POULTRY MICROBIOTA

The poultry gut harbours a complex and dynamic microbiota whose role is essential in digestion and health. Find out more in this series of short articles.

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How to study the microbiome?

The poultry gut microbiota is a complex ecosystem, housing billions of bacteria whose role is essential in the bird's digestion, natural defences, well-being and overall health maintenance. Recently, the advances in DNA sequencing has allowed us to grow our understanding of the poultry microbiome.

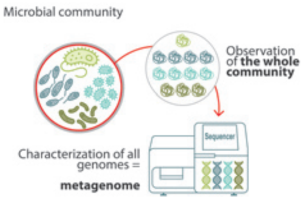
What is metagenomics?

Microbiologists used to apply cultural techniques and microscopy to identify and characterise microorganisms. These techniques are time consuming and provide a limited vision of a microbial community. Lately, thanks to the progress of high-throughput sequencing technologies, microbiologists have made a giant leap with the development of metagenomics.

Metagenomics applies a suite of sequencing technologies and bioinformatics tools to directly access the genetic content of entire communities of microorganisms (Thomas et al., 2012). Cultural and metagenomics approaches are complementary.

Characteristics of metagenomics techniques:

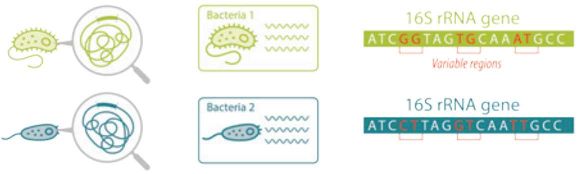
- They allow detection of rare bacteria species and ones that cannot be cultivated
- They enable the analysis of a large number of microbial samples at the same time
- They give a snapshot of a population's diversity within a sample



These techniques require a very specific expertise in biostatistics and important bioinformatics resources in order to translate the millions of DNA sequences generated into a microbial population composition. They also rely on a specific database of known bacteria sequences, while a large proportion are still unknown. Depending on the level of detail needed and the complexity of the experimental design, it can take between one week to several months to analyse a sequencing dataset.

From metagenomics to barcoding

To describe the microbial composition of gut microbiota using sequencing strategy it is not necessary to sequence the full metagenome. A common practice is to target a fragment of the bacterial genome, which is used as a marker or a sort of ID card of a bacteria. This approach, called **amplicon sequencing** or barcoding, drastically reduces the cost and the time of analysis. The 16S ribosomal RNA subunit gene (16S rRNA gene) is the most used marker gene to describe bacteria composition.



The rapid and substantial cost reduction in next-generation sequencing has dramatically accelerated the development of metagenomics and the understanding of host microbiota.

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Poultry Microbiota Insight

In the September issue we described metagenomics and DNA sequencing based techniques used to characterise bacterial communities such as amplicon sequencing. Let's see how these help better understand the effects of challenges and dietary intervention on poultry microbiota.

Unravelling microbiota diversity

Amplicon sequencing, or barcoding, is a technique based on the sequencing of only very small pieces of a bacteria genome, used as a marker or a sort of ID card of a bacteria. **Amplicon sequencing generates thousands of data from a single microbiota sample. How to make sense of these?** Scientists use several indicators to analyse the diversity and richness of a microbial population and to evaluate the effect of an intervention on microbiota diversity, including:

- **Alpha-diversity** represents the diversity within one microbiota sample. Shannon index is one of the most used criteria to describe alpha-diversity. It considers both the total number of different taxa found in the sample, called the **richness,** and the abundance or **predominance** of some taxa among the sample. The higher the Shannon Index, the more diverse and evenly abundant the microbiota.
- **Beta diversity** represents the similarity (or dissimilarity) between two samples. The beta diversity between groups of samples is evaluated using powerful software and specific statistical techniques (ordination). It applies a set of multiple variables to distinguish samples from each other. The shorter the distance between two samples, the lower the difference between the microbiota, the lower the beta-diversity.

Microbiota modulation

Recent metagenomic studies (Massacci et al., 2019) has shown how probiotic yeast interacts with broiler intestinal microbiota under challenging situations (*Campylobacter jejuni* experimental challenge), showing that the bacterial challenge:

- Lowers the richness and alpha-diversity of the birds' gut microbiota (control group).
- Increases the beta-diversity between different birds of the same group: the microbiota composition becomes more disparate, more heterogeneous in the control group.

The broiler diet supplementation with live yeast *Saccharomyces c. boulardii* CNCM I-1079 prior to bacterial challenge appears to soften the gut dysbiosis observed after the bacterial challenge:

- Microbiota richness and diversity (Shannon Index, Fig. 1) is less impacted. There is a higher number of different species and less predominance of certain species in the gut microbiota.
- The beta-diversity is reduced within the group: microbiota is more homogenous after the challenge when compared to the control.

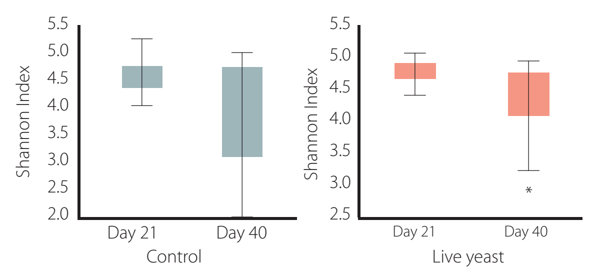

Fig. 1. Shannon Index (alpha-diversity) before (day 21) and after (day 40) bacterial challenge.

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