The effect of host genetics factors on shaping pig gut microbiota

M. Maushammer¹, A. Camarinha-Silva¹, M. Vital², R. Wellmann¹, S. Preuss¹, J. Bennewitz¹

¹University of Hohenheim, Institute of Animal Science, Stuttgart, Germany
²Helmholtz Centre for Infection Research, Braunschweig, Germany
Introduction

• Next Generation Sequencing methods enable to characterize the whole microbiome of an ecosystem

• The gut microbiome plays a major role in the immune system development, state of health and energy supply to the host

• Many factors influence the microbial composition in the gut:
  – feeding
  – housing
  – age
  – host genetic background
Introduction

• The host genetics can influence the microbiota composition due to
  – differences in immunoglobulin and antibacterial molecules secreted into the gut lumen (Wen et al., 2008)
  – differences in the mucosal gut structure (Sommer et al., 2014)
  – differences in bile acid metabolism (Ryan et al., 2014)

• In pig production systems growth performance is of great interest
  ’investigating the influence of the microbial community in the gut on growth performance
Aim of the study:

• Describing the bacterial gut microbiome of purebred Piétrain sows

• Estimating genetic parameters of the microbiota composition in the porcine gut (heritabilities of microbial abundances and genetic correlations)

• Predicting the phenotype (daily gain and feed conversion) from the microbial community (microbial prediction)
Methodology

207 Piétrain sows

→ Colon digesta samples

→ DNA extraction

→ 16S Illumina-amplicon sequencing

Blood samples

→ DNA extraction

→ Illumina 60k genotyping

Genomic relationship matrix

→ Describing bacterial community

→ Estimating genetic parameters

Microbial prediction

Bioinformatic and statistical analysis
Methodology

207 Piétrain sows

- Standardized feeding and housing at LSZ Boxberg (ZDS, 2007)
- Performance testing from 30kg-105kg of daily gain (DG) and feed conversion (FC)
Methodology

207 Piétrain sows → Colon digesta samples

Blood samples

• 14 Slaughter days
Methodology

- 207 Piétrain sows
- Colon digesta samples
- DNA extraction
- 16S Illumina-amplicon sequencing

- Fast DNA spin kit for Soil from MP Biomedicals
- Sequencing of the V1-V2 region of the 16S rRNA gene
207 Piétrain sows → Colon digesta samples → DNA extraction → 16S Illumina-amplicon sequencing

**Methodology**

- Bioinformatic analysis with RPD piepeline
- Statistical analysis with Primer7

- Distribution of MO’s at Phylum level
- Firmicutes:Bacteroidetes
- Relative abundances at Genus level

Bioinformatic and statistical analysis → Describing bacterial community
Methodology

207 Piétrain sows

Blood samples

DNA extraction

Illumina 60k genotyping

Colon digesta samples

DNA extraction

16S Illumina-amplicon sequencing

• Maxwell ® 16 instrument (Promega)

• After quality control and filtering 45181 SNPs remained

Bioinformatic and statistical analysis

Describing bacterial community
Methodology

207 Piétrain sows → Colon digesta samples → DNA extraction → 16S Illumina-amplicon sequencing

Blood samples → DNA extraction → Illumina 60k genotyping

Genomic relationship matrix

Bioinformatic and statistical analysis → Describing bacterial community
• Univariate and pairwise bivariate analysis with ASReml in R for each bacterial Genus (51 Genera with a relative abundance >0.1)
• Fixed effects were estimated separately by fitting a linear mixed model to each Genus
• Genomic mixed linear models were used to estimate genetic parameters:

\[ y = Xb + Z_{SD}SD + Z_a a + e \]

- \( y \): Vector of observations (relative abundances of bacterial Genera)
- \( b \): Vector of fixed effects
- \( SD \): Vector with random slaughter day effects
- \( a \): Vector with random additive-genetic effects of the animal
- \( X, Z_{SD}, Z_{a} \): Corresponding design matrices
- \( e \): Residual term
The covariance structure of the random animal effect was

$$\text{var}(a) = G \times \sigma_a^2$$

- $G$ genomic relationship Matrix (VanRaden, 2008)
- $\sigma_a^2$ additive genetic variance

P-values ofheritabilities were estimated by performing a Likelihood-Ratio test of the random animal effect.
• Prediction of the phenotype (DG, FC) based on bacteria at OTU level
• Less abundant genera were removed from the dataset
• After log transformation the data were standardized to a mean of 0 and a standard deviation of 1
• Calculating the microbial relationship matrix $M$:

$$M = XX'/m$$

$X$  $n \times m$ Matrix  
$n$  Samples  
$m$  OTUs
Fitting a G-BLUP model to predict the phenotype with the package `rrBLUP` in R:

\[ y = Xb + Zg + e \]

- \( y \) Vector of observations (DG, FC)
- \( b \) Vector of fixed effects
- \( X \) Corresponding design matrix
- \( g \) Random effect of the OTUs, with \( g \sim N(0, M\sigma_g^2) \)
- \( Z \) Design matrix containing the individual’s relative abundances of the OTUs
- \( e \) Residual term
Five way cross validation was performed
  - Splitting the data in 5 equally sized groups
  - Predicting each group from the other four groups

Accuracy of prediction was determined by Pearson’s $r$ (correlation between observed and predicted phenotype)
Results: Microbial community

Phylum

- Bacteroidetes 42%
- Firmicutes 54%
- Proteobacteria 2%
- Spirochaetes 1%
- Others 1%

**Box plots:**
- **Nr. OTUs:**
  - 0
  - 200
  - 400
  - 600
  - 800
  - 1000
  - 1200
  - 1400
  - 1600
  - 1800
  - 2000

- **Shannon diversity:**
  - 0
  - 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
Results: Microbial community

2D Stress: 0.22

$R = 0.339$
$p = 0.001$
Results: Microbial community

<table>
<thead>
<tr>
<th>Family</th>
<th>Higher abundance of Bacteroidetes</th>
<th>Higher abundance of Firmicutes</th>
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<tbody>
<tr>
<td>Clostridiaceae 1</td>
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## Results: Genetic Parameter

Table: Estimated heritability ($h^2$), standard error (SE) and p-value for the relative abundances of bacterial genera

<table>
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<tr>
<th>Bacteria</th>
<th>$h^2$</th>
<th>SE</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Alloprevotella</td>
<td>0.36</td>
<td>0.09</td>
<td>0.01</td>
</tr>
<tr>
<td>Blautia</td>
<td>0.36</td>
<td>0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>Catenibacterium</td>
<td>0.39</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>0.34</td>
<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>Unc. Firmicutes</td>
<td>0.28</td>
<td>0.11</td>
<td>0.02</td>
</tr>
<tr>
<td>Unc. Proteobacteria</td>
<td>0.29</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Unc. Spirochaetales</td>
<td>0.52</td>
<td>0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>Unc. Spirochaetes</td>
<td>0.32</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Unc. Succinivibrionaceae</td>
<td>0.57</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>Unc. Veillonellaceae</td>
<td>0.32</td>
<td>0.14</td>
<td>0.01</td>
</tr>
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</table>

Based on 51 bacterial Genera 10 showed significant heritabilities with a p-value < 0.05
Results: Microbial prediction

- To link the phenotype with the microbial community a microbial prediction was performed.
- This is similar to genomic prediction, but instead of using the SNP data as explaining variable microbiota data were included.
- The five way cross validation resulted in a prediction accuracy of 0.39 for daily gain and 0.10 for feed conversion.
Conclusion

• The gut microbiota in Piétrain pigs is influenced by the genetics of the host

• Based on microbial prediction the influence of the gut microbiota on growth performance has been shown

These results show various possibilities, e.g. concerning biological explanations and an improved nutrient supply caused by breeding
Thank you for your attention!

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