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

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Keywords: genomics, lactobacilli, probiotic bacteria, gut microbiota, bifidobacteria

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.

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 administered 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 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.6,11
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. 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 corresponding novel discipline designated as probiogenomics (Table 1), with genome sequences of another 13 strains still unfinished (NCBI source). Notably, for a small number of cases such as B. infantis, B. longum subsp longum,15–17 B. animalis subsp lactis18,19 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 correspondingly novel discipline designated as probiogenomics (Table 1), with genome sequences of another 13 strains still unfinished (NCBI source). Notably, for a small number of cases such as B. infantis, B. longum subsp longum,15–17 B. animalis subsp lactis18,19 two or more genome sequences are publicly available.

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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 metabolic capabilities, genetics and phylogeny of these bacteria.

Table 1. General features of sequenced Bifidobacterium and Lactobacillus genomes

| Species Genome size | %GC Gene numbers |
|---------------------|------------------|
| Bifidobacterium strains | |
| B. longum subsp longum NCC515 2,256,640 60% 1798 |
| B. longum subsp longum DJ010A 2,375,792 59% 2061 |
| B. salivarius ATCC15703 2,089,645 59% 1701 |
| B. animalis subsp lactis AD011 1,933,695 60% 1603 |
| B. animalis subsp lactis BI-04 1,938,709 60% 1631 |
| B. animalis subsp lactis DSM 10140 1,938,463 60% 1629 |
| B. bifidum PR0210 2,214,656 62% 1791 |
| B. bifidum S17 2,186,862 62% 1845 |
| B. dentium Bd1 2,636,367 58% 2197 |
| B. longum subsp infantis 157F 2,400,312 60% 1602 |
| B. longum subsp infantis ATCC 15697 2,632,748 59% 2358 |
| B. longum subsp longum BBNM48 2,265,943 59% 1878 |
| B. longum subsp longum DSM 11317 2,385,164 60% 2009 |
| B. longum subsp longum DSM 118 2,477,838 59% 2035 |
| B. breve UCC2003 2,422,684 59% 1642 |

Lactobacillus strains

| Lactic acidophilus NCFM 1,993,560 34% 1938 |
| L. casei ATCC331 2,855,264 46% 2909 |
| L. gasseri ATCC 53533 1,894,360 35% 1898 |
| L. johnsonii NCC535 1,992,676 34% 1918 |
| L. plantarum WCFS1 3,208,274 44% 3135 |
| L. fermentum IFO 3956 2,098,665 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 3,070,196 46% 3090 |
| L. casei Zhang 1,861,846 46% 2906 |
| L. crispatus ST1 2,043,161 36% 2100 |
| Lactobacillus subsp bulgaricus ATCC11842 1,864,998 49% 2184 |

Lactobacillus subsp bulgaricus ATCC BAA-165 185,695,1 49% 2033 |
| Lactobacillus subsp bulgaricus NDQ2 2,125,753 49% 2177 |
| L. helveticus DPC 4571 2,080,931 37% 1838 |
| L. johnsonii F119785 1,755,993 34% 1780 |
| L. plantarum DSM1 3,197,359 44% 3029 |
| L. plantarum ST-48 3,254,376 44% 3137 |
| L. reuteri DSM 20016 1,999,518 38% 2027 |
| L. reuteri ATCC 1112 2,039,414 38% 1901 |
| L. rhamnosus GG 3,010,111 46% 2985 |
| L. rhamnosus Lc 705 2,968,598 46% 2954 |
| L. saker 23k 1,884,661 41% 1963 |
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-adapted metabolic activities. In this context, the bifidobacterial genome content highlights its relatively broad prototrophy with respect to amino acids, nucleotides and vitamins. In contrast, the genome content of lactobacilli reflects a high level of auxotrophy for such compounds.

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 carbohydrate transporters predicted to be required for the utilization of various plant-derived dietary fibers or complex sugars. Moreover, genetic and biochemical studies have been directed to analyze the capabilities of various bifidobacterial species to utilize dietary-related carbohydrates, such as amylopectin, galactan, starch and pullulan.

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* and the oral cavity (i.e., the case of *B. dentium* Bd17) or to a bifidobacterial strain that can utilize human mucin (i.e., the case of *B. infantis* PRL2010).

### Probiogenomics of Bifidobacteria

As mentioned above, various probiogenomic efforts have been undertaken in order to understand the genetic and metabolic characteristics of selected members of the genus *Bifidobacterium*.

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. This 43 Kb large gene cluster specifies a variety of catalytic enzymes such as fuco- and sialidases, β-hexosaminidase and β-galactosidase activities, as well as extracellular solute binding proteins and permeases predicted to be active on HMOs.

Furthermore, the genome of this microorganism contains additional genetic loci specifying fuco- 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.

Another important member of the bifidobacterial population frequently encountered in the infant gut microbiota is represented by the *B. bifidum* species. Members of this species are, among bifidobacteria, the most capable representatives to metabolize host-derived glycans, such as mucin. Other human gut microbiota members including *Bacteroides* spp, *Ruminococcus* spp, *Clostridium* spp and *Akkermansia muciniphila*, have been identified as major bacterial players in mucin degradation, 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 nutritive components in the gut.

Recently, the genome sequence of *B. infantis* PRL2010 was fully decoded, revealing novel insights into the metabolic strategies followed by this strain to metabolize mucin-derived carbohydrates.
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.31,32 In addition, the action of an endo-β-N-acetylglactosaminidase 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-acetylgalactosaminidase, 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, deacetylation 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 sfp 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. Notably, the Tad pili-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. reuteri) 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 contain an arsenal of genes that encode enzymes dedicated to the metabolism of typical milk-derived sugars and other carbohydrates. 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.33-35 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.36

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.37-39 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.39 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).40

Interaction of Bifidobacteria and Lactobacilli with Their Host

So far, little is known about the genetic basis of interactions between probiotic bacteria and 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.41-43 Moreover, other predicted cell surface-encoding proteins are the sortase-dependent fimbriae-like structures, which are encoded by the genome of enteric,44 as well as oral bifidobacteria.45-47 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.48

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 UCC2003.36,49 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.50 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 ruminis, B. breve, B. dentium, B. weisilii and B. suis), three of which, i.e., B. longum subsp longum, B. longum subsp infantis and B. breve, are commonly encountered within the human gut microbiota.51 The presence of such a protease inhibitor may provide an ecological advantage to bifidobacteria since serpin activity may protect them against host proteases.52 The observation of transcriptional activation of the serpin-encoding gene represents a molecular mechanism for immune-modulation, triggered by particular members of intestinal bifidobacteria.53

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.54 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.55
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 distantly 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 arsenals (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. 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.

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 and bifidobacteria. 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.

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 a priori criterion for successful probiotic therapy.

References

1. Khlebourov M, Vaughan EE. Probiotic and gut lactobacilla and bifidob który molecular approaches to study diversity and activity. Annals for Microbiology 2009; 59:209-98; PMID:19757586; http://dx.doi.org/10.1146/annurev.micro.091208.073341
2. Michels-Knoop F. The proportions of life. Opticism in studies. G.L. Pasteur 1958.
3. Salminen S, Nurmi J, Gueimonde M. The genomics of genomes so far sequenced.21
4. Dunne C, Murphy L, Flynn S, O’Halloran V, Kenny M, et al. Probiotics from myth to reality: Determination of functionality in animal models of disease and in humans. Current Microbiology 1999; 39:270-82; PMID:10532384; http://dx.doi.org/10.1007/s0028299003911970
5. Goldblatt BB. Health benefits of probiotics. Br J Nutr 1996; 86:265-7; PMID:8924995
6. Saxelin M, Tynkkynen S, Mattila-Sandholm T, De Vos WM. Probiotics and other functional microorganisms: markets to mechanisms. Curr Opin Biotechnol 2005; 16:284-11; PMID:15833380; http://dx.doi.org/10.1016/j.copbio.2005.02.010
7. Mahony L, Feeney M, et al. Probiotics: from myth to reality. Determination of functionality in animal models of disease and in humans. Current Microbiology 1999; 39:270-82; PMID:10532384; http://dx.doi.org/10.1007/s0028299003911970
8. 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.
19. Bottacini F, Medini D, Pavesi A, Turroni F, Foroni E, Moras D, Derrien M, Collado MC, Isolauri E, Barrangou R, Buck BL, McAuliffe O, et al. Comparative genomic analysis of the probiotic bacterium Bifidobacterium breve UCC2003. Proc Natl Acad Sci USA 2009; 106:15116-21; PMID:19612347; http://dx.doi.org/10.1073/pnas.0813772106.

20. Turroni F, van Sinderen D, Ventura M. Genomics and environmental niches. Environ Microbiol 2010; 12:284-92; PMID:20065353; http://dx.doi.org/10.1111/j.1462-2920.2009.02119.x.

21. Makarova KS, Koonin EV. Evolutionary genomics – bacillus phylogenomics. Microbiology 2010; 156:324354; PMID:21106887; http://dx.doi.org/10.1111/j.1365-2918.2010.01775.x.

22. Makarova KS, Redzic D, Koonin EV. Genomics and environmental niches. Environ Microbiol 2010; 12:284-92; PMID:20065353; http://dx.doi.org/10.1111/j.1462-2920.2009.02119.x.

23. Altermann E, Russel WM. Anaerococcus and related species. Environ Microbiol 2004; 6:285-306; PMID:15041138; http://dx.doi.org/10.1111/j.1748-7741.2004.00218.x.

24. Derrien M, Collado MC, Isolauri E, de Vos WM, Bryson K, Nicolas P, et al. The complete genome sequence of Lactobacillus bulgaricus reveals extensive horizontal gene transfer. Genomics 2009; 94:260-70; PMID:19518476; http://dx.doi.org/10.1016/j.ygeno.2009.09.007.

25. Pridmore RD, Berger B, Desiere F, Vilanova D, Gerardin E, Chavannac L, et al. Comparative genomics of the lactic acid bacteria. J Bacteriol 2007; 189:1199-54; PMID:17550700; dx.doi.org/10.1128/JB.01515-08.

26. Ventura M, Canchaya C, Tauch A, Chandra G, Boekhorst J, Boles M, et al. Comparative genomics of the lactobacillus reuteri superfamily. J Bacteriol 2007; 189:1199-54; PMID:17550700; dx.doi.org/10.1128/JB.01515-08.

27. Brody DK. Oligosaccharide structures of isolated human colonic mucin osan. J Biol Chem 1985; 260:15510-5; PMID:4066681.

28. Yamaguchi M, et al. Bifidobacterium bifidum lacto-N-bioside, a critical enzyme for the degradation of human milk oligosaccharides with a type-I endo-N-acetylgalactosaminidase. Appl Environ Microbiol 2001; 67:835-42; PMID:11289887; http://dx.doi.org/10.1128/AEM.67.3.835-842.2001.

29. Pokusaeva K, Fitzgerald GF, van Sinderen D. Carbohydrate metabolism and identification of starch-, amylopectin-, and pullulan-degrading activities in bifidobacterial strains. Food Microbiol 2011; 28:374-84; PMID:21129863; http://dx.doi.org/10.1016/j.fm.2010.09.007.

30. Fujii T, Kataoka T, Sato M, Yamasaki K. Activity of alpha-D-galactosidase produced by Enterococcus faecalis ATCC19434. Biosci Biotechnol Biochem 2008; 72:5289-96; PMID:18488729; http://dx.doi.org/10.1271/bbb.070299.

31. Altermann E, Russel WM, Anaerococcus and related species. Environ Microbiol 2004; 6:285-306; PMID:15041138; http://dx.doi.org/10.1111/j.1748-7741.2004.00218.x.

32. Calladine MC, Denier, M. Isolauri E, de Vos WM, Sorman V. Intestinal immunity and Akkermansia muciniphila, a moist-dwelling member of the abundant bacterial mucous associated microbiota, interacts with host immune cells in infants, adults, and the elderly. Appl Environ Microbiol 2007; 73:7767-79; PMID:17933596; http://dx.doi.org/10.1128/AEM.01778-06.

33. Bifidobacterium adolescentis, a natural metabolic engineer. Microb Cell Fact 2011; 10(1):53; PMID:21782257; http://dx.doi.org/10.1186/1475-2859-10-53.

34. Derrien M, Collado MC, Isolauri E, de Vos WM, Sorman V, Tannock GW. Intestinal immunity and Akkermansia muciniphila, a moist-dwelling member of the abundant bacterial mucous associated microbiota, interacts with host immune cells in infants, adults, and the elderly. Appl Environ Microbiol 2007; 73:7767-79; PMID:17933596; http://dx.doi.org/10.1128/AEM.01778-06.

35. Calladine MC, Denier, M. Isolauri E, de Vos WM, Sorman V. Intestinal immunity and Akkermansia muciniphila, a moist-dwelling member of the abundant bacterial mucous associated microbiota, interacts with host immune cells in infants, adults, and the elderly. Appl Environ Microbiol 2007; 73:7767-79; PMID:17933596; http://dx.doi.org/10.1128/AEM.01778-06.

36. Calladine MC, Denier, M. Isolauri E, de Vos WM, Sorman V. Intestinal immunity and Akkermansia muciniphila, a moist-dwelling member of the abundant bacterial mucous associated microbiota, interacts with host immune cells in infants, adults, and the elderly. Appl Environ Microbiol 2007; 73:7767-79; PMID:17933596; http://dx.doi.org/10.1128/AEM.01778-06.
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:21312452; http://dx.doi.org/10.1128/AEM.02480-10

49. Pepe PF, Masson Y, Dabadie EA, De Antoni G. Surface properties of bifidobacterial strains of human origin. Appl Environ Microbiol 1998; 64:21-6; PMID:9555127

50. Ventura M, Carcharu F, Fitzgerald GF, Gupta RS, van Sinderen D. Genomics as a means to understand bacterial phylogeny and ecological adaptations: the case of bifidobacteria. Antonie van Leeuwenhoek 2007; 91:351-72; PMID:17072533; 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: Microwave Cell Fact 2011; 18 (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, Simkova H, et al. A serpin from the gut bacterium Bifidobacterium longum inhibits eukaryotic elastase-like serine proteases. J Biol Chem 2006; 281:17246-52; PMID:1627567; http://dx.doi.org/10.1074/jbc.M601678200

54. Turroni F, Foroni E, O’Connell-Mitchell J, Bottacini F, Godoy-Vázquez V, Zanin A, et al. Characterization of the serpin-expressing strain 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; 75:135-57; PMID:10512375; http://dx.doi.org/10.1023/A:1002854104298

58. Parra AF, Novara R, Parra JC. Design of probiotics for prevention of enteric infections. Nat Rev Microbiol 2006; 4:193-200; PMID:16462752; http://dx.doi.org/10.1038/nrmicro1699

59. Skarzynski K. Peritoneal-sable therapeutic alternative for enteric infections especially in the developing world. Dis Mon 2010; 56:119-26; PMID:20887672

60. Chong TH, Chang CH, Simpson DA, Yu G, Martin PK. Lagunas EP, et al. Inhibition of HIV infectivity by a natural human isolate of Lactobacillus rhamnosus engineered to express functional transmembrane C2D4. Pro Natl Acad Sci USA 2005; 102:13072-7; PMID:16297307; http://dx.doi.org/10.1073/pnas.1508747100

61. Skarzynski K. New frontier in probiotic research. Lett Appl Microbiol 2008; 46:145-7; PMID:18628263; http://dx.doi.org/10.1111/j.1472-765X.2007.02293.x

62. Thrush L, Neijdant S, Maheshwar N, Snoek M, Vermeiren A, Goldin 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