

An adult pig's gut harbours 2kg of microorganisms. Find out more about the key role of this microbiota in livestock performance and health in this series of short articles.

➤ How to study the microbiome?

The swine gut microbiota is a complex ecosystem, housing billions of bacteria whose role is essential in the animal's digestion, natural defences, well-being and overall health maintenance. Until recently, it was poorly described. However, the advances of the DNA sequencing has recently allowed us to grow our understanding of the swine microbiome.

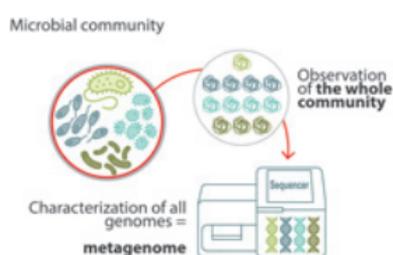
What is metagenomics?

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:

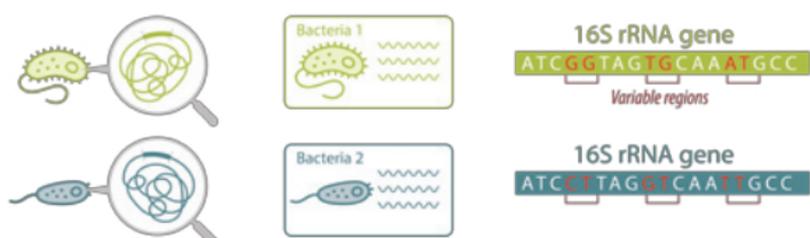
- They allow detection of rare bacteria species and ones that cannot be cultivated
- They enable the analysis of a large number of microbial samples at the same time
- They give a snapshot of a population's diversity within a sample



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 of 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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In the previous 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 swine 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**.

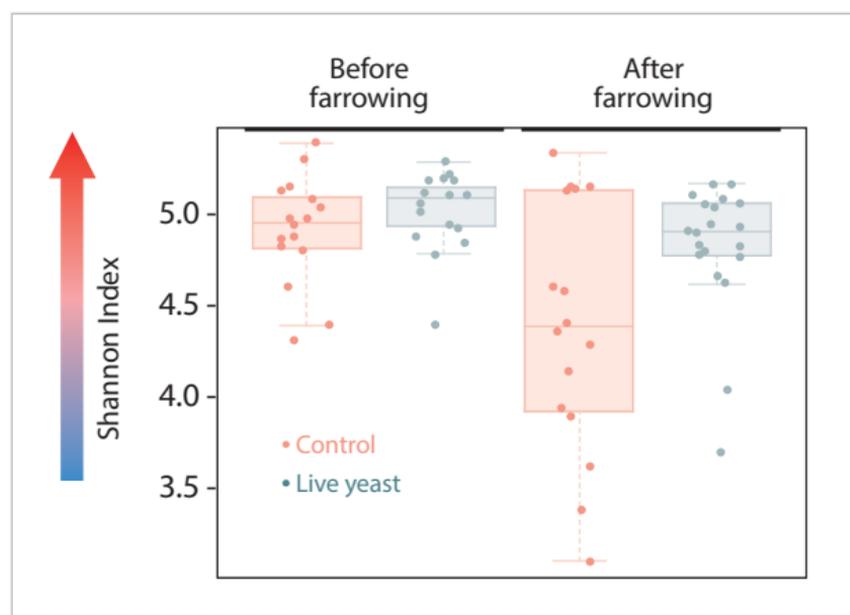
In practice, barcoding can be applied to study the effects of a challenge, dietary change, or probiotic supplementation on the animal microbiota. Using this method allows the structure of the bacteria community to be obtained by giving relative abundance of the different bacteria (called 'Operational Taxonomic Unit' or 'Amplicon Sequence Variants' depending on the method used), expressed in relative percentage. Different diversity indexes can also be calculated.

Among the various indexes, the **Shannon Index** is a parameter used to illustrate the richness of a microbiota sample and the evenness of the organisms' abundance distribution.

* The higher the Shannon Index, the more diverse and evenly abundant the microbiota.

Stress effect

Achard et al. (2019) have shown the detrimental effect of farrowing on sow microbiota diversity with important heterogeneity between the animals, as shown by the drop in the Shannon Index (red control group). The sows' diet supplementation with live yeast *S. c. boulardii* CNCM I-1079 prior to farrowing is able to attenuate this drop in diversity after farrowing. In addition, there is more homogeneity between the sows.



Reference: Achard C., Bravo de Laguna F., Apper E., Castex M. (2019). *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079 modulates the fecal microbiota of sows and subsequently beneficially affects weanling piglets. International Scientific Conference on Probiotics, Prebiotics, Gut Microbiota and Health (IPC), Prague, June 2019