Minireview

The genomics of probiotic intestinal microorganisms

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Abstract

An intestinal population of beneficial commensal microorganisms helps maintain human health, and some of these bacteria have been found to significantly reduce the risk of gut-associated disease and to alleviate disease symptoms. The genomic characterization of probiotic bacteria and other commensal intestinal bacteria that is now under way will help to deepen our understanding of their beneficial effects.

While the sequencing of the human genome [1,2] has increased our understanding of the role of genetic factors in health and disease, each human being harbors many more genes than those in their own genome. These belong to our commensal and symbiotic intestinal microorganisms - our intestinal 'microbiome' - which play an important role in maintaining human health and well-being. A more appropriate image of ourselves would be drawn if the genomes of our intestinal microbiota were taken into account. The microbiome may contain more than 100 times the number of genes in the human genome [3] and provides many functions that humans have thus not needed to develop themselves. The indigenous intestinal microbiota provides a barrier against pathogenic bacteria and other harmful food components [4-6]. It has also been shown to have a direct impact on the morphology of the gut [7], and many intestinal diseases can be linked to disturbances in the intestinal microbial population [8].

The indigenous microbiota of an infant's gastrointestinal tract is originally created through contact with the diverse microbiota of the parents and the immediate environment. During breast feeding, initial microbial colonization is enhanced by galacto-oligosaccharides in breast milk and contact with the skin microbiota of the mother. This early colonization process directs the microbial succession until weaning and forms the basis for a healthy microbiota. The viable microbes in the adult intestine outnumber the cells in the human body tenfold, and the composition of this microbial population throughout life is unique to each human being. During adulthood and aging the composition and diversity of the microbiota can vary as a result of disease and the genetic background of the individual.

Current research into the intestinal microbiome is focused on obtaining genomic data from important intestinal commensals and from probiotics, microorganisms that appear to actively promote health. This genomic information indicates that gut commensals not only derive food and other growth factors from the intestinal contents but also influence their human hosts by providing maturational signals for the developing infant and child, as well as providing signals that can lead to an alteration in the barrier mechanisms of the gut. It has been reported that colonization by particular bacteria has a major role in rapidly providing humans with energy from their food [9]. For example, the intestinal commensal *Bacteroides thetaiotaomicron* has been shown to have a major role in this process, and whole-genome transcriptional profiling of the bacterium has shown that specific diets can be associated with selective upregulation of bacterial genes that facilitate delivery of products of carbohydrate breakdown to the host's energy metabolism [10,11]. Key microbial groups in the intestinal microbiota are highly flexible in adapting to changes in diet, and thus detailed prediction of their actions

and effects may be difficult. Although genomic studies have revealed important details about the impact of the intestinal microbiota on specific processes [3,11-14], the effects of species composition and microbial diversity and their potential compensatory functions are still not understood.

Probiotics and health

A probiotic has been defined by a working group of the International Life Sciences Institute Europe (ILSI Europe) as "a viable microbial food supplement which beneficially influences the health of the host" [15]. Probiotics are usually members of the healthy gut microbiota and their addition can assist in returning a disturbed microbiota to its normal beneficial composition. The ILSI definition implies that safety and efficacy must be scientifically demonstrated for each new probiotic strain and product. Criteria for selecting probiotics that are specific for a desired target have been developed, but general criteria that must be satisfied include the ability to adhere to intestinal mucosa and tolerance of acid and bile. Such criteria have proved useful but cumbersome in current selection processes, as there are several adherence mechanisms and they influence gene upregulation differently in the host. Therefore, two different adhesion studies need to be conducted on each strain and their predictive value for specific functions is not always good or optimal. Demonstration of the effects of probiotics on health includes research on mechanisms and clinical intervention studies with human subjects belonging to target groups.

The revelation of the human genome sequence has increased our understanding of the genetic deviations that lead to or predispose to gastrointestinal disease as well as to diseases associated with the gut, such as food allergies. In 1995, the first genome of a free-living organism, the bacterium *Haemophilus influenzae*, was sequenced [16]. Since then, over 200 bacterial genome sequences, mainly of pathogenic microorganisms, have been completed. The first genome of a mammalian lactic-acid bacterium, that of *Lactococcus lactis*, a microorganism of great industrial interest, was completed in 2001 [17]. More recently, the genomes of numerous other lactic-acid bacteria [18], bifidobacteria [12] and other intestinal microorganisms [13,19,20] have been sequenced, and others are under way [21]. Table 1 lists the probiotic bacteria that have been sequenced. These great breakthroughs have demonstrated that evolution has adapted both microbes and humans to their current state of cohabitation, or even symbiosis, which is beneficial to both parties and facilitates a healthy and relatively stable but adaptable gut environment.

Table 1

Probiotic bacteria with completed genome sequences

Strain	Size (Mb)	Reference
Bifidobacterium longum NCC 2705	2.25	[12]
Lactobacillus plantarum WCFS1	3.30	[18]
Lactobacillus johnsonii NCC 533	2.02	[23]
Lactobacillus acidophilus NCFM	1.99	[22]

Lessons from genomes

Lactic-acid bacteria and bifidobacteria can act as biomarkers of gut health by giving early warning of aberrations that represent a risk of specific gut diseases. Only a few members of the genera *Lactobacillus* and *Bifidobacterium*, two genera that provide many probiotics, have been completely sequenced. The key issue for the microbiota, for probiotics, and for their human hosts is the flexibility of the microorganisms in coping with a changeable local environment and microenvironments.

This flexibility is emphasized in the completed genomes of intestinal and probiotic microorganisms. The complete genome sequence of the probiotic *Lactobacillus acidophilus* NCFM has recently been published by Altermann *et al*. [22]. The genome is relatively small and the bacterium appears to be unable to synthesize several amino acids, vitamins and cofactors. It also encodes a number of permeases, glycolases and peptidases for rapid uptake and utilization of sugars and amino acids from the human intestine, especially the upper gastrointestinal tract. The authors also report a number of cell-surface proteins, such as mucus- and fibronectin-binding proteins, that enable this strain to adhere to the intestinal epithelium and to exchange signals with the intestinal immune system. Flexibility is guaranteed by a number of regulatory systems, including several transcriptional regulators, six PurR-type repressors and nine two-component systems, and by a variety of sugar transporters. The genome of another probiotic, *Lactobacillus johnsonii* [23], also lacks some genes involved in the synthesis of amino acids, purine nucleotides and numerous cofactors, but contains numerous peptidases, amino-acid permeases and other transporters, indicating a strong dependence on the host.

The presence of bile-salt hydrolases and transporters in these bacteria indicates an adaptation to the upper gastrointestinal tract [23], enabling the bacteria to survive the acidic and bile-rich environments of the stomach and small intestine. In this regard, bile-salt hydrolases have been found in most of the sequenced genomes of bifidobacteria and lactic-acid bacteria [24], and these enzymes can have a significant impact on bacterial survival. Another lactic-acid bacterium, *Lactobacillus plantarum* WCFS1, also contains a large number of genes related to carbohydrate transport and utilization, and has genes for the production of exopolysaccharides and antimicrobial agents [18], indicating a good adaptation to a variety of environments, including the

human small intestine [14]. In general, flexibility and adaptability are reflected by a large number of regulatory and transport functions.

Microorganisms that inhabit the human colon, such as *B. thetaiotaomicron* and *Bifidobacterium longum* [12], have a great number of genes devoted to oligosaccharide transport and metabolism, indicating adaptation to life in the large intestine and differentiating them from, for example, *L. johnsonii* [23]. Genomic research has also provided initial information on the relationship between components of the diet and intestinal microorganisms. The genome of *B. longum* [12] suggests the ability to scan for nutrient availability in the lower gastrointestinal tract in human infants. This strain is adapted to utilizing the oligosaccharides in human milk along with intestinal mucins that are available in the colon of breast-fed infants. On the other hand, the genome of *L. acidophilus* has a gene cluster related to the metabolism of fructo-oligosaccharides, carbohydrates that are commonly used as prebiotics, or substrates to enhance the growth of beneficial commensals in the colon [25].

Microbe-host interactions

Genomic information on *B. longum* [12], *L. plantarum* [18], *L. johnsonii* [23] and *L. acidophilus* [22] also gives insight into the adhesive mechanisms of these microorganisms, which provide the basis both for populating the gut and for communicating developmental signals to specific areas and sites in the gut mucosa. In addition, a eukaryotic-type serine protease inhibitor was identified in the genome of *B. longum* which may contribute to the immunomodulatory activity of this species. Operons coding for bacteriocins have been identified in *L. johnsonii* and *L. acidophilus*, and they may have a role in influencing the succession of microbiota in humans over time.

It is obvious that an understanding of the cross-talk between the intestinal microbiota and its host would expand our understanding of the relationship between microbiota and health. Specific imbalances or deviations in the intestinal microbiota may render us more vulnerable to intestinal inflammatory diseases and to diseases beyond the intestinal environment. Genomic information will be important in understanding this cross-talk. Genomic data from *B. longum* and *Bacteroides thetaiotaomicron*, for example, provide information on how these bacteria are specifically adapted to the gut. *B. thetaiotaomicron* contains the largest number of genes related to carbohydrate uptake and metabolism so far reported for a sequenced bacterial genome [13]. It has also been shown to modulate glycosylation of the intestinal mucus and to induce the production of antimicrobials by the mucosa [26], and it attenuates inflammation in an *in vitro* model [27]. These observations suggest mechanisms by which intestinal microbes may influence the gut microecology and shape the immune system. Genomic information from *Bacteroides* has also shown how these intestinal bacteria may be able to evade detection by the immune system by changing the composition of the capsular surface polysaccharide, and therefore their antigenicity [13,20].

The genome sequences now available give some idea of the potential properties of these microorganisms, but give no information about the situation *in vivo*. A full response to the local environment will only be triggered when all the factors, including physicochemical conditions and microbe-host interactions, are present. In this regard, genomic research can be extremely useful, as it should provide the necessary tools, such as DNA microarrays, for unraveling the functions of probiotics and gut-related bacteria *in vivo* [14] and for monitoring the effect of probiotic consumption on gene expression in the host [28]. At the same time, genomics will open avenues to understanding microbe-host and microbe-microbe cross-talk, and will provide mechanisms for the specific effects of probiotics on host gene expression and cell proliferation that have been observed in model systems. The weak messages provided so far by the mass of microarray data will have to be correctly interpreted and bioinformatic approaches developed.

Towards a complete understanding of probiotics

Integrating microbial genomic and transcriptional information with data on host gene expression in the exposed mucosal sites and elsewhere will help in understanding the roles of probiotics, microbiota, and microbe-microbe and host-microbe interactions. Symbiotic microorganisms may dedicate part of their genomes to processes that are beneficial to both the host and the microbe, and identification of such processes will help in the development of new probiotics. Functional redundancy in the ecosystem can guarantee that these key processes are not affected by environmental changes [29]. Again, genomic research will provide the evidence of redundancy which will, in turn, help to identify the key processes.

From a functional point of view, genomic analysis often allows one to assign a possible function to uncharacterized genes that have homology with annotated genes of known or putative function. Genes with known function represent 71% of the genes of *B. longum*, 70% of *L. plantarum*, 40% of *Bacteroides fragilis* and 58% of *B. thetaiotaomicron*, indicating the need to characterize the functions of the remaining, unknown, genes. The availability of probiotic genomes will be very important for predicting the capabilities of the various probiotic microorganisms [30], and will also allow the development of genetic tools to analyze the functionality of these strains as probiotics [31]. This will also provide information about their mechanisms of action, facilitating the development or selection of a new generation of probiotics. Such data will also enable us to know which factors influence the performance

of probiotics, thus allowing a rational approach to strain improvement.

The comparative genomics of probiotic and symbiotic microorganisms and pathogens will provide valuable information on the features of these different lifestyles. This will, in turn, shed light on the detailed functional properties of probiotics and their safety, as well as their evolutionary relationships. In conclusion, genetic studies on the current generation of probiotic microorganisms will increase our understanding of their biological mechanisms and provide an important step toward understanding human biology in its most complete sense.

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