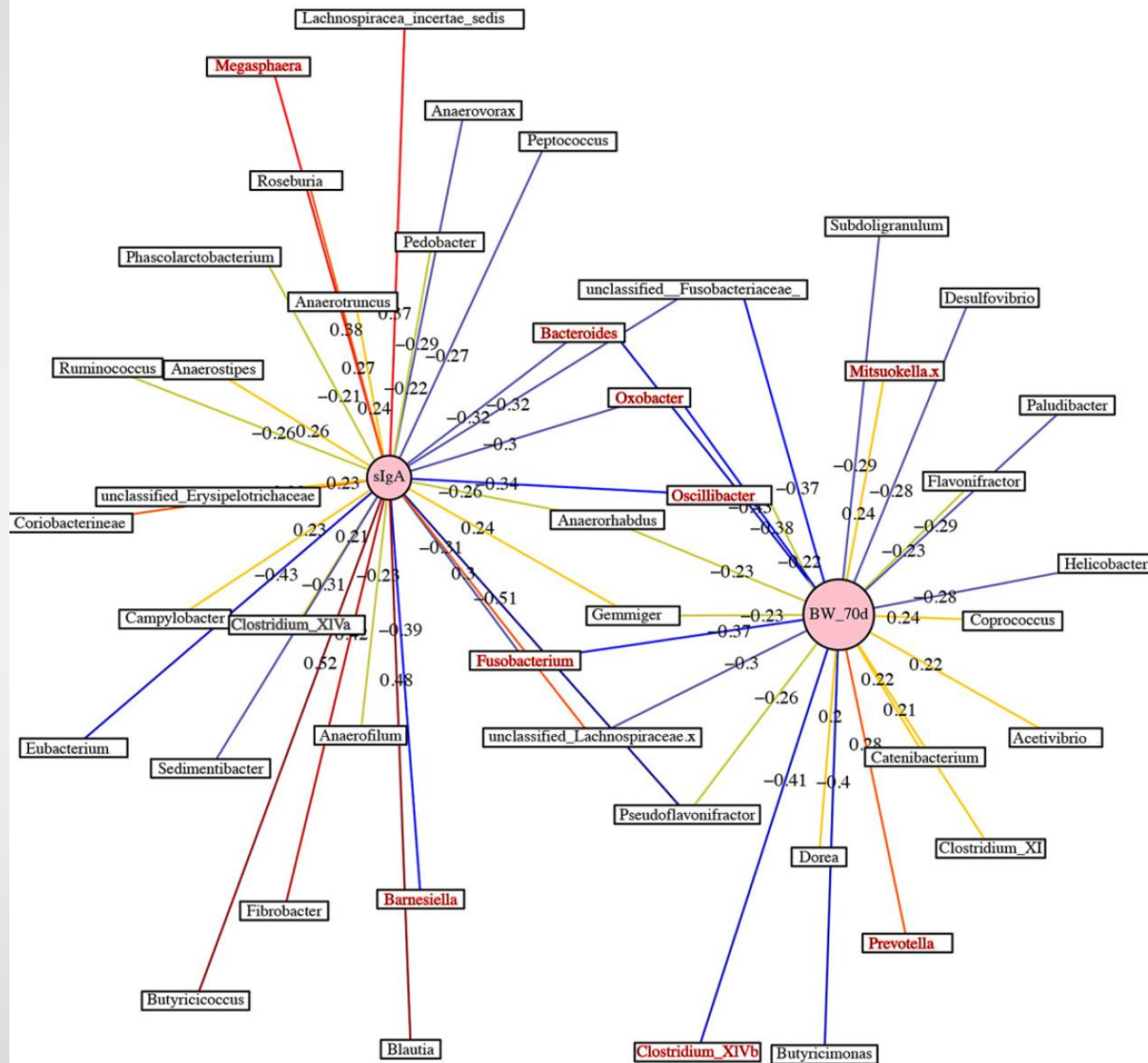
Mach N. et. al., 2015. Early-life establishment of the swine gut microbiome and impact on host phenotypes. *Environ Microbiol Rep* 7:554-69.



## Colour key



-0.52 0.52



# Collrea

Mach N. et al., 2015. Early-life establishment of the swine gut microbiome and impact on host phenotypes. *Environ Microbiol Rep* 7:554-69.