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Probiogenomics as a tool to obtain genetic insights into adaptation of probiotic bacteria to the human gut

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Bifidobacteria and lactobacilli are widely exploited as health-promoting bacteria in many functional foods. However, the molecular mechanisms as to how these bacteria positively impact on host health are far from completely understood. For this reason these microorganisms represent a growing area of interest with respect to their genomics, molecular biology and genetics. Recent genome sequencing of a large number of strains of bifidobacteria and lactobacilli has allowed access to the complete genetic makeup of representative members of these bacteria. Here, we will discuss how the analysis of genomic data has helped us to understand the mechanisms by which these bacteria adapt to the specific environment of the gastrointestinal tract, while also revealing genetic functions that mediate specific host-microbe interactions.

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General Features

The bacterial community living in the human gastrointestinal tract (GIT), also known as GIT microbiota, are composed by a vast collection of microorganisms whose composition differs depending on the different regions of the gut. Bifidobacteria and lactobacilli are common inhabitants of the distal regions of the GIT, i.e., the large and the small intestine, respectively.[1] Interestingly, the intestinal microbiota not only includes naturally resident lactobacilli, also known as autochthonous lactobacilli, but also various lactobacilli that have been acquired by food ingestion.

The genera Bifidobacterium and Lactobacillus belong to the phyla Actinobacteria and Firmicutes, respectively, both representatives of Gram positive microorganisms that ferment carbohydrates to mainly organic acids. Bifidobacteria predominantly produce acetate and lactate as fermentation end products, whereas lactobacilli will produce a variety of organic acids, although all produce a significant amount of lactate. Bifidobacteria and lactobacilli are often grouped together based on the fact that these microorganisms share certain metabolic features (i.e., lactic acid production), while both are also extensively exploited by the food industry as health-promoting or probiotic bacteria in functional foods. Nevertheless, one should keep in mind that lactobacilli and bifidobacteria from a phylogenetic perspective occupy distinctly different positions.

The interplay between the GIT microbiota and the human host can be classified as a continuum involving symbiosis and commensalism to pathogenesis. In the human GIT, co-evolution of such host-microbe interactions is the consequence of commensal relationships in which neither partner is disadvantaged, and symbiotic relationships in which both partners benefit, be it from unique metabolic activities or other advantageous properties.

Probiotics and Health

The probiotic concept dates back to 1908 when Metchnikoff noticed that the consumption of certain fermented foods elicited positive effects on human health.[2] The generally accepted definition of probiotics was proposed by the Food and Agriculture Organization (FAO) World Health Organization (WHO) as follows: "Probiotics are live microorganisms which when administrated in adequate amounts confer a health benefit on the host" (FAO/WHO 2001). This definition implies that safety and efficacy must be demonstrated for each probiotic strain. No specific criteria for selecting new probiotics have been so far proposed, however general criteria that must be satisfied involve the capacity to adhere to the intestinal mucosa and the ability to tolerate acid and bile stress.[3,4] There is accumulating evidence underpinning the capacity of probiotic strains to exert one or more of the following positive activities: anti-inflammatory immune-modulation, reduction of atopic disease symptoms, beneficially influencing the composition and activity of intestinal microbiota, alleviation of acute gastro-enteritis, prevention or suppression of bacterial infections, reduction of lactose intolerance, reduction of intestinal inflammation, production of specific short chain fatty acids, conjugated linoleic acids and vitamins and alleviation of constipation.[5-7]

Although there is suggestive evidence for each of these functional claims, the molecular mechanisms behind such probiotic activities remain largely unknown. The decoding of microbial genome sequences, i.e., microbial genomics, offers the possibility of accelerating research into the mechanisms of action of probiotic bacteria.[8-10]

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Genomics of Probiotic Bacteria

Research in microbiology has remarkably changed during the last decade, largely due to the availability of novel whole-genome sequencing approaches. In fact, the decoding of the genome sequences of more than 1,000 bacteria, as currently present in the NCBI database (www.ncbi.nlm.nih.gov) has greatly advanced our understanding of bacterial biology. The initial microbial genomics efforts were mainly directed toward decoding the genomes of pathogenic bacteria because of their impact on human well-being. The obtained genomic data have opened new avenues of research and even sparked the origin of a new genomics-based discipline, called pathogenomics, which aims to understand the genetic basis of bacterial pathogenesis.[11] Recently, genome sequencing has also directed its interest toward food-related bacteria, intestinal commensals and probiotic bacteria. In 2009, a correspondingly novel discipline designated as probiogenomics was coined, which aims to provide insights into the diversity and evolution of commensal/probiotic bacteria and to reveal the molecular basis for their health-promoting activities.[9] The public availability of full genome sequence data has significantly expanded our understanding of the biology of these microorganisms and has generated an enormous amount of information on metabolic capabilities, genetics and phylogeny of these bacteria.

Currently, the genus Bifidobacterium includes 37 species (for a review see Ref. 10). However, at the time of writing only 11 completely sequenced bifidobacterial genomes were publicly available[12-17] (**Table 1**), with genome sequences of another 13 strains still unfinished (NCBI source). Notably, for a small number of cases such as *B. bifidum*, *B. longum* subsp *longum*,[12,13] *B. animalis* subsp *lactis*[15,16] two or more genome sequences are publicly available.

In contrast, the emphasis on genomics efforts have been firmly placed on the genus Lactobacillus with more than 26 genomes completely decoded (**Table 1**). This larger number of sequenced genomes of lactobacilli (as compared with bifidobacteria) may be a reflection of a larger number of lactobacilli being included as active ingredients in functional foods. Specific probiotic strains have been sequenced, such as those that belong to the species *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus fermentum*, *Lactobacillus gasseri*, *Lactobacillus johnsonii*, *L. plantarum*, *L. salivarius* and *L. reuteri* (for review see refs. 9 and 18). Genomics data has significantly enhanced and will continue to improve our knowledge on the functionality of various Bifidobacterium and Lactobacillus species. Furthermore, the acquired genomic information has also provided clues as to how bifidobacteria and lactobacilli have adapted to the GIT environment and how they interact with their host (see below).

Comparative genome investigations involving the bifidobacterial strains for which the genomes had been completely decoded revealed that the deduced bifidobacterial pan-genome consists of more than 5,000 genes.[19] The function of many bifidobacterial genes is still unknown but one would imagine that some of these have to be crucial for colonization of and survival in the GIT. Moreover, a set of genes shared by all sequenced bifidobacterial genomes was identified and this represents a presumed core

Table 1. General features of sequenced Bifidobacterium and Lactobacillus genomes

Species	Genome size (bp)	%GC	Gene numbers
Bifidobacterium strains			
B. longum subsp *longum* NCC2705	2,256,640	60%	1798
B. longum subsp *longum* DJ010A	2,375,792	59%	2061
B. adolescentis ATCC15703	2,089,645	59%	1701
B. animalis subsp *lactis* AD011	1,933,695	60%	1603
B. animalis subsp *lactis* Bl-04	1,938,709	60%	1631
B. animalis subsp *lactis* DSM 10140	1,938,483	60%	1629
B. bifidum PRL2010	2,214,656	62%	1791
B. bifidum S17	2,186,882	62%	1845
B. dentium Bd1	2,636,367	58%	2197
B. longum subsp *infantis* 157F	2,400,312	60%	2062
B. longum subsp *infantis* ATCC 15697	2,832,748	59%	2588
B. longum subsp *longum* BBMN68	2,265,943	59%	1878
B. longum subsp *longum* JCM 1217	2,385,164	60%	2009
B. longum subsp *longum* JDM 301	2,477,838	59%	2035
B. breve UCC2003	2,422,684	59%	1642
Lactobacillus strains			
L. acidophilus NCFM	1,993,560	34%	1938
L. casei ATCC334	2,895,264	46%	2909
L. gasseri ATCC 33323	1,894,360	35%	1898
L. johnsonii NCC533	1,992,676	34%	1918
L. plantarum WCFS1	3,308,274	44%	3135
L. fermentum IFO 3956	2,098,685	51%	1912
L. salivarius UCC118	1,827,111	32%	1864
L. amylovorus GRL 1112	2,067,702	38%	2126
L. brevis ATCC 367	2,291,220	46%	2314
L. casei BL23	3,079,196	46%	3090
L. casei Zhang	2,861,848	46%	2906
L. crispatus ST1	2,043,161	36%	2100
L.delbrueckii subsp bulgaricus ATCC11842	1,864,998	49%	2184
L.delbrueckii subsp bulgaricus ATCC BAA-365	185,695,1	49%	2033
L.delbrueckii subsp bulgaricus ND02	2,125,753	49%	2177
L. helveticus DPC 4571	2,080,931	37%	1838
L. johnsonii FI9785	1,755,993	34%	1780
L. plantarum JDM1	3,197,759	44%	3029
L. plantarum ST-III	3,254,376	44%	3137
L. reuteri DSM 20016	1,999,618	38%	2027
L. reuteri JCM 1112	2,039,414	38%	1901
L. rhamnosus GG	3,010,111	46%	2985
L. rhamnosus Lc 705	2,968,598	46%	2954
L. sakei 23K	1,884,661	41%	1963

genome of 967 genes, mostly corresponding to housekeeping functions (i.e., cell envelope biogenesis, replication, transcription, translation and signal transduction).[19] This extensive comparative analysis also allowed the identification of genes that were present in just a single genome, while absent in any other currently available bifidobacterial genome [truly unique genes (TUG)]. TUG numbers ranged from 21 to 230 in each of the nine genomes analyzed. The majority of TUG have unknown functions and such genes are thought to perform a specialized function for these bacteria. Thus, the Bifidobacterium genomes display a relative high level of conservation, which is also coupled to a reasonable degree of genome synteny.[9] Conversely, the Lactobacillus chromosomes reflect the high heterogeneity at phylogenetic, phenotypic and ecological levels among the various members of this genus.[20] Sequencing of LAB genomes has indicated that loss and decay of ancestral genes has played a key role in the evolution of Lactobacillales, a taxon that diverged from its Bacillus ancestor with an estimated loss of 600–1,200 genes of its total gene repertoire.[21] A large proportion of these lost genes encoded biosynthetic enzymes and functions involved in sporulation.[21] Nevertheless, in addition to major gene decay occurrences, gene acquisition events also occurred which might be a consequence of the nutrient-rich niches occupied by the LAB, such as milk and the GIT. Gene duplication events in lactobacilli have occurred involving genes encoding peptidases and amino acid transport proteins, as well as involving genes implicated in the transport and metabolism of carbohydrates.[21] Furthermore, comparative analysis between GIT-associated species, such as *L. acidophilus*, *L. gasseri*, and *L. johnsonii*, on the one hand, and dairy species, such as *L. helveticus* and *L. bulgaricus*, on the other, has revealed genetic changes that seem to have occurred as a result of niche-specific selective pressure, which appears to have driven genome evolution of these individual species.[22-24]

In addition to gene duplication, lactobacilli are also believed to have acquired new genetic information through HGT (horizontal gene tranfer), which is obvious from the analysis of such genomes. Genes encoding cell surface factors in *L. johnsonii* and the exopolysaccharide cluster in the *L. acidophilus* complex are clear examples of presumed HGT in probiotic lactobacilli.[23,25]

The differences between the genomes of bifidobacteria and lactobacilli highlight their distinct phylogeny, but also reflect the different niches they occupy and the correspondingly niche-adjusted metabolic activities. In this context, the bifidobacterial genome content highlights its relatively broad prototrophy with respect to amino acids, nucleotides and vitamins.[26] In contrast, the genome content of lactobacilli reflects a high level of auxotrophy for such compounds.[9]

Adaptation of Probiotic Bacteria to the Human Gut

Probiogenomic investigations have highlighted a plethora of genetic features that may explain how bifidobacteria and lactobacilli have so well adapted to the human GIT. A key example of such an adaptation is represented by the carbohydrate-degrading capabilities of bifidobacteria, which consist of a large arsenal of enzymes involved in the metabolism of complex carbohydrates that are not digested by human enzymes and thus are expected to arrive in the lower regions of the GIT in an intact form. Dissection of bifidobacterial genomes suggests that a relatively large proportion of this genetic arsenal is involved in the metabolism and transport of carbohydrates, with several carbohydrolases predicted to be required for the utilization of various plant-derived dietary fibers or complex sugars.[12] Moreover, genetic and biochemical studies have been directed to analyze the capabilities of various bifidobacterial species to utilize diet-related carbohydrates, such as amylopectin, galactan, starch and pullulan.[27-29]

Another example of how bifidobacterial genome data allows us to link the presence of particular genes to a specific ecological niche adaptation has been provided by publications focusing on bifidobacteria isolated from different environments, such as the infant gut (i.e., the case of *B. longum* subsp *infantis* ATCC15697[14]) and the oral cavity (i.e., the case of *B. dentium* Bd1[17]) or to a bifidobacterial strain that can utilize human mucin (i.e., the case of *B. bifidum* PRL2010[30]).

Probiogenomics of Bifidobacteria

As mentioned above, various probiogenomic efforts have been undertaken in order to underpin the genetic and metabolic characteristics of selected members of the genus Bifidobacterium.[12,14,30,31]

The genome sequence of *B. longum* subsp infantis ATCC 15697 contains features that explain the ability of this strain to consume specific human milk carbohydrates known as human milk oligosaccharides (HMO). In particular, the *B. longum* subsp *infantis* ATCC 15697 genome harbours a gene cluster that encodes various glycosyl hydrolases and carbohydrate transporters necessary for importing and metabolizing HMOs.[14] This 43 Kb large gene cluster specifies a variety of catabolic enzymes such as fucosidase, sialidase, β-hexosaminidase and β-galactosidase activities, as well as extracellular solute binding proteins and permeases predicted to be active on HMOs.[14] Furthermore, the genome of this microorganism contains additional genetic loci specifying fucosidases and sialidases, as well as a complete urease operon, predicted to be involved in the utilization of urea, which represents an important nitrogen source of milk.[14]

Another important member of the bifidobacterial population frequently encountered in the infant gut microbiota is represented by the *B. bifidum* species.[32] Members of this species are, among bifidobacteria, the most capable representatives to metabolise host-derived glycans, such as mucin.[33] Other human gut microbiota members including Bacteroides spp, Ruminococcus spp, Clostridium spp and *Akkermansia muciniphila*, have been identified as a major bacterial players in mucin degradation,[34-37] although relatively little is known with respect to the genetic elements required for this property. Mucin is the principal component of mucus gel that covers the GIT epithelium and it represents the first barrier between host and intestinal bacteria, as well as host and nutrients present in the gut.

Recently, the genome sequence of *B. bifidum* PRL2010 was fully decoded,[30] revealing novel insights into the metabolic strategies followed by this strain to metabolize mucin-derived

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carbohydrates. These investigations suggested the existence of specific *B. bifidum* enzymatic pathways involved in the utilization of host-derived glycans, for example by the activity of enzymes that remove sialic acid and fucose moieties from galacto-N-biose (GNB) and its extended derivatives present in various mucin O-glycans.[38-41] In addition, the action of an endo-α-N-acetylgalactosaminidase is predicted to release such galacto-N-biose-containing glycans from the mucin glycoproteins and once released it may undergo further degradation by the extracellular β-galactosidase and β-N-acetylhexosaminidase, before GNB and other degradation products are translocated across the cell membrane to the cell cytoplasm where, depending on their chemical conformation, they are subjected to further hydrolysis, phosphorylation, epimerization, desulphation and/or deacetylation.

Another clear example of how analysis of genomic data underpins specific adaptations of bifidobacteria to the human GIT is represented by the genome sequencing of another key component of the infant gut microbiota, *Bifidobacterium breve* UCC2003.[31] Genome mining of this strain revealed information regarding its genetic adaptation to the colonization and persistence in the human gut through the production of fimbria-like structures belonging to the type IVb (or Tad) pili-family. Mutational analysis demonstrated that the UCC2003 *tad* gene cluster is crucial for efficient in vivo gut colonization in murine models, while immunogold transmission electron microscopy confirmed the presence of Tad pili at the poles of *B. breve* UCC2003 cells.[31] Notably, the Tad pilus-encoding locus was shown to be highly conserved among sequenced Bifidobacterium genomes, thus suggesting the notion of a ubiquitous pili-mediated host colonization and persistence mechanism for bifidobacteria.[31]

Probiogenomics of Lactobacilli

In silico analyses of the genomes between classical intestinal lactobacilli (e.g., *L. rhamnosus*) and plant or milk isolates (e.g., *L. bulgaricus* and *L. helveticus*) have demonstrated functional groups representing their niche adaptation. In this context, the typical milk-adapted *L. bulgaricus* and *L. helveticus* genomes[24,42] contain an arsenal of genes that encode enzymes dedicated to the metabolism of typical milk-derived sugars and other carbohydrates.[9] A clear sign of adaptation of the human GIT is represented by the enrichment of mucus-binding proteins and enzymes that are predicted to be involved in breakdown of complex carbohydrates.[43,44] In addition, specific adaptation to the human intestine is also evident from the existence of a bile salt hydrolase (BSH) encoded by all sequenced intestinal lactobacilli.[45] Gut-adaptation functions are not only encoded by chromosomal DNA but also by large extrachromosomal replicons such as megaplasmids. The first megaplasmid described in lactic acid bacteria was that of *L. salivarius* UCC118, representing almost 11% of the overall coding capacity of the *L. salivarius* genome.[46] This megaplasmid was shown to encode biologically important characteristics including a locus for bacteriocin production, a bile salt hydrolase-encoding gene, and two genes that complete the phosphoketolase pathway.[46]

Comparative genome analyses within the *L. plantarum* species revealed the existence of a DNA region, named life-style cassette, encompassing genes predicted to be involved in sugar metabolism (represented by PEP-PTS systems as well as glycosyl hydrolases).[47]

Interaction of Bifidobacteria and Lactobacilli with Their Host

So far, little is known about the genetic basis of interactions between probiotic bacteria and the intestinal host mucosa. Human gut commensals are known to synthesize cell envelope-associated structures, which are claimed to sustain an important role in determining microbe-host interactions (for a review see ref. 48). All sequenced genomes of bifidobacteria and lactobacilli are predicted to encode an extracellular polysaccharide (EPS) or capsular polysaccharide, and such an extracellular structure may be important in bacterial colonization or adherence to host cells, while it could also contribute to resistance to stomach acids and bile salts.[49,50] Moreover, other predicted cell surface-encoding proteins are the sortase-dependent fimbriae-like structures, which are encoded by the genome of enteric,[12] as well as oral bifidobacteria.[17,31,51] The precise role played by these structures in bifidobacteria has not yet been determined, with the exception of the Tad pili as discussed above. However, in other human GIT commensals, such as *L. rhamnosus* GG, the sortase-dependent pili have clearly been shown to mediate microbial adhesion to and colonization of the epithelial mucus layer.[52]

Other important mediators contributing to the host interaction in the GIT are represented by serpin-like protease inhibitors, which are encoded by *B. longum* subsp *longum* NCC2705 and *B. breve* UCC2003.[31,53] The serpin encoded by *B. longum* subsp *longum* NCC2705 is an efficient inhibitor of human neutrophil and pancreatic elastases, whose release by activated neutrophils at the sites of intestinal inflammation represents an interesting control mechanism of innate immunity.[53] A recent survey on the distribution of the serpin-encoding gene in bifidobacteria indicates the presence of this gene in seven different bifidobacterial species (*B. longum* subsp *longum*, *B. longum* subsp *infantis*, *B. longum* subsp *suis, B. breve*, *B. dentium*, *B. scardovii* and *B. cuniculi*), three of which, i.e., *B. longum* subsp *longum*, *B. longum* subsp *infantis* and *B. breve*, are commonly encountered within the human gut microbiota.[54] The presence of such a protease inhibitor may provide an ecological advantage to bifidobacteria since serpin activity may protect them against host proteases.[53] The observation of transcriptional activation of the serpin-encoding gene represents a molecular mechanism for immune-modulation, triggered by particular members of intestinal bifidobacteria.[54]

The diversity of cell envelope composition and extracellular structures provides species- and strain-specific features that are most likely driving microbe-host responses. For example, genome analysis of *L. plantarum* WCFS1 revealed several secreted proteins that are predicted to be involved in adherence to host components including mucins and collagen.[55] In a similar manner, genome analysis of *L. acidophilus* NCFM suggests the existence of adhesins that may be involved in binding to host glycans such as mucins.[56]

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Genome Evolution of Bifidobacteria and Lactobacilli

In silico analyses of currently available genome sequences of probiotic bacteria has revealed some generally conserved genetic traits (for reviews, see refs. 9 and 10) that may reflect adaptation of these bacteria to the human intestinal niche. Nevertheless, since probiotic bacteria such as bifidobacteria and lactobacilli represent diverse and taxonomically heterogeneous groups of microorganisms, the analysis of gene presence/absence patterns in a particular set of genomes, may be dramatically influenced by the evolutionary distance between these two distant taxa. However, common evolutionary pathways that have been followed by bifidobacterial and Lactobacillus genomes may be identified. These include the loss of genes encoding biosynthetic enzymes, gene duplication and horizontal gene transfer (HGT). From an evolutionary perspective, it must have been crucial for various bifidobacteria and lactobacilli, some of which being exploited as probiotics, to enlarge their genetic arsenal (gene duplication and HGT) in order to successfully colonize the human intestine and to compete with other members of the autochthonous microbiota. Many genes involved in sugar metabolism and transport appear to be duplicated or acquired early in the evolution of bifidobacteria and lactobacilli, including those encoding enolase, β-galactosidase and many other glycosyl hydrolases.[9] Furthermore, the increase of the number of genes encoding peptidases and amino acid transporters has occurred in several species of bifidobacteria and lactobacilli. Another protein family, frequently found in the genomes of lactobacilli, is presented by the gene products that sustain antibiotic resistance vs other bacteria, i.e., β-lactamases.[57]

With the availability of a growing number of whole genome sequences from bifidobacteria and lactobacilli that have probiotic properties, an important future challenge will be to identify the hypothetical core probiogenome, representing core genome functions of probiotic bacteria. Nevertheless, only seven genes present in the bifidobacteria, but absent in the genomes of other members of the Actinobacteria phylum, are shared with lactobacilli. Only one of these genes, which encodes a functionally uncharacterized membrane protein, is present in all the lactobacilli genomes so far sequenced.[21]

Bioengineering of Probiotic Bacteria and Probiogenomics

Recently, the field of probiotics has embraced the application of bioengineering, which aims to develop "designer probiotics," for instance by expressing receptor-mimic structures to circumvent pathogens by blocking crucial ligand-receptor interactions.[58] A good example of such a novel type of bioengineered probiotic is represented by an *Escherichia coli* strain producing a lipopolysaccharide coupled to a shiga toxin receptor that is able to bind and neutralize toxins in the lumen of the intestine, thus avoiding/limiting adhesion of pathogens to the gut mucosa.[59] Engineered probiotics include also genetically manipulated *Streptococcus gordonii* strains and *Lactobacillus jensenii* strains that have been applied to combat HIV.[60] Furthermore, engineered probiotics provide a useful way to solve technical problems such as low rate of cell survival of probiotics during the shelf life of the product due to environmental stresses such as cold stress or oxidative stress.[61]

However, despite a higher functionality of engineered probiotics as compared with their natural counterparts, the use of such recombinant bacteria in the food chain will meet with significant reluctance expressed by consumers, while they will also have to overcome regulatory requirements as imposed by governmental authorities. As outlined by Steidler et al.[62] more rigorous scientific studies are required, which should include a careful evaluation of the genetic contents of engineered bacteria and a thorough functional genomics examination. In this context, probiogenomics should represent a mandatory step in the procedure to achieve development and regulatory approval of novel engineered probiotic bacteria.

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Conclusions

Almost all probiotic lactobacilli and bifidobacteria that are currently on the market were originally selected based on technological stability, such as resistance and stability during food processing and storing, or on some easily measurable phenotype like the ability to tolerate bile salts or survive GIT passage, but not necessarily for their ability to impart health benefits on the human host. At this point in time, the regulatory requirements regarding probiotic products have shifted toward the need for understanding the precise molecular mechanisms by which probiotic bacteria beneficially influence human health. Characterization through so-called "omics" approaches involving genomics and functional analyses may be a route to satisfy such a regulatory requirement. Moreover, the in-depth knowledge on the composition and functionality of the human gut microbiota will provide molecular criteria that predict susceptibility of individual subjects to specific probiotic supplementation and may be utilized as an a priori criterion for successful probiotic therapy.

References

1. Kleerebezem M, Vaughan EE. Probiotic and gut lactobacilli and bifidobacteria: molecular approaches to study diversity and activity. Annu Rev Microbiol 2009; 63:269-90; PMID:19575569; http://dx.doi.org/10.1146/annurev.micro.091208.073341
2. Metchnikoff E. The prolongation of life. Optimistic studies. G.P. Putnam Sons 1908.
3. Salminen S, Nurmi J, Gueimonde M. The genomics of probiotic intestinal microorganisms. Genome Biol 2005; 6:225; PMID:15998456; http://dx.doi.org/10.1186/gb-2005-6-7-225
4. Dunne C, Murphy L, Flynn S, O'Mahony L, O'Halloran S, Feeney M, et al. Probiotics: from myth to reality. Demonstration of functionality in animal models of disease and in human clinical trials. Antonie van Leeuwenhoek 1999; 76:279-92; PMID:10532384; http://dx.doi.org/10.1023/A:1002065931997
5. Goldin BR. Health benefits of probiotics. Br J Nutr 1998; 80:S203-7; PMID:9924285
6. Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM. Probiotic and other functional microbes: from markets to mechanisms. Curr Opin Biotechnol 2005; 16:204-11; PMID:15831388; http://dx.doi.org/10.1016/j.copbio.2005.02.003

7. Ouwehand AC, Salminen S, Isolauri E. Probiotics: an overview of beneficial effects. Antonie van Leeuwenhoek 2002; 82:279-89; PMID:12369194; http://dx.doi.org/10.1023/A:1020620607611
8. Turroni F, Ribbera A, Foroni E, van Sinderen D, Ventura M. Human gut microbiota and bifidobacteria: from composition to functionality. Antonie van Leeuwenhoek 2008; 94:35-50; PMID:18338233; http://dx.doi.org/10.1007/s10482-008-9232-4
9. Ventura M, O'Flaherty S, Claesson MJ, Turroni F, Klaenhammer TR, van Sinderen D, et al. Genome-scale analyses of health-promoting bacteria: probiogenomics. Nat Rev Microbiol 2009; 7:61-71; PMID:19029955; http://dx.doi.org/10.1038/nrmicro2047
10. Turroni F, van Sinderen D, Ventura M. Genomics and ecological overview of the genus Bifidobacterium. Int J Food Microbiol 2011; 149:3744; PMID:21276626; http://dx.doi.org/10.1016/j.ijfoodmicro.2010.12.010
11. Pallen MJ, Wren BW. Bacterial pathogenomics. Nature 2007; 449:835-42; PMID:17943120; http://dx.doi.org/10.1038/nature06248
12. Schell MA, Karmirantzou M, Snel B, Vilanova D, Berger B, Pessi G, et al. The genome sequence of Bifidobacterium longum reflects its adaptation to the human gastrointestinal tract. Proc Natl Acad Sci USA 2002; 99:14422-7; PMID:12381787; http://dx.doi.org/10.1073/pnas.212527599
13. Lee JH, Karamychev VN, Kozyavkin SA, Mills D, Pavlov AR, Pavlova NV, et al. Comparative genomic analysis of the gut bacterium Bifidobacterium longum reveals loci susceptible to deletion during pure culture growth. BMC Genomics 2008; 9:247; PMID: 18505588; http://dx.doi.org/10.1186/1471-2164-9-247
14. Sela DA, Chapman J, Adeuya A, Kim JH, Chen F, Whitehead TR, et al. The genome sequence of Bifidobacterium longum subsp. infantis reveals adaptations for milk utilization within the infant microbiome. Proc Natl Acad Sci USA 2008; 105:18964-9; PMID: 19033196; http://dx.doi.org/10.1073/pnas.0809584105
15. Barrangou R, Briczinski EP, Traeger LL, Loquasto JR, Richards M, Horvath P, et al. Comparison of the complete genome sequences of Bifidobacterium animalis subsp. lactis DSM 10140 and Bl-04. J Bacteriol 2009; 191:4144-51; PMID:19376856; http://dx.doi.org/10.1128/JB.00155-09
16. Kim JF, Jeong H, Yu DS, Choi SH, Hur CG, Park MS, et al. Genome sequence of the probiotic bacterium Bifidobacterium animalis subsp. lactis AD011. J Bacteriol 2009; 191:678-9; PMID:19011029; http://dx.doi.org/10.1128/JB.01515-08
17. Ventura M, Turroni F, Zomer A, Foroni E, Giubellini V, Bottacini F, et al. The Bifidobacterium dentium Bd1 genome sequence reflects its genetic adaptation to the human oral cavity. PLoS Genet 2009; 5:e1000785; PMID:20041198; http://dx.doi.org/10.1371/journal.pgen.1000785
18. Siezen RJ, Tzeneva VA, Castioni A, Wels M, Phan HT, Rademaker JL, et al. Phenotypic and genomic diversity of Lactobacillus plantarum strains isolated from various environmental niches. Environ Microbiol 2010; 12:758-73; PMID:20002138; http://dx.doi.org/10.1111/j.1462-2920.2009.02119.x
19. Bottacini F, Medini D, Pavesi A, Turroni F, Foroni E, Riley D, et al. Comparative genomics of the genus Bifidobacterium. Microbiology 2010; 156:324354; PMID:20634238; http://dx.doi.org/10.1099/mic.0.039545-0
20. Claesson MJ, van Sinderen D, O'Toole PW. Lactobacillus phylogenomics–towards a reclassification of the genus. Int J Syst Evol Microbiol 2008; 58:2945-54; PMID:19060088; http://dx.doi.org/10.1099/ijs.0.65848-0
21. Makarova KS, Koonin EV. Evolutionary genomics of lactic acid bacteria. J Bacteriol 2007; 189:1199-208; PMID:17085562; http://dx.doi.org/10.1128/JB.01351-06
22. van de Guchte M, Penaud S, Grimaldi C, Barbe V, Bryson K, Nicolas P, et al. The complete genome sequence of Lactobacillus bulgaricus reveals extensive and ongoing reductive evolution. Proc Natl Acad Sci USA 2006; 103:9274-9; PMID:16754859; http://dx.doi.org/10.1073/pnas.0603024103
23. Altermann E, Russell WM, Azcarate-Peril MA, Barrangou R, Buck BL, McAuliffe O, et al. Complete genome sequence of the probiotic lactic acid bacterium Lactobacillus acidophilus NCFM. Proc Natl Acad Sci USA 2005; 102:3906-12; PMID:15671160; http://dx.doi.org/10.1073/pnas.0409188102
24. Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E, et al. Comparative genomics of the lactic acid bacteria. Proc Natl Acad Sci USA 2006; 103: 15611-6; PMID:17030793; http://dx.doi.org/10.1073/pnas.0607117103
25. Pridmore RD, Berger B, Desiere F, Vilanova D, Barretto C, Pittet AC, et al. The genome sequence of the probiotic intestinal bacterium Lactobacillus johnsonii NCC 533. Proc Natl Acad Sci USA 2004; 101:2512-7; PMID:14983040; http://dx.doi.org/10.1073/pnas.0307327101
26. Ventura M, Canchaya C, Tauch A, Chandra G, Fitzgerald GF, Chater KF, et al. Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. Microbiol Mol Biol Rev 2007; 71: 495-548; PMID:17804669; http://dx.doi.org/10.1128/MMBR.00005-07
27. Ryan SM, Fitzgerald GF, van Sinderen D. Screening for and identification of starch-, amylopectin-, and pullulan-degrading activities in bifidobacterial strains. Appl Environ Microbiol 2006; 72:5289-96; PMID: 16885278; http://dx.doi.org/10.1128/AEM.00257-06
28. O'Connell Motherway M, Fitzgerald GF, van Sinderen D. Metabolism of a plant derived galactose-containing polysaccharide by Bifidobacterium breve UCC2003. Microb Biotechnol 2011; 4:403-16; PMID:21375716; http://dx.doi.org/10.1111/j.1751-7915.2010.00218.x
29. Pokusaeva K, Fitzgerald GF, van Sinderen D. Carbohydrate metabolism in Bifidobacteria. Genes Nutr 2011; 6:285-306; PMID:21484167; http://dx.doi.org/10.1007/s12263-010-0206-6
30. Turroni F, Bottacini F, Foroni E, Mulder I, Kim JH, Zomer A, et al. Genome analysis of Bifidobacterium bifidum PRL2010 reveals metabolic pathways for host-derived glycan foraging. Proc Natl Acad Sci USA 2010; 107:19514-9; PMID:20974960; http://dx.doi.org/10.1073/pnas.1011100107
31. Motherway MO, Zomer A, Leahy SC, Reunanen J, Bottacini F, Claesson MJ, et al. Functional genome analysis of Bifidobacterium breve UCC2003 reveals type IVb tight adherence (Tad) pili as an essential and conserved host-colonization factor. Proc Natl Acad Sci USA 2011; 108:11217-22; PMID:21690406; http://dx.doi.org/10.1073/pnas.1105380108
32. Turroni F, Foroni E, Pizzetti P, Giubellini V, Ribbera A, Merusi P, et al. Exploring the diversity of the bifidobacterial population in the human intestinal tract. Appl Environ Microbiol 2009; 75:1534-45; PMID: 19168652; http://dx.doi.org/10.1128/AEM.02216-08
33. Ruas-Madiedo P, Gueimonde M, Fernandez-Garcia M, de los Reyes-Gavilan CG, Margolles A. Mucin degradation by Bifidobacterium strains isolated from the human intestinal microbiota. Appl Environ Microbiol 2008; 74:1936-40; PMID:18223105; http://dx.doi.org/10.1128/AEM.02509-07
34. Derrien M, Collado MC, Ben-Amor K, Salminen S, de Vos WM. The Mucin degrader Akkermansia muciniphila is an abundant resident of the human intestinal tract. Appl Environ Microbiol 2008; 74:1646-8; PMID:18083887; http://dx.doi.org/10.1128/AEM.01226-07
35. Collado MC, Derrien M, Isolauri E, de Vos WM, Salminen S. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. Appl Environ Microbiol 2007; 73:7767-70; PMID:17933936; http://dx.doi.org/10.1128/AEM.01477-07
36. Derrien M, Vaughan EE, Plugge CM, de Vos WM. Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. Int J Syst Evol Microbiol 2004; 54:1469-76; PMID:15388697; http://dx.doi.org/10.1099/ijs.0.02873-0
37. Martens EC, Chiang HC, Gordon JI. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. Cell Host Microbe 2008; 4:447-57; PMID:18996345; http://dx.doi.org/10.1016/j.chom.2008.09.007
38. Fujii T, Kitaoka HB, Luo ZP, Kura H, An KN. Analysis of ankle-hindfoot stability in multiple planes: an in vitro study. Foot Ankle Int 2005; 26:633-7; PMID:16115421
39. Wada J, Ando T, Kiyohara M, Ashida H, Kitaoka M, Yamaguchi M, et al. Bifidobacterium bifidum lacto-N-biosidase, a critical enzyme for the degradation of human milk oligosaccharides with a type 1 structure. Appl Environ Microbiol 2008; 74:3996-4004; PMID: 18469123; http://dx.doi.org/10.1128/AEM.00149-08
40. Lloyd KO, Burchell J, Kudryashov V, Yin BW, Taylor-Papadimitriou J. Comparison of O-linked carbohydrate chains in MUC-1 mucin from normal breast epithelial cell lines and breast carcinoma cell lines. Demonstration of simpler and fewer glycan chains in tumor cells. J Biol Chem 1996; 271:33325-34; PMID:8969192; http://dx.doi.org/10.1074/jbc.271.52.33325
41. Podolsky DK. Oligosaccharide structures of isolated human colonic mucin species. J Biol Chem 1985; 260:15510-5; PMID:4066681
42. Callanan M, Kaleta P, O'Callaghan J, O'Sullivan O, Jordan K, McAuliffe O, et al. Genome sequence of Lactobacillus helveticus, an organism distinguished by selective gene loss and insertion sequence element expansion. J Bacteriol 2008; 190:727-35; PMID: 17993529; http://dx.doi.org/10.1128/JB.01295-07
43. Boekhorst J, Helmer Q, Kleerebezem M, Siezen RJ. Comparative analysis of proteins with a mucus-binding domain found exclusively in lactic acid bacteria. Microbiology 2006; 152:273-80; PMID:16385136; http://dx.doi.org/10.1099/mic.0.28415-0
44. Siezen R, Boekhorst J, Muscariello L, Molenaar D, Renckens B, Kleerebezem M. Lactobacillus plantarum gene clusters encoding putative cell-surface protein complexes for carbohydrate utilization are conserved in specific gram-positive bacteria. BMC Genomics 2006; 7:126; PMID:16723015; http://dx.doi.org/10.1186/1471-2164-7-126
45. Lambert JM, Siezen RJ, de Vos WM, Kleerebezem M. Improved annotation of conjugated bile acid hydrolase superfamily members in Gram-positive bacteria. Microbiology 2008; 154:2492-500; PMID:18667582; http://dx.doi.org/10.1099/mic.0.2008/016808-0
46. Claesson MJ, Li Y, Leahy S, Canchaya C, van Pijkeren JP, Cerdeno-Tarraga AM, et al. Multireplicon genome architecture of Lactobacillus salivarius. Proc Natl Acad Sci USA 2006; 103:6718-23; PMID:16617113; http://dx.doi.org/10.1073/pnas.0511060103
47. Siezen RJ. Johan, ET van Hylckama Vlieg. Genomic diversity and versatility of *Lactobacillus plantarum*, a natural metabolic engineer. Microb Cell Fact 2011; 10 (Suppl 1):S3; PMID:21995294; http://dx.doi.org/10.1186/1475-2859-10-S1-S3

48. Sánchez B, Gonzalez-Tejedo C, Ruas-Madiedo P, Urdaci MC, Margolles A. Lactobacillus plantarum Extracellular Chitin-Binding Protein and Its Role in the Interaction between Chitin, Caco-2 Cells, and Mucin. Appl Environ Microbiol 2011; 77:1123-6; PMID:21131525; http://dx.doi.org/10.1128/AEM.02080-10
49. Pérez PF, Minnaard Y, Disalvo EA, De Antoni GL. Surface properties of bifidobacterial strains of human origin. Appl Environ Microbiol 1998; 64:21-6; PMID:9435057
50. Ventura M, Canchaya C, Fitzgerald GF, Gupta RS, van Sinderen D. Genomics as a means to understand bacterial phylogeny and ecological adaptation: the case of bifidobacteria. Antonie van Leeuwenhoek 2007; 91:351-72; PMID:17072531; http://dx.doi.org/10.1007/s10482-006-9122-6
51. Foroni E, Serafini F, Amidani D, Turroni F, He F, Bottacini F, et al. Genetic analysis and morphological identification of pilus-like structures in members of the genus *Bifidobacterium*.. Microb Cell Fact 2011; 10 (Suppl 1):S16; PMID:21995649; http://dx.doi.org/10.1186/1475-2859-10-S1-S16
52. Kankainen M, Paulin L, Tynkkynen S, von Ossowski I, Reunanen J, Partanen P, et al. Comparative genomic analysis of Lactobacillus rhamnosus GG reveals pili containing a human- mucus binding protein. Proc Natl Acad Sci USA 2009; 106:17193-8; PMID:19805152; http://dx.doi.org/10.1073/pnas.0908876106
53. Ivanov D, Emonet C, Foata F, Affolter M, Delley M, Fisseha M, et al. A serpin from the gut bacterium Bifidobacterium longum inhibits eukaryotic elastase-like serine proteases. J Biol Chem 2006; 281:17246-52; PMID:16627467; http://dx.doi.org/10.1074/jbc.M601678200
54. Turroni F, Foroni E, O'Connell Motherway M, Bottacini F, Giubellini V, Zomer A, et al. Characterization of the serpin-encoding gene of Bifidobacterium breve 210B. Appl Environ Microbiol 2010; 76:3206-19; PMID:20348296; http://dx.doi.org/10.1128/AEM.02938-09
55. Boekhorst J, Wels M, Kleerebezem M, Siezen RJ. The predicted secretome of Lactobacillus plantarum WCFS1 sheds light on interactions with its environment. Microbiology 2006; 152:3175-83; PMID:17074889; http://dx.doi.org/10.1099/mic.0.29217-0
56. Buck BL, Altermann E, Svingerud T, Klaenhammer TR. Functional analysis of putative adhesion factors in Lactobacillus acidophilus NCFM. Appl Environ Microbiol 2005; 71:8344-51; PMID:16332821; http://dx.doi.org/10.1128/AEM.71.12.8344-8351.2005
57. Teuber M, Meile L, Schwarz F. Acquired antibiotic resistance in lactic acid bacteria from food. Antonie van Leeuwenhoek 1999; 76:115-37; PMID:10532375; http://dx.doi.org/10.1023/A:1002035622988
58. Paton AW, Morona R, Paton JC. Designer probiotics for prevention of enteric infections. Nat Rev Microbiol 2006; 4:193-200; PMID:16462752; http://dx.doi.org/10.1038/nrmicro1349
59. Sleator RD. Probiotics–a viable therapeutic alternative for enteric infections especially in the developing world. Discov Med 2010; 10:119-24; PMID:20807472
60. Chang TL, Chang CH, Simpson DA, Xu Q, Martin PK, Lagenaur LA, et al. Inhibition of HIV infectivity by a natural human isolate of Lactobacillus jensenii engineered to express functional two-domain CD4. Proc Natl Acad Sci USA 2003; 100:11672-7; PMID:12972635; http://dx.doi.org/10.1073/pnas.1934747100
61. Sleator RD, Hill C. New frontiers in probiotic research. Lett Appl Microbiol 2008; 46:143-7; PMID:18028323; http://dx.doi.org/10.1111/j.1472-765X.2007.02293.x
62. Steidler L, Neirynck S, Huyghebaert N, Snoeck V, Vermeire A, Goddeeris B, et al. Biological containment of genetically modified Lactococcus lactis for intestinal delivery of human interleukin 10. Nat Biotechnol 2003; 21:785-9; PMID:12808464; http://dx.doi.org/10.1038/nbt840

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