

Metagenomics and probiotics

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Abstract

The development of extensive sequencing methods has allowed metagenomic studies on the human gut microbiome to be carried out. This has tremendously increased our knowledge on gut microbiota composition and activity, allowing microbiota aberrations related to different diseases to be identified. These aberrations constitute targets for the development of probiotics directed to correct them. Probiotics are extensively used to modulate gut microbiota. Nevertheless, metagenomic studies on the effects of probiotics are still very scarce. In the near future, the use of metagenomics promises to expand our understanding of probiotic action.

Keywords: Probiotics, Metagenomics, Microbiota, Microbiome

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Human intestinal microbiota

The human gastrointestinal tract (GIT) harbours a very complex and dynamic microbial community which is specific for each person depending on environmental and genetic factors [1]. Each individual harbours about 1.5 kg of bacteria that can account for up to 60% of faecal weight. This microbial community, containing hundreds of different species, varies along the GIT, the stomach being sparsely populated (10^3 – 10^4 CFU/g contents) whilst the colon is heavily populated (10^{11} – 10^{12} CFU/g contents).

This gut microbiota plays an important role in human health, not only due to its participation in the digestion process, but also for its critical function in the development of the gut and the immune system [2,3]. A role of the GIT microbiota on the regulation of absorption and storage of lipids has also been demonstrated, constituting a promising area of research on the fight against obesity [4]. Moreover, recent studies suggest that specific aberrations in gut microbiota composition are associated with different diseases [5]. The

rationale for modulating the gut microbiota by means of probiotics derives from this demonstration on the importance of gut microbiota on host health.

Probiotics

Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a benefit on the host' [6]. According to this definition the term probiotic refers to strains for which specific beneficial effects have been scientifically demonstrated. Therefore, the properties and characteristics of each individual strain should be well defined and the effect of each strain demonstrated in a case by case manner. Some of the beneficial effects attributed to specific probiotic strains are supported by good scientific evidence from human intervention studies whilst other effects, although promising, are merely hypothetical at the moment [7].

Different microorganisms have been used as probiotics, among the most frequently used being *Lactobacillus* and *Bifidobacterium*. Most of the currently used strains have been isolated from the intestinal microbiota of healthy humans, which establishes a strong relationship between probiotics and gut microbiota research. In addition, most of their effects on human health are thought to be mediated through

modulation of the intestinal microbiota composition and/or activity.

Metagenomics in microbiome and probiotics research

Until the last decade our knowledge on microbiota composition and development was largely based on the use of traditional culture-based methods. The use of culturing has provided interesting data but unfortunately also a very biased view of the gut microbiota composition. The recent development of molecular culture-independent techniques has demonstrated that only a minority of the members of the microbiota are culturable, and therefore most of the microbes present in the gut had escaped scientific scrutiny until recently.

During the last 20 years several qualitative and quantitative culture-independent techniques, mostly based on the PCR and DNA hybridization, have been developed and applied to microbiota assessment. More recently, the development of fast and low cost DNA sequencing methods has allowed these modern techniques to be applied to the study of microbial communities directly in their environment without the need for species cultivation and isolation (so-called metagenomics) to characterize the human gut microbiome [8,9]. These techniques, based on extensive sequencing of DNA, have increased enormously our knowledge on microbiome composition and activity. The study of entire microbial communities using metagenomic approaches has revealed a much greater diversity than was previously thought to exist and has helped to determine the community structure of several previously unknown ecosystems. The development of these techniques is also contributing enormously to many different aspects of probiotics research (Fig. 1).

Over 3 million genes from the intestinal microbiome have been sequenced so far [9] and the existence of different human enterotypes has been identified [10]. The process of microbiota establishment in the newborn has been characterized in detail by using metagenomics [11] and the effect of diet has also been assessed [12]. Determining the microbiome of healthy compared with diseased individuals has allowed aberrations related to different human diseases to be identified, such as inflammatory disease or obesity [4,9]. In the probiotics field these aberrations constitute clear targets for the future development of products directed to counteract them.

Similarly, several projects have sequenced genomes of probiotics and intestinal commensal bacteria. This is allowing an understanding of the role of microbiota and probiotics in

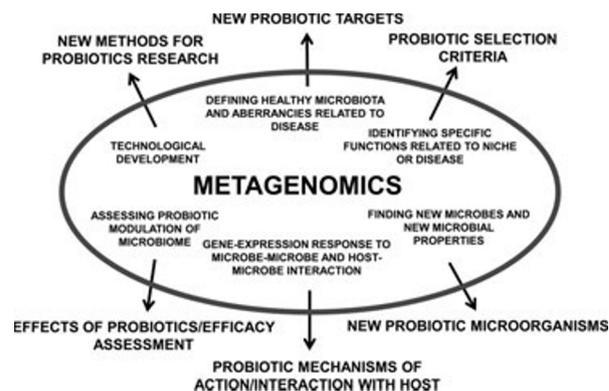


FIG. 1. Some contributions of metagenomics to the field of probiotics.

influencing human health. Genomic data on important intestinal microbiota members, such as *Bifidobacterium* or *Bacteroides*, have shown the genetic basis of the adaptation of these microorganisms to the intestinal environment. Genome sequencing of specific probiotic strains has also provided data on interesting properties of probiotics, including mechanisms of adhesion to the gut mucosa or interaction with the immune system, among others [13].

Challenges in microbiome and probiotics research

One of the major challenges in this field is to define the composition and activity of the healthy normal microbiota. Recent studies have shown differences in the microbiota composition of healthy subjects from different geographical locations [14], underlining the difficulties in defining the normal microbiota at microbial species level. However, metagenomic studies have provided new insight, indicating that the normal microbiota may be better defined at functional than at phylogenetic level. Thus, in order to understand the functional shift and restoration of the healthy condition induced by the probiotic, metagenomic studies should go beyond the taxonomic composition as inferred by the 16S rRNA gene and try to characterize the full genetic repertoire of the community [9]. Once the healthy human gut microbiota is defined the next step will be to develop intervention strategies targeted to modulating aberrant microbiota states towards healthy composition and activity. In this regard, probiotics constitute a promising option to achieve this aim. A deeper understanding of the microbiome-modulating abilities of specific probiotic strains is thus needed. So far, the metagenomic data available from human intervention studies with probiotics are very limited. Nevertheless, a plethora of such data is expected in the near future and some studies

are already under way. Incorporating such data with studies on host gene response to changes in microbiota, and probiotic administration, will allow us to understand microbe–microbe and host–microbe interactions and therefore how the gut microbiota affects our well-being and, in broader terms, our physiology.

It is important to underline that, as with any other technique, metagenomics may be subject to certain biases. Sampling, storage of samples and DNA extraction methods are likely to have an influence on the results obtained, and therefore comparative studies in this field are very welcome. When phylogenetic studies are carried out the amplification of the 16S rRNA gene, or a region of it, is often needed. It has been shown that this PCR amplification step is critical and the use of different primers may lead to a different representation of certain bacterial groups [15]. In this regard, it is surprising that in numerous metagenomic studies sequences from bifidobacteria are either absent or present at low levels, whilst using other methods these microorganisms appear to be present at relatively high levels in the same samples [1]. This example points out the need for further fine tuning of metagenomic techniques in order to avoid methodological biases that may have a profound effect on the results obtained. Another important point that should always be considered before running metagenomic studies is the sequencing depth, as it correlates directly with the cost, as well as the management and analysis of the massive amount of data generated, which requires the availability of storage capability and bioinformatics tools.

Conclusions

It has been known for a long time that alterations in gut microbiota are related to certain diseases, e.g. antibiotic-associated diarrhoea. However, until recently the beneficial effects of the microbiota on health have been less widely known. These benefits associated with an appropriate microbiota composition and activity constitute the ‘proof of concept’ for probiotics. Beneficial intestinal microbes constitute the main source for isolation of potential probiotic strains targeted at modulating the human microbiome to restore health.

The normal healthy microbiota cannot be easily defined but the development of metagenomics is rapidly extending our understanding of the role the microbiota plays in our health. This is allowing new potential areas of application of probiotics to be identified, including body sites other than the gut, as well as development of an understanding of mechanisms of action.

Combination of genome, microbiome and host-response data promises to bring probiotic research to a new level, in which scientific criteria for selection of effective strains for different conditions will be available worldwide for the scientific community to the benefit of consumers.

Transparency Declaration

The authors declare no conflict of interest.

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