

# Fecal microbiome profiling of piglets during post- weaning diarrhea outbreaks

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# Post-weaning diarrhea (PWD) in pigs



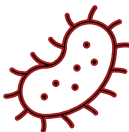
Occurs the first two weeks after weaning



Low growth performance



High treatment costs



Bacterial (enterotoxigenic *E. coli* - ETEC) and viral pathogens (Rotavirus)

## Our interest in PWD includes

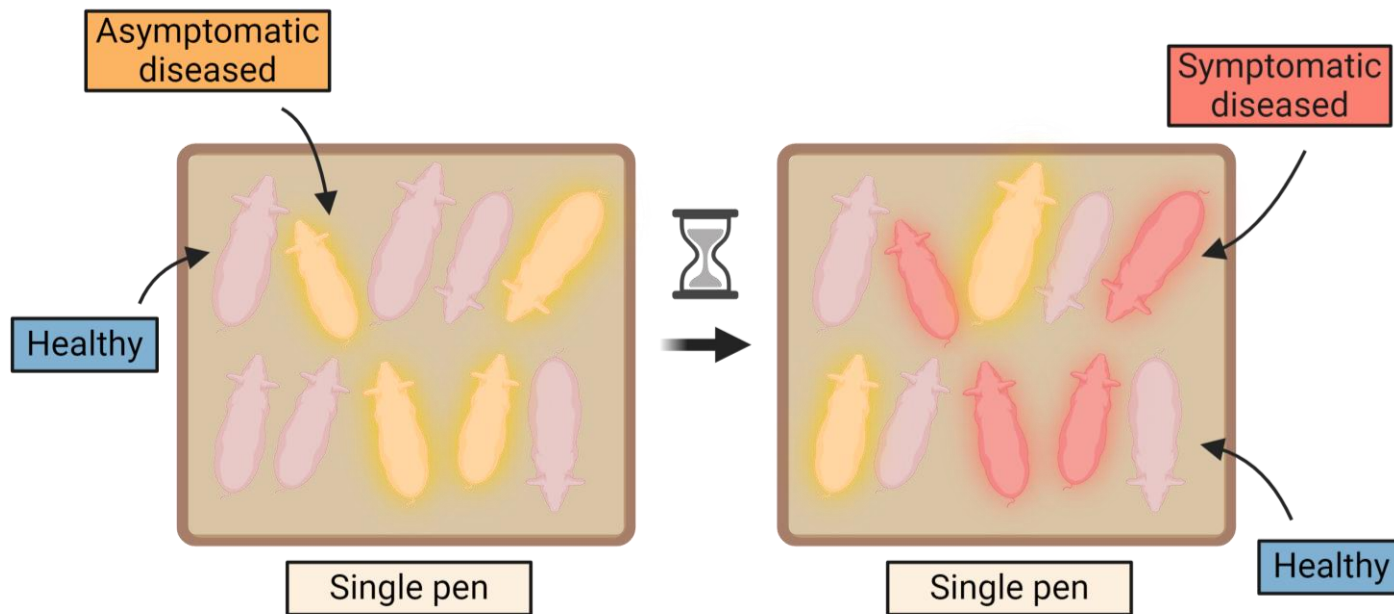
Improve diagnosis

Identify effective non-antibiotic treatments to reduce AMU

Understand PWD aetiology

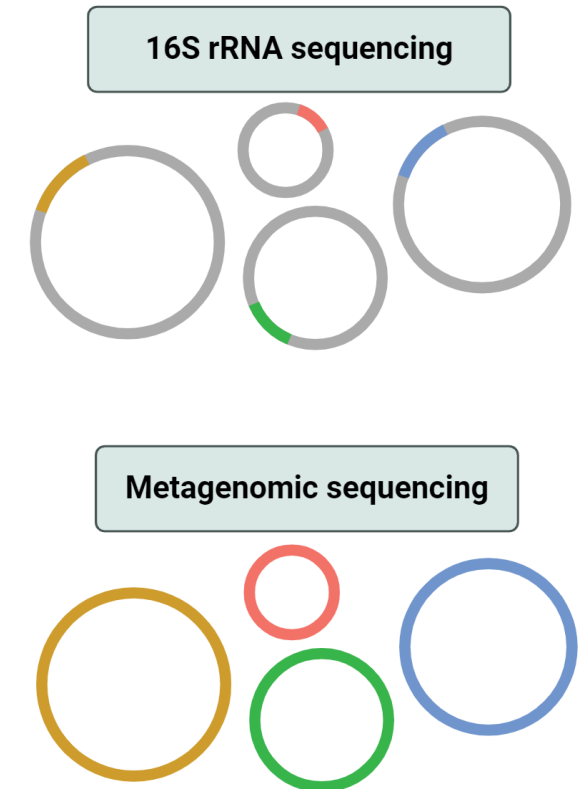
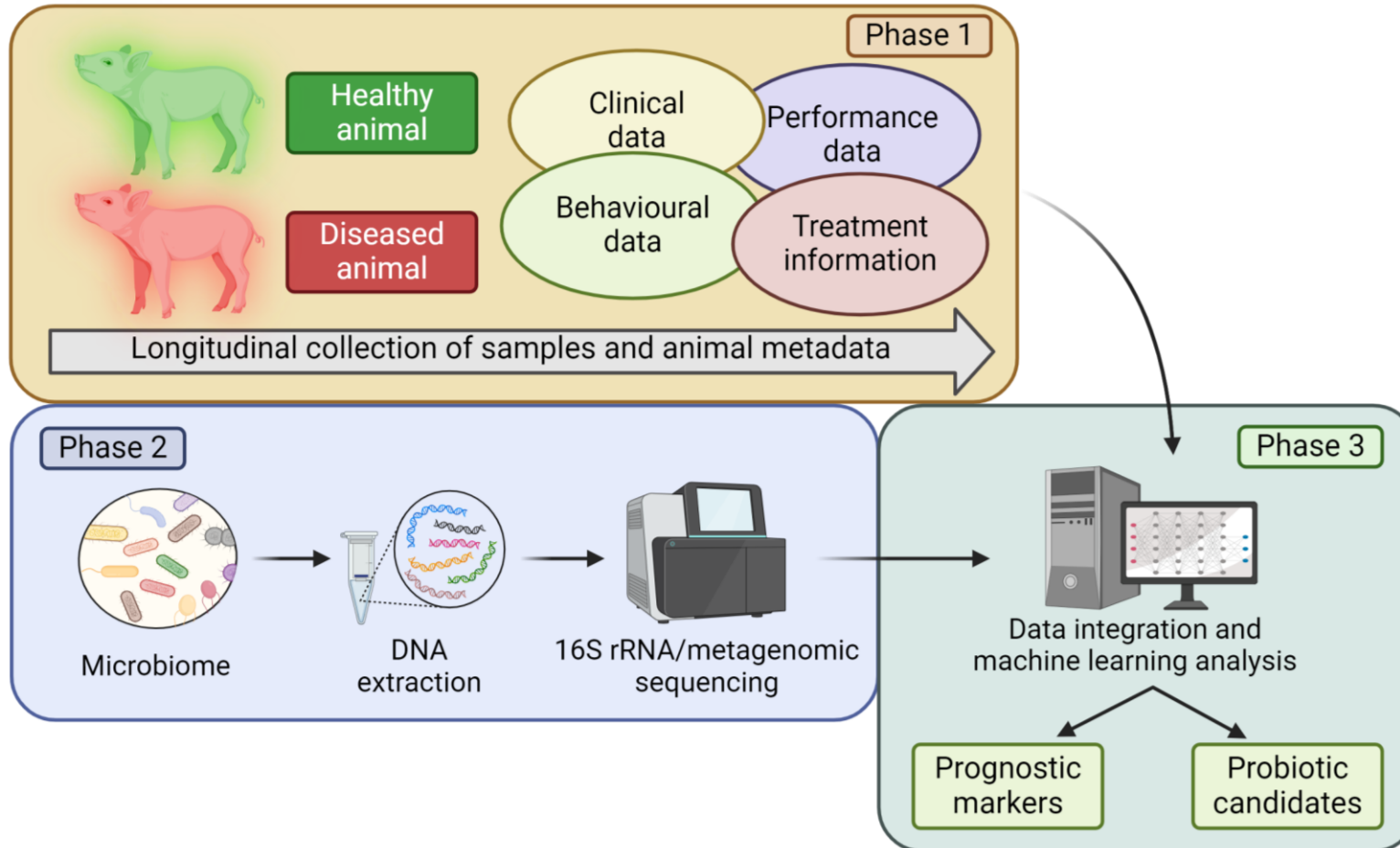
# Our hypothesis on PWD development

Within the same pen some piglets develop PWD while others remains healthy



**Our hypothesis:** the development of PWD is facilitated by a pre-existing state of dysbiosis

# Our approach to study PWD development



# Study background data

Eriksen et al. *Porc Health Manag* (2021) 7:54  
<https://doi.org/10.1186/s40813-021-00232-z>

Porcine Health Management

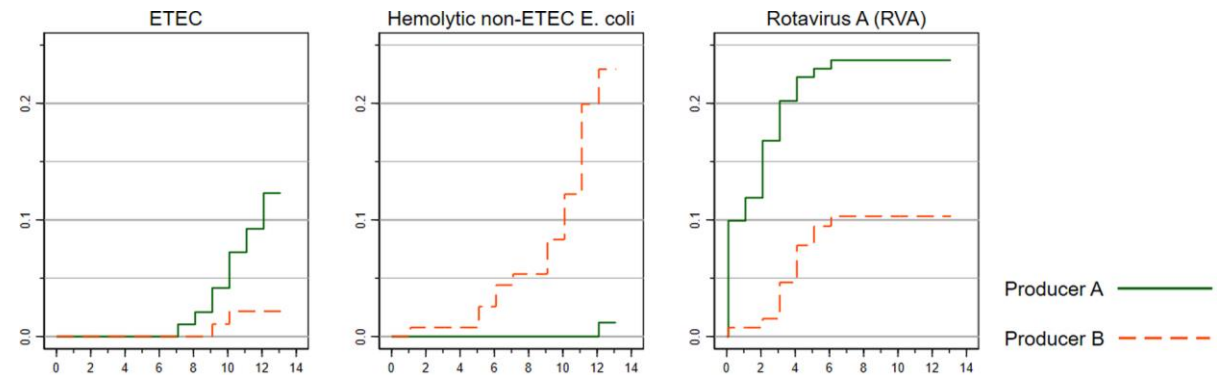
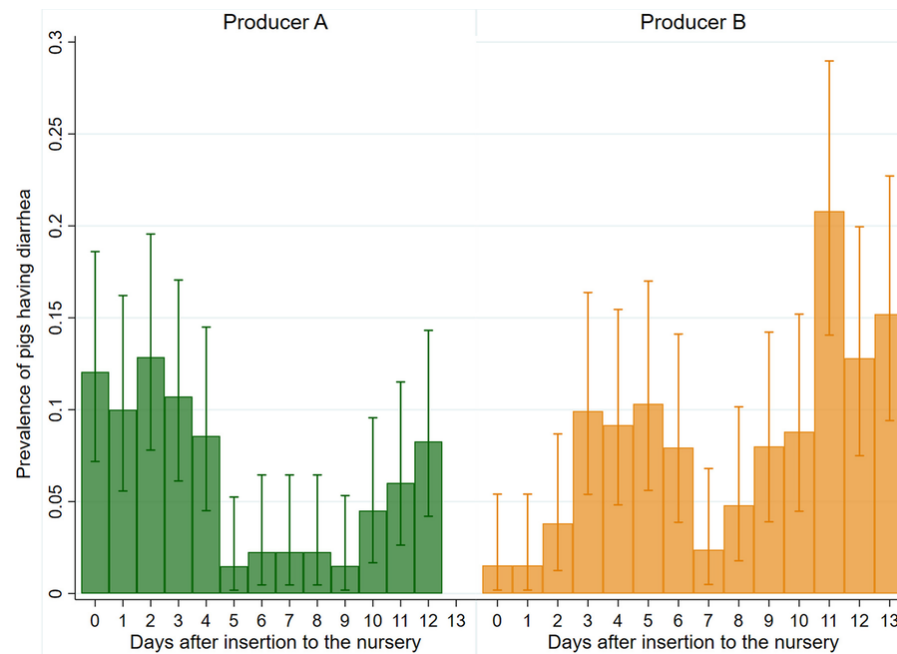
RESEARCH

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## Post-weaning diarrhea in pigs weaned without medicinal zinc: risk factors, pathogen dynamics, and association to growth rate

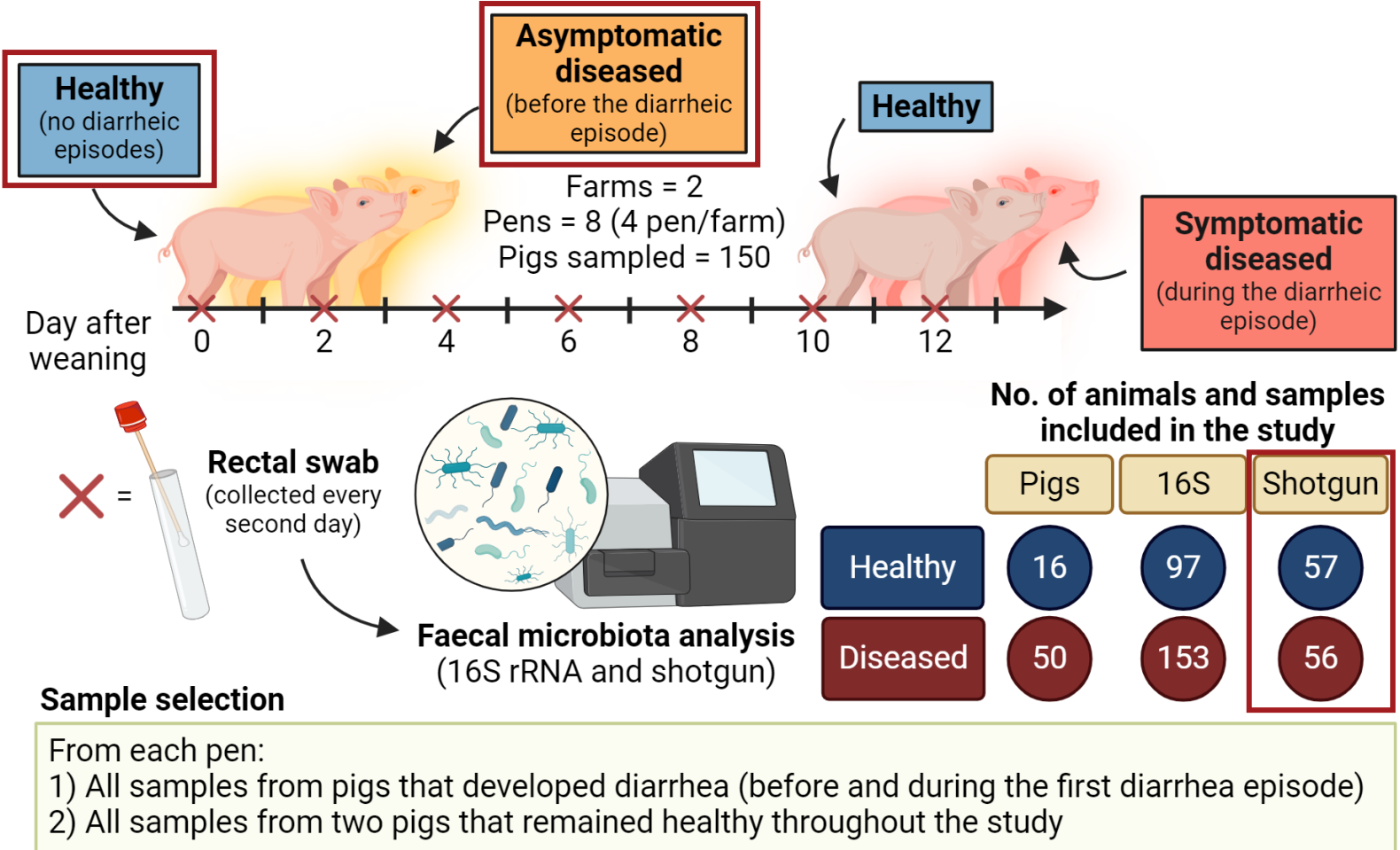
Esben Østergaard Eriksen<sup>1\*</sup>, Egle Kudirkienė<sup>1</sup>, Anja Ejlersgård Christensen<sup>2</sup>, Marianne Viuf Agerlin<sup>3</sup>, Nicolai Rosager Weber<sup>3</sup>, Ane Nødvedt<sup>4</sup>, Jens Peter Nielsen<sup>1</sup>, Katrine Top Hartmann<sup>1</sup>, Lotte Skade<sup>3</sup>, Lars Erik Larsen<sup>1</sup>, Karen Pankoke<sup>1</sup>, John Elmerdahl Olsen<sup>1</sup>, Henrik Elvang Jensen<sup>1</sup> and Ken Steen Pedersen<sup>1,2</sup>



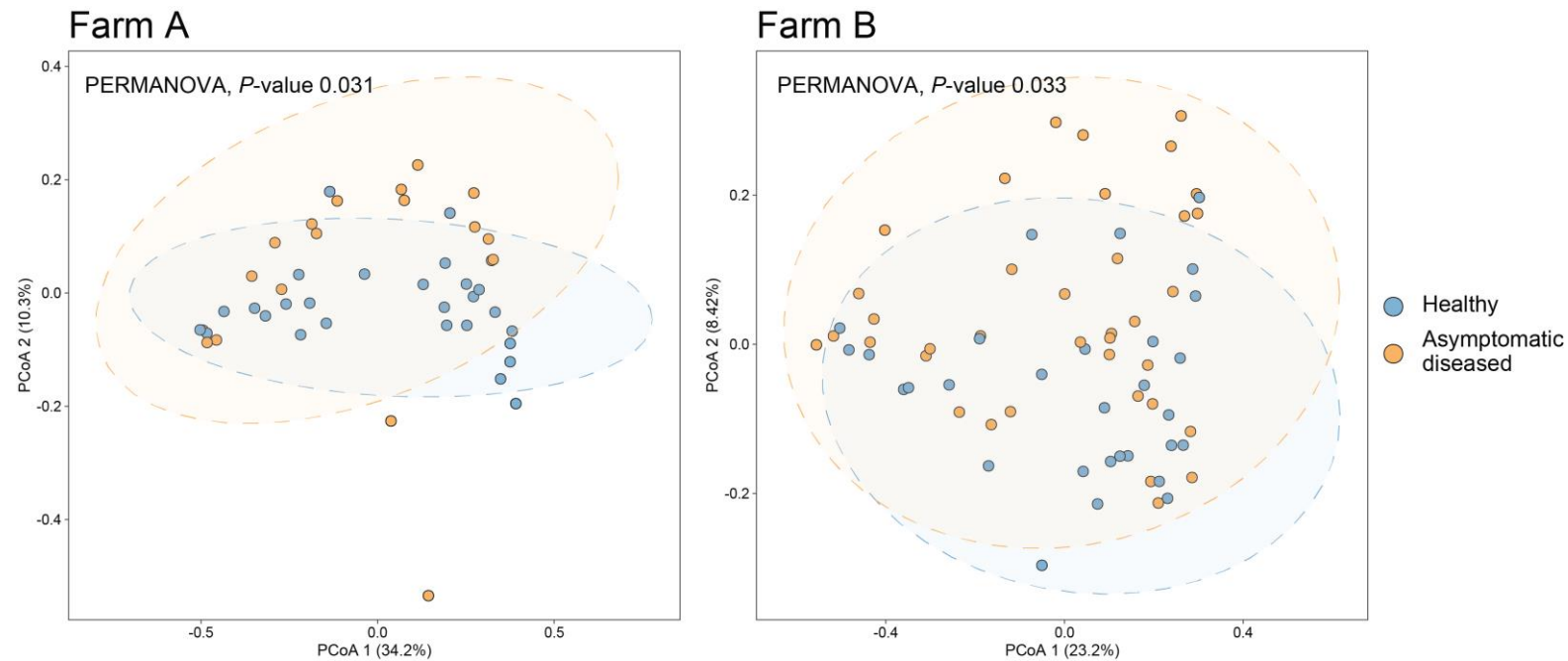
Cumulative incidence of diarrhoea by microbial aetiology

Prevalence of diarrhoea during the first 14 days after insertion

# Study design



## Community structure (beta-diversity) results



Significantly differences (PERMANOVA,  $p < 0.05$ ) were observed in the community structure of healthy and asymptomatic diseased animals in both farms

## Machine learning analysis to classify samples by health status

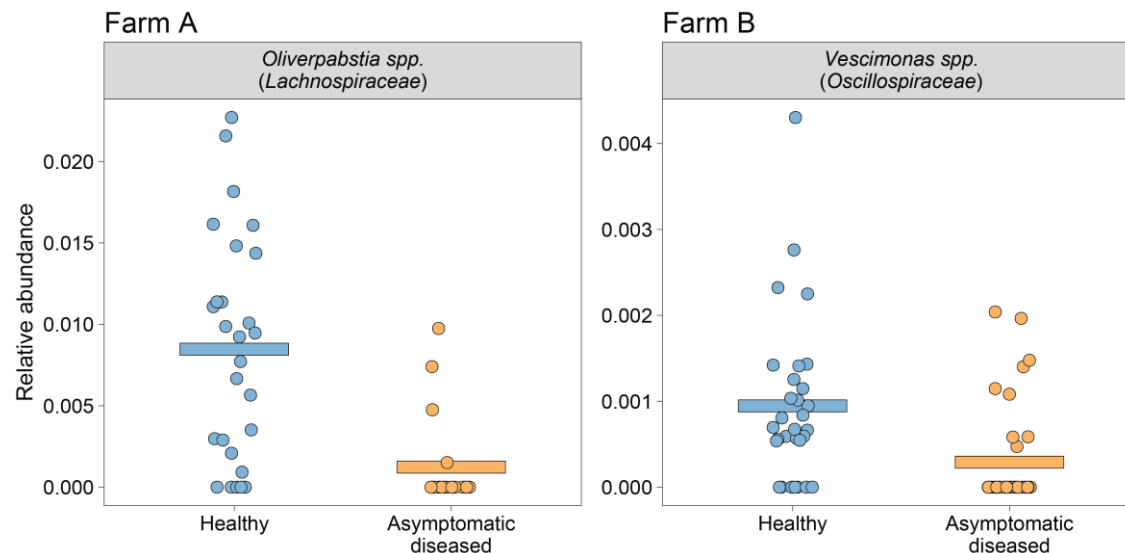
### Farm A: 10 MAGs

MAG ID	Family	Species
MAG0001	<i>Lachnospiraceae</i>	<i>Oliverpabstia spp.</i>
MAG0058	<i>Oscillospiraceae</i>	<i>Vescimonas spp.</i>
MAG0119	<i>Oscillospiraceae</i>	<i>Faecousia spp.</i>
MAG0434	<i>Bacteroidaceae</i>	<i>Prevotella spp.</i>
MAG0622	<i>Oscillospiraceae</i>	<i>Faecousia spp.</i>
MAG0882	<i>Lachnospiraceae</i>	<i>Oliverpabstia intestinalis</i>
MAG0886	<i>Lactobacillaceae</i>	<i>Limosilactobacillus reuteri</i>
MAG1019	<i>Lachnospiraceae</i>	<i>Blautia spp.</i>
MAG1020	<i>Oscillospiraceae</i>	<i>Faecousia spp.</i>
MAG1028	<i>Oscillospiraceae</i>	<i>Limivacinus spp.</i>

### Farm B: 9 MAGs

MAG ID	Family	Species
MAG0301	<i>Anaerovoracaceae</i>	<i>RUG099 spp.</i>
MAG0357	<i>Acidaminococcaceae</i>	<i>Phascolarctobacterium spp.</i>
MAG0461	<i>Butyricicoccaceae</i>	<i>Butyricicoccus spp.</i>
MAG0851	CAG-138	<i>UBA1685 spp.</i>
MAG0872	<i>Oscillospiraceae</i>	<i>F23-B02 spp.</i>
MAG0877	<i>Ruminococcaceae</i>	<i>SFIE01 spp.</i>
MAG0880	<i>Oscillospiraceae</i>	<i>F23-B02 spp.</i>
MAG0983	<i>Butyricicoccaceae</i>	<i>Butyricicoccus spp.</i>
MAG1090	<i>Oscillospiraceae</i>	<i>Vescimonas spp.</i>

ML analysis predicted different metagenomic-assembled genomes (MAG) to be associated with healthy animals in both farms





# Lactobacillaceae, Lachnospiraceae and Oscillospiraceae as probiotic candidates

## Effects of probiotics on treating PWD

Microorganism Name	Treatment	Host Health Influence	Reference
<i>Lactobacillus acidophilus</i> , <i>Lactobacillus casei</i> , <i>Bifidobacterium thermophilum</i> and <i>Enterococcus faecium</i>	Piglets weaned at 28 d of age were fed the basal diet mixed the probiotics ( $0.25 \times 10^8$ CFU/g for each strain) for 25 days, and orally administered with ETEC F18+ ( $2 \times 10^9$ CFU/g) on day 13 postweaning	Decreasing serum TNF- $\alpha$ ; increasing jejunal villus height, and especially villus height-to-crypt depth ratio in piglets	(Sun et al., 2021b)
<i>Lactobacillus delbrueckii</i>	The piglets were orally administrated with <i>Lactobacillus delbrueckii</i> ( $50 \times 10^8$ CFU/mL) at amounts of 1, 2, 3, and 4 mL per animal at 1, 3, 7, and 14 d of age	Increasing the height of intestinal villi of piglets; promoting the expression of intestinal TJs proteins, and reducing the incidence of diarrhea by more than 50%	(Li et al., 2019b)
<i>Enterococcus faecalis</i>	Piglets weaned at 26 d of age were fed basal diet supplemented with <i>Enterococcus faecalis</i> ( $2.5 \times 10^9$ CFU/kg) for 28 days	<i>Enterococcus faecalis</i> and neomycin sulfate decreased diarrhea index and improve growth performance, <i>Enterococcus faecalis</i> increased <i>Lactobacillus</i> in feces	(Hu et al., 2015)
<i>Lactobacillus plantarum</i>	Piglets (4 d of age) were orally administrated with <i>Lactobacillus plantarum</i> ( $5 \times 10^{10}$ CFU/kg) for 15 days and then orally administrated with ETEC F4 ( $1 \times 10^8$ CFU per pig)	Improving performance and effectively preventing the diarrhea; improving function of the intestinal barrier by protecting intestinal morphology and intestinal permeability and the expression of genes for TJs proteins	(Yang et al., 2014)
<i>Lactobacillus zeae</i> and <i>Lactobacillus casei</i>	Piglets weaned at 28 d of age were fed corn-soybean meal mixed feed fermented by <i>Lactobacillus zeae</i> and <i>Lactobacillus casei</i> for 3 days, and then orally challenged with 1 mL <i>Salmonella</i> ( $1 \times 10^6$ CFU/mL)	Decreasing pro-inflammatory cytokine expression and alleviating <i>Salmonella</i> infection	(Yin et al., 2014)
<i>Enterococcus faecium</i> , <i>Bacillus subtilis</i> , <i>Saccharomyces cerevisiae</i> and <i>Lactobacillus paracasei</i>	Piglet weaned at 28 d of age were fed the basal diet mixed the probiotics ( $>1 \times 10^8$ CFU/g for each strain) for 21 days	Increasing fecal acetic acid and propionic acid; increasing growth performance and significantly reducing PWD	(Lu et al., 2018)

Su et al., 2022 – *Front Cell Infect Microbiol*

GUT MICROBES  
2021, VOL. 13, NO. 1, e1987783 (18 pages)  
<https://doi.org/10.1080/19490976.2021.1987783>



REVIEW

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## Oscillospira - a candidate for the next-generation probiotics

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Microbiology and Microbiome

MICROBIOLOGY AND MICROBIOME

## Identification of the relationship between the gut microbiome and feed efficiency in a commercial pig cohort

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### Abstract

**Feed efficiency (FE)** is an economically important trait in pig production. Gut microbiota plays an important role in energy harvest, nutrient metabolism, and fermentation of dietary indigestible components. Whether and which gut microbes affect FE in pigs are largely unknown. Here, a total of 208 healthy Duroc pigs were used as experimental materials. Feces and serum samples were collected at the age of 140 d. We first performed 16S rRNA gene and metagenomic sequencing analysis to investigate the relationship between the gut microbiome and **porcine residual feed intake (RFI)**. 16S rRNA gene sequencing analysis detected 21 operational taxonomic units showing the tendency to correlation with the RFI ( $P < 0.01$ ). Metagenomic sequencing further identified that the members of Clostridiales, e.g., *Ruminococcus flavefaciens*, *Lachnospiraceae bacterium 28-4*, and *Lachnospiraceae phytofermentans*, were enriched in pigs with low RFI (high-FE), while 11 bacterial species

Members of these bacterial families are culturable!

## What's next?

- Integrate other relevant metadata in the ML analysis
- Identify microbiome markers associated with PWD

Application to DFF Research Project 2:

### **Pig culturomics to understand and limit diarrhea (PIG-CULT)**

- ? When do these beneficial bacteria colonize the piglet's intestinal microbiota after birth?
- ? Can these beneficial bacteria be isolated and used for probiotic development?



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