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Intestinal microbiota and its host: harmony or discord?

# Structural and functional exploration of gut microbiota in 2018: how can this be used to improve health outcomes? As a diagnostic tool? As a source to identify new drugs? The pros and cons of these approaches

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## INTRODUCTION

Since the "microbiota revolution", the human intestinal microbiota has been considered a major contributor to the control of key physiological functions of the host. Its role is crucial, especially in the barrier function of the gut and the maturation of the immune system. The first studies that showed a "dysbiosis" of the intestinal microbiota and identified pathological signatures have raised great interest, not only among scientists but also among health industrialists.

Subsequent studies, conducted most often in animal models, have demonstrated a contributing role of the microbiota in various diseases. This new science suggested the existence of a new diagnostic means, made it possible to define new intervention strategies targeting the microbiota, and even saw in microorganisms a new source of treatments for various diseases, such as microbiota transplantation, new generation probiotics or bacterial metabolites.

The debate is open between the pros and cons of such approaches. Skeptics consider it as a transient micro-bio-mania, expensive (next-generation sequencing) and of little interest. Supporters, conversely, see it as a complete paradigm shift that invites us to review health monitoring, prevention and therapeutics, especially regarding chronic diseases, the incidence of which has steadily increased, uncontrolled, over the past 60 years.

## DEVELOPMENT OF THE INTESTINAL MICROBIOTA

How human beings and our constitution are considered has changed considerably since the discovery by the scientific community of the significant microbial nature of our composition, which is close to 50%.[1] Furthermore, it is now widely thought that there is a strong and symbiotic relationship between the purely human dimension of the individual and his/her microbial dimension. This unique symbiosis is established at the time of birth, develops with the maturation of immunity and, dependent on diet, remains relatively stable as long as the individual is in good health (Figure 1).[2]

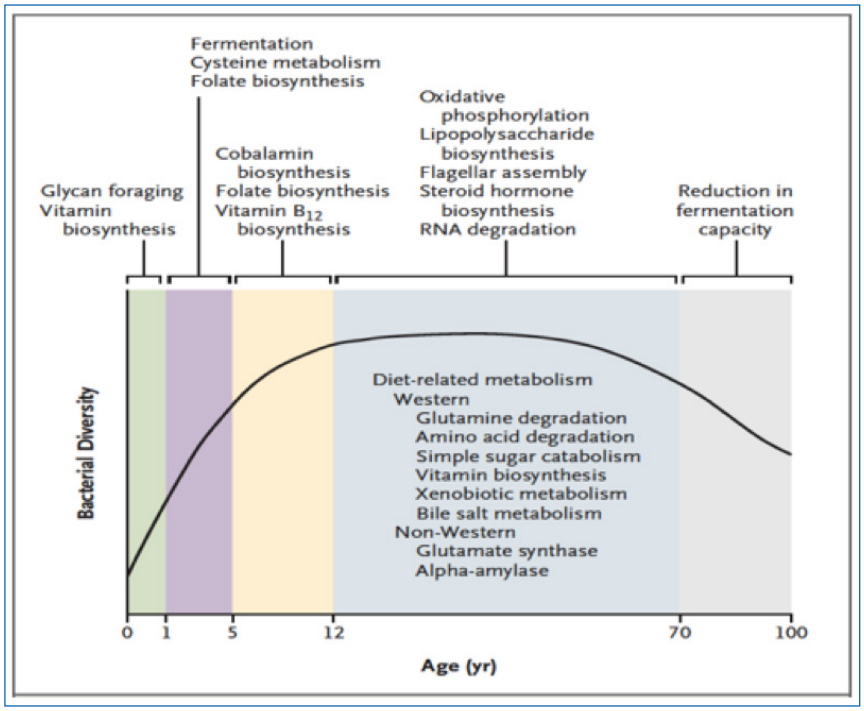



Figure 1. Development of the intestinal microbiota with age.[2]

## HOW TO STUDY THE MICROBIOTA? WHAT TOOLS DO WE HAVE?

### Bacterial culture

Despite the fact that bacterial culture makes it possible to isolate and assess a large part of the elements present in the microbiota, this modality presents a number of difficulties, including determining the appropriate culture medium. In fact, characterization of the composition of the intestinal microbiota, long limited only to the cultivated fraction, was often carried out by anaerobic cell culture methods, thus encountering the problem of most commensals, i.e. their high sensitivity to oxygen (*F. prausnitzii*). In addition, traditional culture methods do not allow for exhaustive description of the microbiome, and due to some bacteria being recalcitrant to culture, many of their functions and phenotypes remain unknown.[3] Nevertheless, new tools for analyzing "normal" and pathological microbiota allow a better phenotypic approach. "Culturomics" at least doubles the number of species isolated from the gut and allows for the culture of organisms corresponding to previously unassigned sequences.[4] The same is true for targeted phenotypic culture.[5]

### Metagenomics and sequencing

Currently, two techniques are conventionally used for sequencing of the microbiota. One focuses on a single gene that encodes 16S ribosomal RNA, amplifies a portion of it, and sequences it to identify the "50% of microbial inhabitants" taxonomy. The second, the "shotgun" method (or overall random sequencing of the metagenome), is more expensive and complex, and consists of sequencing the whole DNA of the microorganisms present. Both techniques require the use of powerful bioinformatics tools.

The shotgun technology has, notably, been adopted as part of the European project MetaHIT (Metagenomics of the Human Intestinal Tract).[6] The aim of this program was to study the genomes of all the bacteria constituting the human intestinal flora by establishing correlations between the genes of the microbiota and the state of health or the pathological state of the host. This sequencing provides information on bacterial richness and makes it possible to establish a catalog of the reference genes, identifying the functions of an individual's ecosystem. Many research studies have used these technologies for metagenomic characterization of the microbiome. Some of these have led to major advances in the understanding of the gut microbiota (Table 1).[7-23]

These tools have become more accessible since governmental support has helped develop and make them available to academic and industrial partners via the creation of MetaGenoPolis. This program supports platforms designed to promote the quantitative and functional investigation of the microbiome and introduce the microbiota approach in the management of human health.[24]

Table I. Lessons from the first metagenomic studies of the intestinal tract.[7-23]

| Reference | Publication focus |
|---|---|
| **Qin J, 2010 [7]** | Catalogue of 3.3 million genes; common core of functional genes and rare genes |
| **Arumugam M, 2011 [8]** | 3 enterotypes; selective ecological organization |
| **Schloissnig S, 2013 [9]** | Stability at the level of single nucleotide polymorphism (strains) |
| **Qin J, 2012 [10]** | Metagenomic approach of dysbiosis in type 2 diabetes |
| **Le Chatelier E, 2013 [11]** | Metagenomic approach of dysbiosis in obesity; diagnostic signatures |
| **Cotillard A, 2013 [12]** | Metagenomic approach of dysbiosis in obesity; importance of taking into account gene richness in stratification |
| **Qin N, 2014 [13]** | Metagenomic approach of dysbiosis in liver cirrhosis |
| **Li J, 2014 [14]** | Catalogue of 10 million genes; non modified common core of genes |
| **Nielsen HB, 2014 [15]** | Clustering by co-abundance of genes and metagenomic species |
| **Xiao L, 2015 [16]** | Catalogue of murine genes; correlation with environment |
| **Shoaie S, 2015 [17]** | Nutrition and intestinal metabolome |
| **Forslund K, 2015 [18]** | Signature of metformin treatment in type 2 diabetes |
| **Xiao L, 2016 [19]** | Catalogue of reference genes of porcine gut microbiome |
| **Plichta DR, 2016 [20]** | Metatranscriptomics, segregation of ecological niches |
| **Pedersen HK, 2016 [21]** | Impact of the microbiota on insulin sensitivity |
| **Costea PI, 2017 [22]** | Standard procedures for the metagenomic analysis of fecal samples |
| **Routy B, 2018 [23]** | Microbiota and effectiveness of anticancer immunotherapy |

## Standardisation of procedures

Characterization of the gut microbiota requires the use of procedures for which instructions, including hygiene and handling, are to be strictly applied. The maximum standardization of these procedures is crucial and guarantees the prevention of bias or loss of information.[22,25]

## The catalogs

The first published catalog of the genes present in the human gut microbiota came from information arising from the microbiome sequencing of 124 Spanish and Danish individuals, which nonetheless gave rise to a catalog of about 3 million genes.[7] Subsequently, a more comprehensive catalog of almost 10 million genes from 1267 individuals was published, in 2014.[26] These studies represented a significant advance in the knowledge of the human gut microbiota, showing in particular the existence of common genes and rare genes.

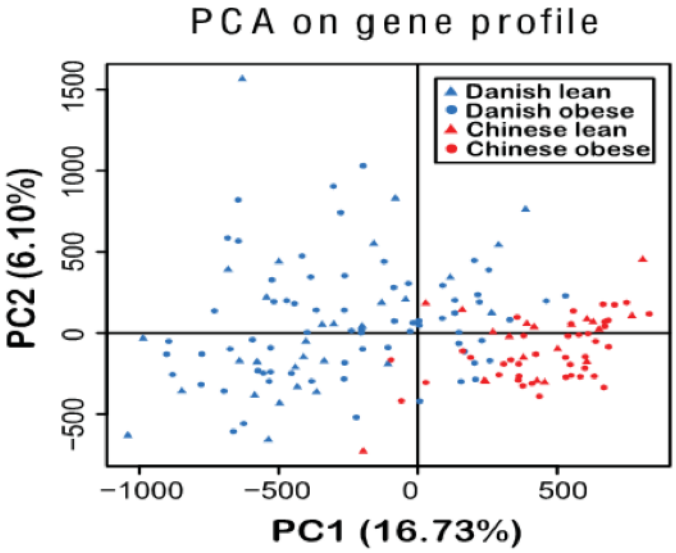
What is the use of this information, and how can the knowledge of the microbiota be used to manage health? Can the microbiota be a stratification tool, a diagnostic aid or a predictor of response? Although many questions arise when considering the microbiota and using it in the management of diseases, it is now accepted that it can help to identify a state of symbiosis or dysbiosis.

## HOW TO DEFINE SYMBIOSIS?

In contrast to dysbiosis – a more or less transient change in the composition of the gut microbiota depending on external conditions (diet, viral or bacterial infections, and antibiotics), which could indicate a pathological state – symbiosis, or eubiosis, means a state of equilibrium of the microbial ecosystem. However, this state is not easy to understand, and data are still fragmentary.

In the study by Li et al[14] that established a catalog of 10 million genes, a principal component analysis was performed on a set of samples from obese and non-obese Danish and Chinese subjects. This analysis revealed specific intestinal microbial signatures in both countries, showing the "country" parameter as more discriminating than the "obesity" parameter (Figure 2).[14] This result, which shows that the microbiota in obesity is not the same depending on whether the subject is Chinese or Danish, suggests that the importance of parameters defining the state of eubiosis or dysbiosis may vary according to ethnicity and geographical context.

Figure 2. Metagenomic shotgun sequencing of 60 Chinese and 100 Danish subjects. Country-specific signature of nutrient and xenobiotic metabolism.[14]




A study by Falony et al[26], conducted in a Belgian Flemish population, showed that the first element of variation of the intestinal microbiota was drug treatment (10%), followed by blood parameters, intestinal motility (transit), diet, health status, anthropometric characteristics and lifestyle. Thus, in type 2 diabetes, metformin could potentially be a confounding factor, with a greater impact on the microbiota than diabetes itself.

These results indicate that defining a state of dysbiosis or eubiosis in an individual requires taking into account a series of parameters identified in these studies as elements of microbiome variation.

A recent study by He et al[27], conducted in an ethnically homogeneous Chinese population, found that the most discriminating factors at the level of the microbiota were first the region and the place of life, followed by occupation, education and age. Anthropometric parameters, sex, obesity, existence of metabolic syndrome, alcohol consumption and smoking status, among other factors, showed less influence. The authors conclude that regional variability is a limitation to the diagnostic application of healthy intestinal microbiome reference genes and genes related to pathological conditions.[27]

A Dutch team studied a geographically homogeneous population (subjects who were all living in Amsterdam, The Netherlands) that was ethnically heterogeneous.[28] The different origins appeared as one of the major determinants separating the populations into three groups, each defined by an enterotype: *Prevotella* (Moroccans, Turks, Ghanaians), *Bacteroides* (Surinamese from Africa and South Asia), and *Clostridiales* (Dutch). Despite having shared the same environment for a long time, the study subjects had gut microbiota profiles specific to their birthplace. This geographical imprint probably reflected the composition they had acquired before migration (94% arrived in The Netherlands in adulthood).[28]

Conversely, in a US study of 550 Thai subjects living in Thailand or who were immigrants to the United States, microbiota analysis by 16S and shotgun sequencing showed that migration from a non-Western country to the United States was associated with a gradual loss of diversity, a transition from a *Prevotella*-dominated microbiota to a *Bacteroides*-dominated microbiota, and functions of the initial intestinal microbiome shifting to a microbiota much closer to the American standard microbiota.[29] This change increased with length of residence in the US, was complicated by obesity, and was increasingly evident over generations.[29]

These studies, which indicate, for some, the strong imprint of the ethnic group or country of birth or, on the contrary, for others, the loss of this imprint and its replacement by that of the "current" geographical context, highlight the difficulty of defining symbiosis and dysbiosis.

### Gene richness

The richness of the microbiome is another dimension identified as particularly important in defining symbiosis. In European populations with a bimodal distribution between gene-poor and gene-rich individuals, a relationship has been identified between the enterotype and health status, with gene poverty appearing associated with an altered health status.[11,12] This statement seems somewhat controversial, since a Chinese study observed, in diabetic patients eating a very high fiber diet, that there was both an improvement in their diabetes and a reduction in gene richness.[30]

Gene richness is a relatively constant factor of influence in various diseases. This is the case in Crohn's disease, which is marked by a loss of richness, especially a loss of *Faecalibacterium*, during the disease but also during the remission phase.[31]

Given the difficulty of defining symbiosis and the abundance of information generated by research on the microbiota, which has shown great differences between populations, the relevance of integrating a microbiota approach into the management of diseases, for example, for disease stratification, as an aid to diagnosis or as a predictor of response, remains in question.

## MICROBIOTA, A DIAGNOSTIC TOOL? A STRATIFICATION TOOL?

Nevertheless, disturbances of the microbiota have been described in some diseases, including: Crohn's disease, ulcerative colitis, irritable bowel syndrome, colorectal cancer, obesity, diabetes, liver, kidney and cardiovascular diseases, autism and depression, and Alzheimer's and Parkinson's diseases.[32]

With regards to the potential interest of using the microbiota as a stratification tool and predictor of response, several studies clearly show that it is possible to determine, by analysis of the microbiota, whether a patient is going to be a responder or a non-responder to a treatment. In a French cohort of obese patients, it appeared possible to predict, on examination of the microbiota, whether a patient was going to respond to dietary treatment or not.[33] Similarly, Routy et al[23] found in their study that metagenome analysis of fecal samples made it possible to predict the response to anti-PD1 antibody immunotherapy in patients with cancer.

With regards to the use of the microbiota for diagnosis, the study by Qin et al[13] in particular suggests that it may be a useful tool for some indications. This study identified several species that can diagnose quite effectively the presence or absence of liver cirrhosis. Microbiota analysis in this study also provided functional

information, such as the differences observed between the microbiota of sick individuals and that of healthy volunteers. Nevertheless, given the findings of He et al[27], showing that there is large regional variability in the microbiota, its diagnostic use may be limited as it may be questioned whether the powerful diagnostic tool developed in the study by Qin et al[13] can show the same performance in another geographical context.

Despite these questions regarding the validity of using the microbiota as a diagnostic tool, kits for self-assessment of intestinal microbiota are already marketed. These aim to "diagnose", "encourage", "choose a diet" or "improve self-awareness", or to serve as a screening tool for self-diagnosis of various diseases. Similarly, some companies propose, in response to the microbiota analysis of their customers, adapted diets and nutritional advice.

## MICROBIOTA AS A TREATMENT IN ITSELF? SOURCE OF NEW MEDICINES OR NEW TARGETS?

A number of trials present the microbiota as an interesting therapeutic tool.[34-36] Two studies of microbiota transplantation in *Clostridium difficile* infection found high response rates in transplant patients.[34,35] A Dutch study also reported that the transfer of microbiota from patients with a normal body mass index (BMI) to patients with metabolic syndrome improved insulin sensitivity in recipients, although this effect was transient.[36] However, microbiota transplantation has not shown convincing results in other diseases, and this modality remains under study. Several companies are interested in the potential benefits of microbiota transplantation, after the identification of bacteria of interest likely to be potentially used as drugs.

In fact, the gut microbiota may be a source of new drugs. Various research studies on butyrate show effects of this short-chain fatty acid that are comparable with those of a drug.[37-39]

It also helps to define new targets, as shown by the current development of a small molecule oral (and non-systemic) drug designed to block FimH adhesin from overabundant enterobacteria in patients with Crohn's disease.

A French platform for functional metagenomics has been set up in order to identify interesting microbial elements that can provide new molecules with potential therapeutic effects. The use of high-performance sampling, DNA extraction, cloning, metagenomic library and sequencing technologies has yielded encouraging results.[32] Using this same technology, an American team has recently discovered a bacterial compound ligand of a receptor in humans, showing the potential of this approach to identify in the gut microbiota molecules with therapeutic potential.[40]

The microbiota can also be a target of modulation: a specific nutritional modality or a diet rich in prebiotics, can have an impact on some diseases. With regard to probiotics, despite a demonstrated interest in some therapeutic applications, their impact on the microbiota and their effectiveness in some pathological conditions remain to be documented by clinical studies.

## COMMENTS, CONCLUSIONS

Despite the abundant and valuable information we have on the intestinal microbiota, additional knowledge is still needed. Beyond the wide interest and the fashionable phenomenon aroused by this topic, scientific research must remain very active in this highly promising field.

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