METHODS TO STUDY THE INTESTINAL MICROBIOTA TO ADDRESS THE DISEASE IN A MORE PRECISE AND PERSONALIZED WAY

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Introduction

Today, precision medicine needs new analytical techniques that allow for more efficient results in less time on an individual basis. Before the use of molecular techniques, the study of microbial diversity was carried out using culture techniques. These techniques were not very precise and did not allow us to identify all microorganisms that make up a given microbiome. The appearance of molecular techniques has allowed the identification of practically all microorganisms, even those that cannot be cultivated. The ribosomal DNA is used as a molecular marker, due to its presence in all bacteria, easy detection and characterization. It contains conserved regions being not very variable. However, other complementary techniques are needed to avoid some limitations.

Objectives

This work aims to review the different techniques used in the study of microbiota to address the disease in a more precise and personalized way

Methods

A PubMed review was carried out using the keywords "microbiota, precision medicine, health, disease, techniques".

Results

Fluorescence in Situ Hybridization (FISH)

Fluorochrome-labeled DNA probes are used for the specific identification and quantification of microorganisms by fluorescence microscope. Specific probes can be designed that can reach the order, genus or species level. Its main limitation is that there are no specific probes for all bacterial genera and species, and their design is not simple. This methodology is used to study the alterations in the intestinal microbiota produced by the consumption of drugs, probiotics or to look for the presence of *Clostridium* in children.

Clone collection

It is a technique that combines PCR with the subsequent cloning and sequencing of the fragments obtained, which has allowed direct access to a great phylogenetic diversity. Universal primers are used that make it possible to amplify the DNA of practically any microorganism, without the need to cultivate it. The PCR products, cloned to form a database, permits the comparison of the obtained sequences. This methodology allows to perform a very precise taxonomic study. In contrast, it is expensive and needs specialized personnel. This technique is used to characterize the small intestine microbiota to look for differences in Crohn's disease and ulcerative colitis.

Denaturing gradient gel electrophoresis (DGGE)

DGGE allows the separation of DNA molecules according to a gradient of urea and formamide concentrations. DNA samples run through the gel, stopping when the concentration of urea and formamide is sufficient to cause denaturation. This process causes a change in the structure of the double strand, making it no longer soluble, preventing it from continuing to advance through the gel. To avoid the completely separation of DNA molecules, some cytokine and guanine sequences, called GC-Clamp, are attached to one of their ends. This technique makes it possible to study the possible modifications of the microbiota after the intake of antibiotics, probiotics and symbiotics, the colonization dynamics of bacteria in the gastrointestinal tract, and the different bacterial compositions in patients with inflammatory bowel disease. The most important limitation is that only bacteria in high concentrations are detected. The use of specific primers has enhanced the detection of minority bacterial populations in the gastrointestinal tract, mainly *Lactobacillus* and *Bifidobacterium*.

Polymerase chain reaction (PCR)

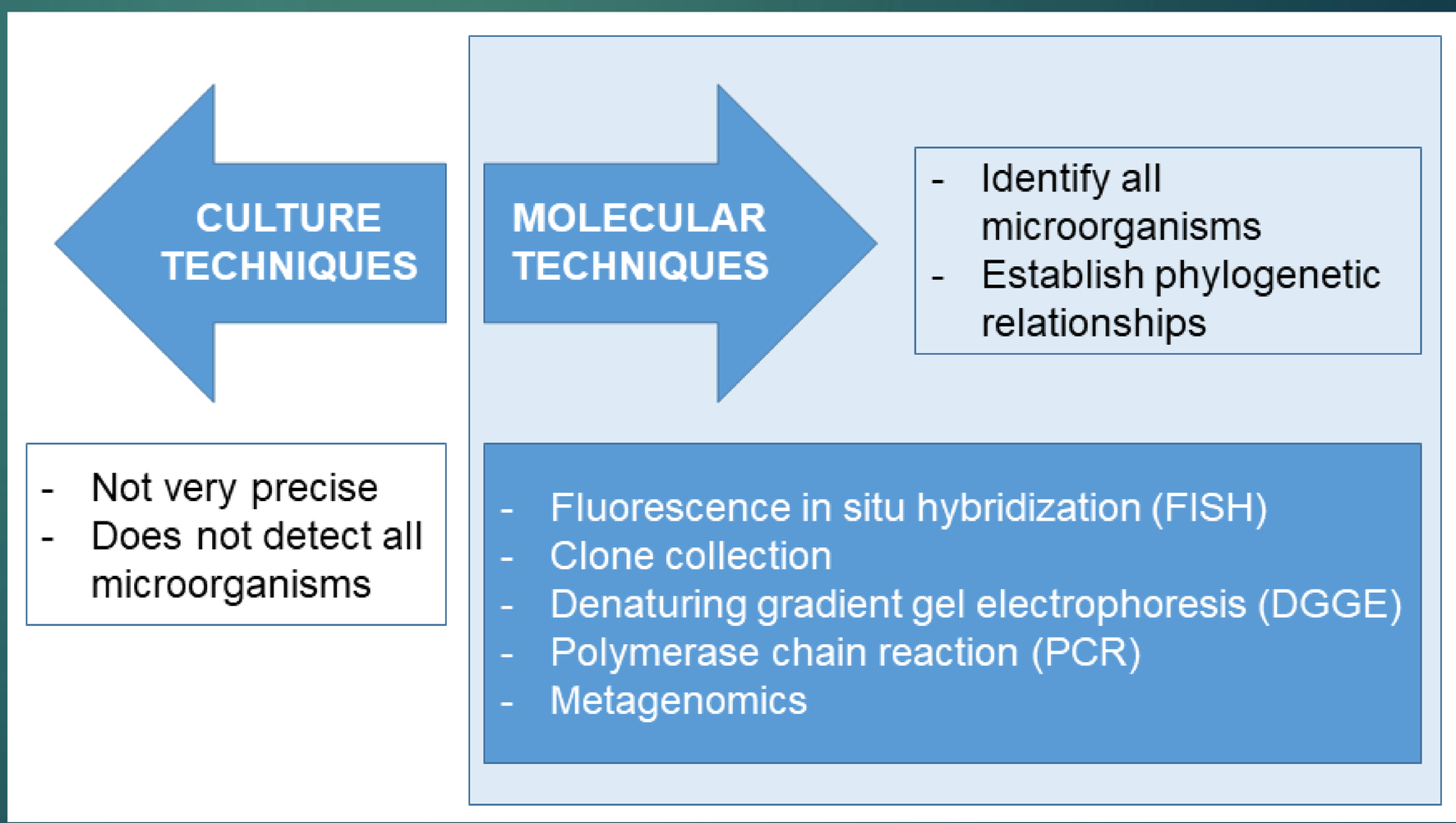
PCR is a quantitative technique used in the study of the composition of the microbiota. It is based on the analysis of the bands obtained in each cycle of the PCR, to be able to further compare them with a database. PCR constitutes the most widely used technique. It allows the quantification of variations in bacterial genera, alterations produced by the consumption of probiotics, symbiotics and prebiotics, and also the study of the whole intestinal microbiota in the study of pathological situations that affect the gastrointestinal tract.

Metagenomics

This technique allows the study of microbial communities from a genomic point of view, from genomic DNA extracted from non-cultured samples. Mass sequencing technologies, such as pyrosequencing or the Shotgut method, have made it possible to carry out the sequencing of large amounts of DNA. The main advantage is to analyze a microbial community from a taxonomic and functional point of view, with a minimal taxonomic bias. Furthermore, it not only provides information on the composition of the intestinal microbiota, but also reveals certain metabolic routes, such as the synthesis of antibiotics or vitamins.

Conclusions

FISH, clone collection, DGGE, PCR and metagenomics techniques contribute to the study of the human microbiota in a precise and effective way, constituting very useful diagnostic tools in precision medicine to address patient treatment in a personalized way.



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