Proceedings, 10th World Congress of Genetics Applied to Livestock Production

The influence of host's genetics on the gut microbiota composition in pigs and its links with immunity traits

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ABSTRACT: The objective of this study was to estimate the genetic parameters of the gut microbiota composition and study the links with immunity traits (ITs) in French Large White pigs. A cohort of 60-days old piglets was assessed for fecal microbiota composition by pyrosequencing of the 16S rDNA. First results on 299 piglets showed a predominance of *Prevotella* followed by *Oscillibacter*, *Dialister*, *Roseburia* and *Treponema*. Among a set of 63 genera, 7 had low (0.1<h2<0.2), 15 medium (0.2<h2<0.4) and 8 high (h2>0.4) heritabilities for abundance variations. At the genetic level, the relative abundance of *Prevotella*, *Oribacterium*, *Selenomonas*, *Dialister* and *Megasphaera* are correlated positively with each other and tend to be negatively correlated to other genera. Finally, canonical correlation (rCCA) and sPLS analyses highlighted both positive and negative correlations between various ITs (e.g. monocytes, eosinophils, platelets) and genera such as *Prevotella, Roseburia* and *Dialister*.
Keywords: pig gut; microbiota; immunity

Introduction

The intestinal microbiome plays a major role in host's physiology and homeostasis. It creates a barrier to infections, helps to develop and mature the immune system, and contributes to extract nutriments and energy from food (Prakash et al. 2010). Despite large scale studies in human, little is known on gut microbiota composition and potential associations with individual traits in livestock species. The onset of high throughput sequencing methods have paved the way toward an unprecedented characterization of the microbiota by producing data on the non cultivable bacteria set (Foster et al. 2012), which represents a vast majority of the gut microbiota. The scientific and technological context now provides strong opportunities to characterize the individual gut microbiota composition as new a trait to explore livestock animal phenotype shaping.

In order to get new insights into health traits and immunocompetence in swine, we have launched a national program (SUS_FLORA project) to study the links between gut microbial composition and a wide range of innate and adaptive immunity traits (ITs) in French Large White pigs. Here we report the genetic parameters of the microbiota composition (heritabilities of microbial genera abundance, phenotypic and genetic correlations), and a first analysis to explore links with a set of ITs.

Materials and Methods

Animals sampling and phenotyping. A population of 533 piglets was produced in an INRA experimental farm at Le Magneraud from 90 families. Animals were weaned at 28 days of age and vaccinated against *Mycoplasma hyopneumoniae* at 36 and 42 days old (Stellamune vaccine, Pfeizer). Blood and feces were sampled on 60 days old piglets. Fecal samples were collected in cryotubes, frozen into liquid nitrogen immediately after sampling and further stored at -80° before processing. Immune parameters were measured as reported (Flori et al., 2011), and in this report we have focused on blood formula parameters (i.e. total white blood cells, lymphocytes, monocytes, eosinophils, platelets, red cells and hemoglobin parameters).

DNA preparation and sequencing for microbiota studies. DNAs form fecal samples were prepared by following in-laboratory procedures set up for human microbiota studies (Qin et al. 2010). The microbial diversity was studied by sequencing the variable region of the v3-v4 segment of the 16s ribosomal DNA by 454 Roche pyrosequening (Genoscreen, Lille, France). Sequences were converted into operational taxonomic units (OTUs) for further taxonomic assignation and quantification by using Qiime (Caporaso et al. 2010). In brief, two sequences showing similarity equal to or higher than 97% on the whole length were considered the same OTU. Only sequences longer than 300 bases were included in the analysis. DNA samples with more than 1000 sequences harboring the previous quality criteria were further processed for analyses, thus reducing the subset of animals to 299 for statistical analyses. In the present report we focus on the analyses performed at the level of genera quantification.

Data and statistical analyses. For visualization and analysis of microbiota in association with the set of ITs, multivariate statistical analysis such as regularized canonical correlation (rCCA) and sparse Partial Least Square (sPLS) analyses were applied by using the R software.For genetical analyses, from an initial set of 210 genera a subset of 63 was considered for heritability estimates. Since the relative abundances of microbiota taxomical data do not follow a normal distribution and are very sparse, all genera with more than 200 null values (absence of count) from the total group of 299 animals were removed from the analysis of the genetic parameters. Relative abundances were log-transformed in order to normalize the data. Model included

fixed effects of "sequencing run", post-weaning pen, batch, and weight at 21 days, as a covariate, and animal as a random additive effect. Litter effect was considered as too low to be kept in the final model. Heritability values were first estimated in univariate analyses and genetic correlations between the 40 genera with non null heritability value were estimated in bivariate analyses. Genetic parameters were estimated with VCE6 software (Neumaier & Groeneveld (1998); Groeneveld et al. (2010))

Results and discussion

Phylogenetic composition of the pig gut microbiota. The sequence data included 1.4 million reads with an average of 1,927 sequences per sample and a mean read length of 453 bases. These data were quality filtered and taxonomically assigned. Main genera in 60 days old piglets related to *Prevotella*, *Oscillibacter*, *Dialister*, *Roseburia* and *Treponema,* the *Prevotella* genus being the most predominant. Other well represented genera include *Lactobacillus*, *Mitsuokella*, *Faecalibacterium*.

Heritabilities of relative abundances of bacterial genera. Heritabilities ranged from 0 (23 genera have null heritability values) to 0.82 (*Gemmiger*). As shown in Table 1, 7 genera had low h^2 ($0.1<h^2<0.2$), 15 genera had medium h^2 ($0.2<h^2<0.4$) and 8 genera had high h^2 ($h^2>0.4$). Interestingly, most genera identified as predominantly represented in the gut microbiota showed medium to high h^2 (*Prevotella*: $h^2=0.45$; *Treponema*: $h^2=0.30$; *Faecalibacterium*: $h^2=0.54$; Lactobacillus: $h^2=0.37$; Mitsuokella: $h^2=0.30$; Dialister: $h^2=0.22$). These results clearly demonstrate that the host genetics influences the gut microbiota composition in pigs. Intriguingly, two abundant genera *Oscillibacter* and *Roseburia*, , have very low heritability values (0.08 and 0, respectively).

Table 1. Heritabilities (h2) and standard deviation (SD) estimated for the relative abundances of bacterial genera that characterize individual variability of the gut microbiota composition.

Genus	h2	SD
Gemmiger	0.82	0.13
uncl_Rikenellaceae	0.55	0.15
Faecalibacterium	0.54	0.14
Coprococcus	0.51	0.15
Clostridium_XlVa	0.45	0.14
Prevotella	0.45	0.13
Coriobacterineae	0.44	0.14
Oribacterium	0.41	0.14
Phascolarctobacterium	0.39	0.13
Lactobacillus	0.37	0.13
Dorea	0.33	0.12
Anaerovibrio	0.31	0.52
Mitsuokella	0.30	0.48
Treponema	0.30	0.13
Blautia	0.25	0.12
Butyricicoccus	0.25	0.12
Streptococcus	0.25	0.12
Oxobacter	0.24	0.52
Selenomonas	0.24	0.12
uncl_Ruminococcaceae	0.23	0.12
Dialister	0.22	0.37
Acidaminococcus	0.21	0.46
Anaerovorax	0.20	0.11

[§]Genera identified as the most predominant are in bold letters.

Genetic correlations between relative abundances of genera. Numerous strong positive and negative genetic correlations were found between the relative abundances of genera (Figure 1). At the genetic level, the relative abundance of *Prevolla*, *Oribacterium*, *Selenomonas*, *Dialister* and *Megasphaera* are correlated strongly and positively to each other. *Prevotella* and *Dialister* are strongly correlated (rg=078) and are both negatively correlated to *Treponema* (rg=-0.9 and rg=-0.97, respectively) and to *Oscillibacter* (rg=-0.38 and rg=-0.32, repectively). *Oscillibacter* and *Treponema* are correlated moderately (rg=0.36). These results confirm that the microbiota composition reflects a functional ecosystem with a likely importance of the relative abundances of the various microbial components.

Microbiota diversity and immunity traits. Multivariate statistical analysis was applied to study microbiota in association with immune traits. Results highlighted both positive and negative correlations between monocytes, eosinophils, platelets, haemoglobin and red blood cell parameters, and genera such as *Prevotella, Roseburia* and *Dialister* (Figure 2). The strongest correlations were found with *Dialister*.

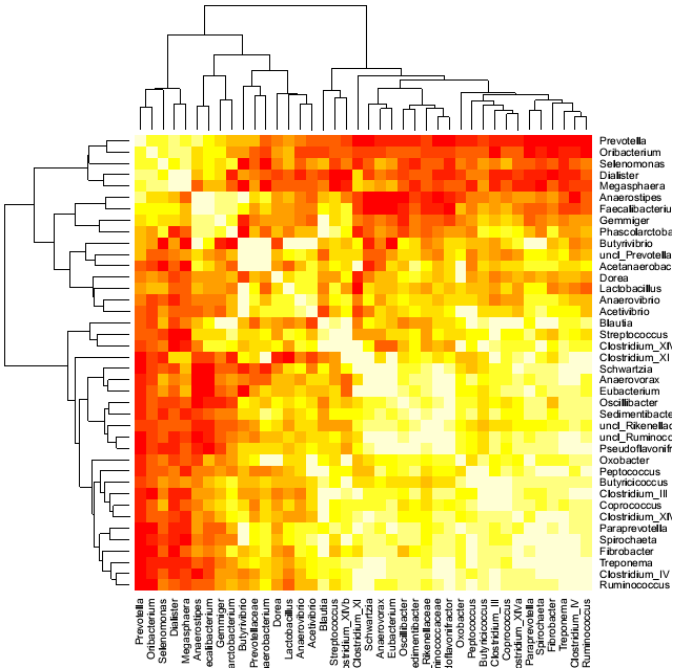

Figure 1: Genetic correlations between relative abundances of genera (positive correlations are shown in white, negative correlations in red and yellow colors indicate no correlation).

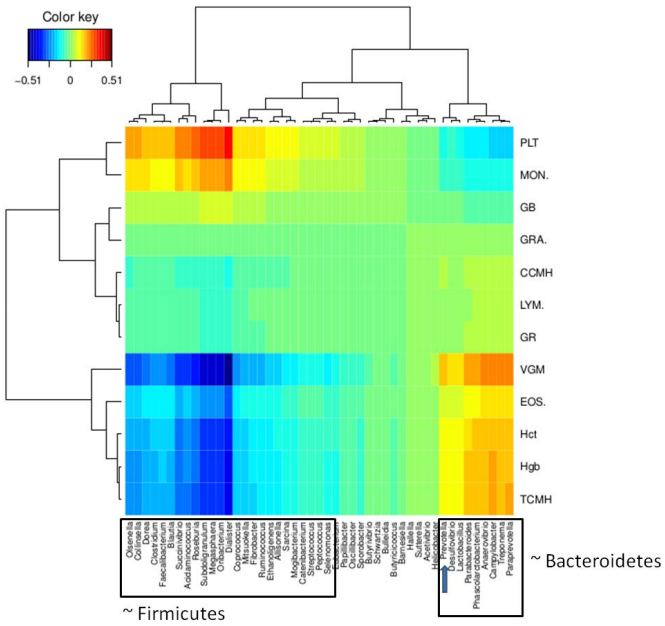

Figure 2: Canonical correlation analyses between bacterial genera and immune traits (PLT: platelets; MON: monocytes; GB: white blood cells; GRA: granulocytes; CCMH: mean cell hemoglobin concentration; LYM: lymphocytes; GR: red blood cells; VGM: red cell distribution width; EOS: eosinophils; Hct: hematocrit; Hgb: hemoglobin; TCMH: mean cell hemoglobin). The blue arrow points to the predominant *Prevotella* genus.

Conclusion

We have characterized the composition of the gut microbiota on a cohort of 60 days old piglets and have estimated the genetic parameters of the relative abundance of bacterial genera. In addition, we have evidenced significant links between the composition of the gut microbiota and few ITs, suggesting that the gut microbiota interplays with the host immunity in swine.

In a perspective of safe, competitive and sustainable animal breeding systems, deepening animal phenotyping and defining new selection goals are identified as major concerns. In this report we demonstrate for the first time that the gut microbiota composition in swine is influenced by the genetics of the host. These results pave the way for studying the microbiota as a new phenotype to measure in pigs. Microbiota parameters, together with zootechnical and immunity traits, will help to better decipher the driving forces that shape animal performances and robustness.

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