The ENTEROTYPIG project

Enterotypes of the intestinal microbiota in pigs: characterization and influence of the host genetics to assemble the holobiont

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https://www6.jouy.inrae.fr/gabi

@UMR_GABI
Gut microbiota: a dynamic ecosystem over lifetime

- Links with production traits?
- Links with health and welfare traits?
- Links with adaptative and resilience traits?
Stratification of pigs according to two enterotypes

60-day-old Large White piglets (N=518)

Enterotype PM

*Prevotella*

*Mitsuokella*

Enterotype RT

*Ruminococcus*

*Treponema*

Enterotypes reflect microbial networks (ecosystems)

Ramayo-Caldas et al., 2016, ISME Journal
## Relationships between enterotypes and growth rate

<table>
<thead>
<tr>
<th>Enterotype</th>
<th>Body weight (Kg)</th>
<th>Average Daily Gain (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Prevotella - Mitzuokella</em></td>
<td>26.7 ± 0.1**</td>
<td>456.3 ± 4.4*</td>
</tr>
<tr>
<td><em>Ruminococcus - Treponema</em></td>
<td>25.8 ± 0.2</td>
<td>438.3 ± 6.7</td>
</tr>
</tbody>
</table>

Mach et al., 2015, Environ Microbiol Rep Ramayo-Caldas et al., 2016, ISME Journal

A higher body weight gain in 60-day-old animals with fecal microbiota containing more *Prevotella*
Interactions between host and microbiome

What influence of the host genetics?

A controversial issue!
Objectives of ENTEROTYPPIG

1. To estimate the heritabilities of the composition of pig gut microbiota at 60 and 120 days of age

2. To demonstrate the possibility of acting on the host genome to modify and orientate the composition of the gut microbiota

3. To study enterotype functionalities

4. To create pig genetic lines as models to further study host gut microbiota interactions, dynamics of microbiota over life and links with host phenotypes (holobiont assembly)
Selection criteria: microbiota at 60 days of age

Fecal microbiota characterization

Data production
DNA extraction
16S rRNA gene sequencing

Sequence data processing
QIIME2
16S alignment database:
SILVA138 → ASV* Identification
Rarefaction set to 10 000 counts

Enterotype determination
Genus relative abundance
Cluster based on JSD* distance

* amplicon sequence variant
* Jensen-shannon divergence

Birth  Weaning at D28  D60 fecal sample  D120 fecal sample
> Selection criteria
Selection experiment: 2 lines referred to as PM and RT

Founders: generation G0

« 30 families »

♂ X ♀

Large White
N = 316

line PM:
Prevotella ++ Mitsuokella +

N = 6
N = 30

♂ X ♀

line RT:
Treponema ++ Ruminococcus +

N = 6
N = 30

♂ X ♀

Generation G1

N = 133

N = 139
Enterotypes at D60 in the base population (G0)

Enterotype RT: *Ruminococcus* and *Treponema*
Enterotype PM: *Prevotella* and *Mitsuokella*

1/3 animals with enterotype RT
– 52 females and 51 males

2/3 animals with enterotype PM
– 87 females and 126 males

*Prevotella* is always dominant.

<table>
<thead>
<tr>
<th></th>
<th>Prevotella (%)</th>
<th>Mitsuokella (%)</th>
<th>Ruminococcus (%)</th>
<th>Treponema (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>51.80</td>
<td>0.52</td>
<td>0.97</td>
<td>3.25</td>
</tr>
<tr>
<td>RT</td>
<td>34.43</td>
<td>0.17</td>
<td>1.62</td>
<td>7.83</td>
</tr>
<tr>
<td>PM</td>
<td>60.05</td>
<td>0.69</td>
<td>0.66</td>
<td>0.08</td>
</tr>
</tbody>
</table>
PM vs RT enterotypes: differences and individual variabilities

Individual variability between and within enterotypes in G0 population at J60

- **Bacteroidota**
  - Prevotella
  - Prevotellaceae NK3B31
  - Alloprevotella
  - Muribaculaceae
  - Rikenellaceae RC9
  - Autres bacteroidota

- **Firmicutes**
  - Clostridia_UCG-014
  - Eubacterium coprostanoligenes
  - Unknown genus
  - Oscillspiraceae UGC002
  - Oscillospirales UGC10
  - Faecalibacterium
  - Ruminococcus
  - Streptococcus
  - Dialister
  - Mitsuokella
  - Autres Firmicutes

- **Spirochaetota**
  - Treponema

- **Campilobacterota**
  - Campylobacter

- **Proteobacteria**
  - Succinivibrio
Half pigs changed their enterotype between 60 and 120 days of age.

316 Large White pigs
60 and 120 days of age

Enterotype PM

Enterotype RT
Statistical analyses: focus on four genera
*Prevotella, Mitsuokella, Ruminococcus, Treponema*

Differences between the two pig lines PM and RT
- Data from G1 generation
- 4 genera under selection
- Pigs at 60 and 120 days of age

Estimation of genetic parameters
- Data from G0 and G1 generations
- Fixed effects: sex, batch
- Heritabilities and genetic correlations between data from samples collected at 60 and 120 days of age
Response to selection: D60 microbiota

**Generation G1**

<table>
<thead>
<tr>
<th>Line</th>
<th>Prevotella (%)</th>
<th>Mitsuokella (%)</th>
<th>Ruminococcus (%)</th>
<th>Treponema (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>57.43</td>
<td>1.03</td>
<td>0.73</td>
<td>2.05</td>
</tr>
<tr>
<td>RT</td>
<td>46.96</td>
<td>0.51</td>
<td>1.32</td>
<td>3.88</td>
</tr>
</tbody>
</table>

*What differences?*

- PM: % Prevotella 57.43, % Mitsuokella 1.03, % Ruminococcus 0.73, % Treponema 2.05
- RT: % Prevotella 46.96, % Mitsuokella 0.51, % Ruminococcus 1.32, % Treponema 3.88

*p < 0.0001*
Response to selection: D120 microbiota

Generation G1

What differences?

N = 133
N = 139

Difference partially maintained at 120 days for *Prevotella* only
Genetic parameters – 4 genera under selection

- **prevotella**: Heritability values of medium range at 60 days.
- **mitsuokella**: Higher heritability values at 60 days compared to 120 days.
- **ruminococcus**: Medium genetic correlation (rg) between 60 days and 120 days.
- **treponema**:
Conclusion and perspectives

- Heritability of moderate values and in same range for the four genera (~0.3-0.4)

- Evidence that fecal microbiota can be modified by directional selection

- Enterotypes may change with age

- A good model to study the respective influences of the host genetics and of the environment to assemble the pig holobiont
Thanks for your attention

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Partnership

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