[Frontiers in Bioscience 14, 4673-4684, January 1, 2009]

Bifidobacteria: from ecology to genomics

Francesca Turroni[1], Douwe van Sinderen[2], Marco Ventura[1]

[1] Department of Genetics, Biology of Microorganisms, Anthropology and Evolution University of Parma, Italy, [2] Alimentary Pharmabiotic Centre and Department of Microbiology, Bioscience Institute, National University of Ireland, Western Road, Cork, Ireland

TABLE OF CONTENTS

1. ABSTRACT

Bifidobacteria are high G+C Gram positive bacteria belonging to the phylum *Actinobacteria*. In recent years bifidobacteria have attracted a lot of attention because of their perceived positive contribution to the functionality of the human gastro intestinal tract. For this reason, scientific research on these bacteria has been rapidly expanding, in particular in areas such as genomics, molecular ecology and genetics. Ecological studies together with genome-based sequencing efforts have provided scientific evidence for the considerable contribution of bifidobacteria to the human gut microbiome. Furthermore, bifidobacterial genomics has revealed various genetic adaptations of these bacteria to the gastrointestinal niche.

2. INTRODUCTION

Bifidobacteria represent high G+C Gram positive microorganisms belonging to the *Actinobacteria* phylum. The *Bifidobacterium* genus forms a coherent phylogenetic unit within the *Actinobacteria* as illustrated by the 16S rDNA sequences of its members, which share over 93% similarity (1). Notably, recent evidence from several taxonomic attempts (e.g. phylogenetic trees from concatenated sequences as well as from protein indel analyses) has demonstrated that bifidobacteria are related to the *Micrococcineae* order (e.g., *Tropheryma*, *Leifsonia* and *Kinenococcus*) where they form a deep branching lineage within the *Actinobacteria* phylum (2; 3).

Bifidobacteria were first described by Tissier at the beginning of the last century, and their name is a reference to the unusual bifid morphology they may display (4). In fact, bifidobacteria are morphologically bifid, rod-shaped or multiple branching rods, depending on their environmental circumstances. The *Bifidobacterium* genus belongs to the *Bifidobacteriaceae* family which includes four other genera, i.e. *Scardovia*, *Parascardovia*, *Aeroscardovia* and *Gardnerella* (5). All the so far recognized 30 *Bifidobacterium* species have been arranged into six different phylogenetic clusters, i.e. *B. longum*, *B. adolescentis*, *B. pullorum*, *B. boum*, *B. pseudolongum* and *B. asteroides*, to the latter representing the most closely related relative of the ancestral progenitor of this genus (6).

3. BIFIDOBACTERIA AND ECOLOGY

The ecological distribution of bifidobacteria is mainly restricted to the gastro-intestinal tract (GIT) of mammals and birds where they colonized the distal tract (e.g., large intestine) or the oral cavity (for review see 3). A few exceptions to this exist and are represented by *Bifidobacterium scardovii*, which was found in human blood, and three other bifidobacterial species, *Bifidobacterium animalis* subsp. *lactis*, *Bifidobacterium subtile* and *Bifidobacterium minimum*, which were isolated from food or sewage. However, the ecological origin of these latter bifidobacteria raises the question as to whether this was its original environment or rather the logical result of a transfer from the original source. Recently, ecological studies involving faecal samples from different animals (e.g., birds, ungulates, lagomorphs and rodents) as well as humans revealed that some bifidobacterial species (e.g. *B. animalis*, *Bifidobacterium adolescentis*, *Bifidobacterium dentium* and *Bifidobacterium catenulatum*) enjoy a cosmopolitan lifestyle, whereas other bifidobacterial species showed a highly specialized ecological adaptation to life in the GIT of a particular animal (e.g., *Bifidobacterium cuniculi* for rabbits, *Bifidobacterium angulatum* for cows and *Bifidobacterium gallinarum* for chickens) or in the human GIT (e.g. *Bifidobacterium breve* and *Bifidobacterium longum*) (7). Bifidobacteria living in the human intestine are subject to growing interest due to their probiotic and prebiotic features. In fact, bifidobacteria as well as most of the enteric bacterial microflora are capable of hydrolyzing a large variety of oligosaccharides in the GIT, some of which are not digested by their human host and which are commercially used to improve bifidobacterial numbers and/or activity in the GIT, a practice that is referred to as the prebiotic concept (8).

The intestinal microflora is an extremely complex ecosystem whose composition is still far from fully understood and explored. It has been stated that the complete intestinal bacterial community, i.e., intestinal microbiota, constitutes another organ within the human body (9) that provides various metabolic and physiological functions for the host such as nutrient processing, immune modulation and anti-infection (10). Ecological investigations based on both cultivation methods as well as culture-independent assays have been performed in order to investigate the microbial diversity encountered in the human GIT (11; 12). All the above mentioned reports highlighted the huge complexity of the gut-associated microbiota, with a high inter-individuality variability, but with a considerable stability of this microbial community over time in a given single individual (13). In fact, host development age, health condition, diet (cultural influences of e.g. a more protein- or carbohydrate based diet), and the adaptability of each bacterial species influences the overall composition of the microflora in the intestine. It has been shown that bifidobacteria become the prominent bacteria in the faeces of breast-fed infants within the first week after birth, then drop following the weaning period but are stable during adolescence, followed by a reduction within the elderly population (14). In breast-fed infants bifidobacteria predominate whereas in formula-fed infants a more diverse microbiota develops. The most frequently detected species in faeces of breast-fed infants are *B. breve*, followed by *B. infantis*, *B. longum* and *B. bifidum*, whereas in the faeces of adults the most detected species are *B. adolescentis* and *B. catenulatum (*15). It has been demonstrated that the human faecal flora differs quantitatively and qualitatively from the cecal flora (84). Bifidobacteria and *Bacteroides* were found to be consistently lower in cecal samples compared to those of the faeces (84).

The gut regions are active sites of microbial breakdown of dietary plant polysaccharides. In particular bacteria that colonize the large intestine such as bifidobacteria have only access to dietary residues that escape digestion and uptake by host enzymes in the upper gut. The amount and extent of such non-digestible sugars in the diet is likely to determine the variety in bacterial populations and associated metabolic activities within the gut microbiota (16). Specific carbohydrates are used as prebiotic dietary food components to manipulate gut metabolism and bacterial populations in a direction that is deemed to be beneficial to the host. The success of prebiotic applications relies on exploiting differences in substrate preferences and competitive abilities between the various members of the gut microbiota. The variety of poly-, oligo- and mono-saccharide substrates that arrive in the distal regions of the GIT from the diet is enormous but generally includes a significant amount of insoluble plant-derived polysaccharides together with oligosaccharides and complex storage sugars such as inulin and a fraction of starch, known as resistant starch, which has escaped hydrolysis in the upper gut. These sugars present themselves in the colon in an insoluble particulate form, protected by cell wall polymers, or in colloidal solution, e.g. xylans and pectins. This extraordinary substrate diversity in turn creates a vast number of different ecological niches that can be exploited by GIT bacteria. In this context, it is not surprising that some species possess the capacity to switch readily between different substrates, e.g., between those of dietary and host origin (17), other species, such as those associated with insoluble substrates, appear to be far more specialized (17).

Knowledge on the identity, organization and regulation of the genes encoding these sugar-degrading enzymes as well as the genes for their corresponding transport systems in a particular bacterial species will allow

Table 1: Genes coding for glysoside hydrolase in the genomes of bifidobacteria according to CAZy database

Glycoside hydrolase family	B. longum subsp. longum NCC2705	B. adolescentis ATCC15703
1	2	-
2	3	2
3	6	3
5	2	3
13	13	8
20	-	1
25	1	-
26	1	-
27	1	1
30	1	-
31	1	-
32	2	1
35	1	-
36	2	1
38	1	3
42	4	2
43	7	9
51	2	5
53	-	1
Total	50	40

Source: ref 19

us to investigate its capacity to compete for a given substrate and thus its ecological niche. The availability of genome data from symbiotic and commensal bacteria colonizing the mammalian intestine might provide considerable insights into such characteristics and the roles of different groups within the GIT community.

4. BIFIDOBACTERIA AND THEIR ROLE IN THE HUMAN GUT

Bifidobacteria are considered to play a key role in carbohydrate breakdown in the human colon. In fact, oligo- and polysaccharides are hydrolized to monosaccharides and these are then channeled into the so-called fructose-6-phosphate or bifid shunt to be fermented to short chain fatty acids, mainly lactic acid and acetate (18). Bifidobacteria may first hydrolize polysaccharides to low molecular weight oligosaccharides that are then degraded to monosaccharides. This step-wise breakdown is achieved by a wide spectrum of depolymerising enzymes called glycosidases or glycosyl hydrolases (GHs), which are either located intracellularly or secreted, in which case they may be attached to the cell envelope.

The number of GHs found in the genome of a given bifidobacterial strain is variable (Table 1). GH classification is based on amino acid sequence similarities, where homologous GHs are assigned to the same GH family. Glycosidases belonging to a single GH family may exhibit different substrate specificities but also different modes of action (e.g., exo or endo). In the genome of *B. longum* subsp. *longum* NCC2705 the percentage of annotated genes that code for predicted carbohydrate-modifying enzymes such as GHs and glycoside esterases is around 4.4%, which is very similar (4.8%) to that of *B. adolescentis* ATCC 15703 (NCBI source NC_008618). This percentage is higher (7.8%) only in the genome of *Bacteroides thetaiotaomicron*, also a gut commensal. In the case of bifidobacteria a number of differences between the predicted carbohydrate-degrading abilities of these sequenced genomes could be noticed. For example, the genome of *B. longum* subsp. *longum* NCC2705 possesses a higher number of arabinofuranosidases, whereas *B. adolescentis* ATCC15703 genome is richer in GHs predicted to be involved in the degradation of α-glucosidic bonds, thus suggesting different preferences for carbon sources between bifidobacteria. Furthermore, with respect to the localization of GH the genome sequences show that only few GHs possess a signal peptide thus suggesting an intracellular localization. The utilization of diet-derived oligosaccharides requires sugar transport systems that transfer such oligosaccharides inside the cell.

Bacteria typically use a number of different enzymes to metabolize a given carbohydrate, which may involve an extracellular enzyme to degrade a high molecular weight polysaccharide into oligosaccharides and/or monosaccharides, which are then imported into the cell using one or more sugar-specific transporters. The internalized sugars may then undergo further processing (phosphorylation, hydrolysis or conversion) before they are ready to enter the cell's major energy-generating metabolic pathway, which is the bifid shunt in the case of bifidobacteria (see above). So far only fragmentary information exists regarding sugar import systems in bifidobacteria, being limited to glucose and arabinose in *B. breve* (20), lactose and glucose in *B. longum* (21; 22), and sucrose and galacto-oligosaccharides in *B. animalis* subsp. *lactis* (23). With respect to the carbohydrate fermentation capabilities of *B. longum* subsp. *longum* NCC2705, genome analysis has indicated that this strain possesses all the gene homologs to encode enzymes needed to feed fructose, galactose, N-Ac-glucosamine, N-Ac-galactosamine, arabinose, xylose, ribose, sucrose, lactose, cellobiose, melibiose into the fructose-6-phosphate shunt (24). The genome sequences of *B. longum* subsp. *longum* NCC2705 contains eight high-affinity MalEFG-type oligosaccharide transporters and one phosphoenolpyruvate-phosphotransferase system (PEP-PTS) (24). This latter system acts through the simultaneously internalization and phosphorylation of sugars. The transfer of phosphate from PEP to the incoming carbohydrate is mediated via a phosphorylation chain that includes two enzymes, i.e., EI and EII, and a histidine-containing protein (HPr). Recently, it has been shown that bifidobacterial genomes vary with respect to the number of EII-encoding homologs present on a given chromosome (25), suggesting differential sugar-harvesting capabilities.

Genome analysis of *B. longum* subsp. *longum* NCC2705 revealed that this organism is equipped with a number of modular glycanases, an example of this is constituted by a putative endo-xylanase that contains a signal peptide, indicating that this enzyme is secreted, and a C-terminal transmembrane domain suggesting that the enzyme is anchored in the cell membrane. Moreover, the genome *B. longum* subsp. *longum* NCC2705 contains an endogalactanase capable of liberating galacto-trisaccharides from type I galactan in an exo-fashion (e.g., by means of a processive mechanism where following an initial endo cleavage the enzyme stays attached to the galactan and releases galacto-trisaccharides) (26).

5. BIFIDOBACTERIA AND HEALTH-PROMOTING EFFECTS

Gut colonization of bifidoacteria has been associated with many positive effects on the host's health, such as modulation of intestinal microflora, immune-modulation, reduction of allergic disease symptoms, alleviation of acute gastro-enteritis, reduction of lactose intolerance, attenuation of inflammatory bowel disease symptons, and alleviation of constipation.

5.1. Modulation of harmful intestinal microflora

The presence of microorganisms, such as bifidobacteria and Lactic Acid Bacteria (LAB) in the GIT, may lead to the reduction of certain harmful bacteria, e.g. coliforms and clostridia. Such a positive effect exerted by probiotic bifidobacteria may be due to competition for adhesion sites or receptors on epithelial cells, and/or for nutrients. In addition, bifidobacteria may produce antimicrobial agents, like various acid or bacteriocins, which can counteract the colonisation of pathogens (27). Moreover, these microorganisms have been shown to prevent and repair mucosal damage thus reducing the translocation of harmful bacteria (28; 29; 30). However, so far very little is known about the molecular basis underlying this protective effect.

5.2. Immune modulation

The microbiota of the GIT is in close contact with the intestinal epithelial cells from birth and this may directly or indirectly influence the host's immune system. Intestinal microorganisms are believed to bind to certain receptors located on the surface of epithelial cells and consequently stimulate defence mechanisms, such as the production of anti-inflammatory cytokines.

During inflammatory conditions the natural microflora stability appears to be disturbed in a way that causes an increased stimulation of the immune system (31). A probiotic therapy based on the intake of bifidobacteria is proposed to alleviate inflammatory diseases with the use of specific strains (32). Notably, it has been noticed that the consumption by elderly people of fermented drinks containing probiotic improved specific immune functions, such as natural killer cell activity and seem to reduce the incidence in tumor necrosis factor-α by macrophages (33). Moreover, some *Bifidobacterium* strains have recently been studied for their capability to exert specific immunomodulating effects, e.g. *B. bifidum*, *B. longum* subsp. *infantis* (34). In the gut, antigen-presenting cells (APC), in particular dendritic cells (DC), play a crucial role in innate as well as adaptive immune response against microbial antigens, and they are the main stimulators of naive T cells (35). It was also demonstrated that *B. bifidum* promoted a consistent effect in the modulation of the immune responses of neonatal cells in contrast to other probiotic bacteria (e.g., *Lactobacillus salivarius* and *B. infantis*) (35).

5.3. Allergic disease

The improved hygiene in Western societies, which in particular leads the reduced exposure to microbes at an early age, has been suggested to be the cause of the increase in allergic diseases (36). Probiotics, with their production of anti-inflammatory cytokines, alleviate the atopic diseases in allergic individuals (37). Probiotics supplemented to children at high risk of atopic diseases were shown to lead to a reduced prevalence of atopic eczema (27). So far very little information is available on the role of probiotic bifidobacteria in lowering allergic diseases (27).

5.4. Acute gastro-enteritis and diarrhoea caused by viral and bacterial infections

Acute gastro-enteritis may be caused by bacteria or viruses, in particular rotaviruses. Several studies have shown that certain probiotic strains belonging to bifidobacteria and LAB can alleviate and shorten the duration and reduce the severity of diarrhoea (38; 39). Diarrhoea may also be caused by antibiotic treatment, which disturbs the equilibrium of the normal GIT microflora. Thus, an intake of bifidobacterial cells during and following antibiotic treatment may lead to a quick restoration of the normal intestinal microflora and thus alleviate or prevent diarrhoea. The presence of *Clostridium difficile* which is responsible of specific cases of severe and chronic diarrhoea may thus be counteracted by the daily supply of probiotic bifidobacteria such as *B. bifidum* (40).

5.5. Lactose intolerance

Under normal conditions adult mammals possess β-galactosidase activity, which allows lactose digestion. However, in the few decades a remarkable increase in the number of people has been observed that suffer from lactose intolerance caused by the reduction or lack of this activity. Populations that suffer from this deficiency experience an increase in osmotic load after the consumption of lactose in the small intestine resulting in loose stool, abdominal unease and general abdominal pain. It is hypothesized that the colonic microbiota may influence the effects of lactose intolerance due to the breakdown of this disaccharide by microbial β-galactosidases (41; 42; 43; 44).

5.6. Inflammatory bowel disease

The clinical manifestations of inflammatory bowel disease (IBD) include the Crohn's disease (CD), ulcerative colitis (UC) and pouchitis. All these diseases affect the colon and the distal part of the small intestine, and involve an inflammation of the mucosa and submucosa, although the ethiology of these diseases is still not known (45). It has been proposed that IBD is caused by an abnormal communication between gut microflora and the mucosal immune system, with additional contributory elements that relate to the environment, genetic factors and immunoregulatory features (46). During the development of this pathology the composition and the natural activity of the normal microflora of the GIT is greatly affected. A metagenomic study revealed a striking reduction in *Firmicutes* members (e.g., the *Clostridium* group) in patients suffering of Crohn's disease as compared to healthy controls (46). Therefore a selection of probiotics and especially prebiotics (such as inulin and FOS), which favour growth of certain components of the indigenous microflora, might reduce the disease and alleviate symptoms (46).

Table 2. Genome sequencing projects of bifidobacterial strain

Microorganism	Genome size (bp)	ORF number	G+C%	rRNA operons	Reference
B. longum subsp. *longum* NCC2705[1]	2,266,000	1730	60	4	(24)
B. longum subsp. *longum* DJO10A[1]	2,375,800	1811	59	4	(87)
B. longum BORI	NA	NA	NA	NA	Korea Research Institute of Bioscience and Biotechnology
B. longum subsp. *infantis* ATTC 15697	NA	NA	NA	NA	DOE Joint Genome Institute, USA
B. adolescentis ATCC 15703[1]	2,084,445	1564	59	5	Gifu University, Japan
B. adolescentis L2-32	NA	NA	NA	NA	Washington University, USA
B. breve UCC2003[1]	2,422,668	1868	59	NA	National University of Ireland, Cork
B. dentium Bd1	~2,600,000	~2270	59.2	NA	National University of Ireland, Cork; University of Parma, Italy
B. dentium ATCC 27678	NA	NA	NA	NA	Washington University, USA
B. animalis subsp. *lactis* AD011	NA	NA	NA	NA	Korea Research Institute of Bioscience and Biotechnology
B. animalis subsp. l*actis* HN019	~1,915,892	NA	60	NA	Fonterra Institute, New Zeland

[1] Genome completed, NA= not available

5.7. Constipation

Constipated bowel movement is a noteworthy problem for many people, and is particularly prevalent among the elderly. Functional constipation is a common and debilitating phenomenon, which is frequently observed in children.

Probiotics have been suggested to relieve constipation (47) and regarding this, some *Bifidobacterium* strains have been widely used in this context. In fact, the production of lactic, acetic and other organic acids by bifidobacteria promotes a reduction of pH in the colon, enhances peristalsis of the colon and consequently decreases colonic transit time, which is beneficial in the treatment of constipation (48).

6. GENOMICS AND BIFIDOBACTERIA

For many years health-promoting bacteria, i.e., probiotic bacteria, have been widely added as live components to many food preparations (e.g., in so-called functional foods). However, the precise impact of the use of probiotic bacteria on the functioning of the human GIT is not fully understood. The elucidation of the precise mechanisms by which probiotics influence human health as well as a guaranteed biosafety, are essential for the development of novel and effective probiotic products. This will have to be considered in current and future research endeavours when discovering and developing the next generation of probiotic bacteria. In this context, genomics has been shown to be a powerful means to fulfil these new requirements of bacteria in order to be considered as probiotic. The development of a branch of genomics science, i.e., probiogenomics, may be considered as a clear indication to mark this evolution in the probiotic research field. In fact, genome sequencing may be considered the gold standard for analysing the full genetic complement of an organism, thereby providing predictive clues regarding the genetic determinants that specify adaptive functions to the environment in which the organism lives (e.g., ecological adaptation to a specific niche).

Of the so far recognized bifidobacterial species only four strains that represent the *B. longum* and *B. adolescentis* phylogenetic groups have been sequenced to completion (Table 2).

Recently, an additional ten bifidobacterial genome sequencing projects have been started or completed including both intestinal commensal strains (Table 2) and putative oral pathogens (*B. dentium* Bd1 and *B. dentium* ATCC27678). All these bifidobacterial genomes display genomic features typically found in prokaryotes (Table 2). With the exception of *B. dentium* Bd1 which possesses a genome of around 2.7 Mb, the remaining genomes displays an average size of 2.2 Mb.

7. PLASMIDS IN BIFIDOBACTERIA

These extrachromosomal DNA elements are not widely distributed in bifidobacteria (for review see 3). Large part of bifidobacteria plasmids so far described display typical genetic features for plasmid replication through a rolling-circle replication system. i.e., *repB*, *traA* and *mob* genes. Exceptionally, in pDOJH10S from *B. longum* subsp. *longum* DJO10A and pBC1 from *B. catenulatum* posses theta-type replicating plasmids (82; 83). Notably, all bifidobacterial plasmids do not encode for any obvious phenotypic trait except for the plasmid identified from *B. bifidum* NCFB 1454 which encodes the bacteriocin named bifidocin B (84).

8. BIFIDOBACTERIA AND GENETIC ADAPTATION TO THE HUMAN GUT

The current genetic content of a bacterial genome is the consequence of an adaptive evolution of such a microorganism to its ecological niche (3). In this context, various genetic events, such as gene duplication, horizontal gene transfer, gene decay and chromosomal rearrangements, are shaping bacterial genomes and thus leading to the genetic complement which is indispensable for a microorganism to efficiently cope with its ecological niche. In the case of bifidobacteria, it was noted that the gene composition of the *B. longum* subsp. *longum* NCC2705 genome reflects an intrinsic adaptation to the intestinal niche (3, 24). Notably, in this microorganism many of the genes (e.g., α-mannosidases and endo-β-NAc-glucosaminidase encoding genes) involved in carbohydrate utilization are clustered in a specific "life style adaptation" region in its chromosome that is likely to contribute to the

ecological fitness of the NCC2705 strain in the GIT by increasing the metabolic flexibility of this strain with regards to complex carbohydrates (49).

9. BIFIDOBACTERIA AND PREBIOTICS

A wide range of prebiotic compounds involving sugars such as fructooligosaccharides (FOS), β-galacto-oligosaccharides, α-galacto-oligosaccharides, α-gluco-oligosaccharides are naturally present in many vegetable-based foods or they have been applied as ingredients to various foods (e.g., cereal and formula milk).

9.1. Fructo-oligosaccharides (FOS)

In nature, FOS are generally found in plants or can be synthesised from sucrose by the action of transfructosylation enzymes named β-fructofuranosidase, which in bifidobacteria are located intracellularly (50; 51). When FOS are present in a mixture of chains with different chain lengths, bifidobacteria preferentially hydrolize those that have a shorter degree of polymerization (52). Moreover, β-fructofuranosidase can be differentiated on the basis of the type of sugar on which they act. For example, Ryan *et al.*, (50), cloned and expressed the gene encoding the β-fructofuranosidase of *B. breve* UCC2003, an enzyme which catalyzes the hydrolysis of the β— (2-1) glycosidic bond between glucose and its neighboring fructose moiety in sucrose, whereas no detectable activity was noticed towards the β— (2-1) glycosidic bonds between fructose moieties.

9.2. β-galactosidases

β-galactosidases are crucial enzymes for bifidobacteria in order to be able to grow on milk or milk-based substrates, such as lactose and lactose-derived (trans)galacto-oligosaccharides (TOS/GOS), which all contain β-galactosidic-linkages. The β-galactosidase activity has been studied in several bifidobacterial strains, including *B. longum* (53), *B. longum* subsp. *infantis* (54; 55), *B. bifidum (*56) and *B. adolescentis* (57). Originally these studies focused on the hydrolytic degradation of lactose and later on their transferase activity towards lactose for the synthesis of TOS.

Comparative analysis of the different β-galactosidase-encoding genes present in bifidobacterial genomes revealed a clear separation of these in two groups that represent β-galactosidases belonging to GH family 2 or family 42. Notably, the evolutionary diversification of these β-galactosidase groups corresponds to different functional properties of these enzymes: the members of the GH family 2 display greater lactase and transferase activities as compared to those belonging to GH family 42 (54; 55).

Many bifidobacterial β-galactosidases show transferase activity (54; 57). In *Bifidobacterium bifidum* DSM20215 the efficiency of transferase activity of a particular β-galactosidase enzyme can be increased by removal of the C-terminal end of the protein that includes a galactose-binding domain (56). It is suspected that the molecular mechanism behind this is that the truncated β-galactosidase possesses a more open structure to facilitate transglycosylation.

9.3. Arabinoxylan and arabinogalactan

Plant cell wall-containing arabinofuranosyl oligosaccharides such as arabinan, arabinogalactan are fermented by bifidobacteria (57). In such oligosaccharides arabinose is present as single unit side chain. Arabinoxylan-degrading enzymes were found in *B. adolescentis* DSM20082 (57), *B. longum* subsp. *longum* NCC2705 (24), *B. longum* subsp. *longum* B667 (58), and *B. breve* K110 (59). Arabinoxylan has been postulated to represent a valuable carbon source for bifidobacteria. However, these enzymes do not appear to be generally distributed among bifidobacteria. In fact preliminary analysis of the genome of *B. dentium* Bd1 (Ventura *et al.*, unpublished data) highlighted the absence of arabinoxylan degrading enzymes.

9.4. α-Galacto-oligosaccharides and galactomannan

α-Galacto-oligosaccharides from soymilk (e.g., raffinose and stachyose) represent a validated pabulum for growth of bifidobacteria (60). The α-galactosidase responsible for hydrolysis of these sugars has been investigated in *B. breve* (61), *B. longum* subsp. *longum* (62) and *B. adolescenti*s (63; 64). Besides raffinose and stachyose, other sugars such as melibiose and verbascose can be degraded by the α-galactosidase of bifidobacteria (63; 64; 65).

9.5. Starch

Bifidobacteria are known to utilize starch (66), however, only the purification of one amylase from *B. adolescentis* Int-57 has been described so far (67; 68). Therefore, very little is known about the molecular basis allowing starch degradation in bifidobacteria. Van den Broek demonstrated the existence of two different α-glucosidases in *B. adolescentis* DSM20083 which are highly active against isomaltose but not towards starch. Furthermore, two cell-wall associated α-glucosidases were identified and purified from *B. pseudolongum* but none of them were able to hydrolize starch (85).

In a recent study, it was shown that starch, amylopectin and pullulan were utilized by 11 out of 42 different strains representing different species of bifidobacteria (69). Interestingly, all *B. breve* strains analyzed possessed amylopullulanase activity, which may suggest that the combined extracellular α- (1-4) and α- (1-6) glucosidase activities may be characteristic for this *B. breve* strain and may have some biological relevance for this organism in the gut (69). The biological significance of this finding is unknown but tone may speculate that, since *B. breve* constitutes one of the dominant bifidobacteria in the infant microbiota (18), this enzyme is important during weaning when non-milk foods are supplemented to the diet and infants are, for the first time, exposed to complex carbohydrates different from those present in mother's milk.

9.6. Mucin

Enteric bifidobacteria have been demonstrated to utilize complex carbohydrates such as hog gastric mucin

(70; 86), pectin (71) and other complex plant oligosaccharides (64). Thus, such bifidobacteria have acquired adaptations to access a rich repertoire of otherwise indigestible components of the human or animal diet. With respect to host-produced sugar-containing substances, bifidobacteria show the capacity to hydrolize a variety of host-derived glycoconjugates including mucin oligosaccharides and glycosphingolipids through the action of sialidases (70). In this way the host itself may provide substrates to bifidobacteria, thereby representing a remarkable synergistic relationship.

Besides sugars other prebiotic substances eliciting bifidogenic effects are represented by small peptides, which are derived from the digestion of milk protein with the gastric protease pepsin. These compounds contain a pair of cysteine residues forming a disulfide bond and two small hydrophobic domains located C-terminally to the two cysteines (72**)**. The presence of such peptides was shown to be more successful on a molar basis in stimulating bifidobacterial growth as compared to certain prebiotic-sugars, and it has been suggested that the bifidogenic activity of breast milk through the presence of specific peptides far exceeds that of milk oligosaccharides (e.g. GOS) (72).

10. BIFIDOBACTERIAL GENOMICS: BIOSYNTHETIC AND METABOLIC CAPABILITIES

In order to better understand the biology of bifidobacteria the so far available bifidobacterial genomes provide valuable data, which will help us to investigate not only the metabolic capabilities of these bacteria, but also the molecular determinants of the probiotic action exploited by bifidobacteria.

Genomic information clearly pointed out that bifidobacteria are prototrophic for amino acid, and for some vitamins (73). In fact, genome analysis of *B. longum* subsp. *longum* NCC2705 revealed the presence of genes coding for biosynthetic precursors like phosphoenolpyruvate, oxaloacetate, oxoglutarate and fumarate and for the synthesis of at least 19 amino acids from ammonia (24). Moreover, this strain possesses all of the enzymes required for the biosynthesis of purine and pyrimidine nucleotides from glutamine as well as those needed for the synthesis of folic acid, nicotine and thiamine. In contrast, the metabolic pathways responsible for the biosynthesis of riboflavin, cobalamin, biotin, lipoate, pyroxidoxine and pantothenate are incomplete or absent from the genome of *B. longum* subsp. *longum* NCC2705 (24).

In addition, it was noted that bifidobacteria need a reduced sulphur source in order to grow on synthetic media, as the entire repertoire of genes for the sulphate/sulphite pathway appears to be absent from the genome of *B. longum* subsp. *longum* NCC2705 (24). So, it is supposed that *in vivo,* bifidobacteria utilize reduced sulphur compounds produced by other enteric bacteria with which it has established a strict symbiontic relationship.

11. BIFIDOBACTERIA GENOMICS AND HOST INTERACTIONS

Genomic data has provided very useful insights into the genetic adaptation of bifidobacteria to the GIT, while also giving clues as to the molecular interactions between bifidobacteria and its host, as well as other enteric bacteria (e.g., *Bacteroidetes*).

As discuseed above, the most obvious sign of genetic adaptation of bifidobacteria to the GIT is reflected by their extensive capacity to ferment complex carbohydrates.

In order to elucidate possible mechanisms of molecular interaction between bifidobacteria and their host cells, it is crucial to expand our knowledge on proteins that are exported by this group of bacteria. The protein export machinery has been described for *B. breve* UCC2003 (74) and consists of proteins containing either a signal peptide or a number of transmembrane regions in their amino acid sequences.

In many pathogenic bacteria (e.g., *Salmonella*, *Escherichia* and *Listeria*) bacteria-bacteria interactions as well as bacteria-host interactions are expected to be mediated by extracellular structures such as fimbriae and exopolysaccharides (EPS) (75). In contrast, the role of EPS produced by non-pathogenic colonic bacteria (e.g., bifidobacteria) is poorly understood, but it is likely that these extracellular structures may be important for bacteria to establish themselves within the host (75). Notably, EPS-encoding genes have been detected in all bifidobacterial genomes examined so far and display particular sequence features (e.g., divergence in the G+C content) which may suggested that these sequences have been acquired by horizontal gene transfer events (HGT).

Another important protein that could be involved in the bifidobacteria-host interaction is represented by the serine-like protease (also known as serpin proteins) which have been detected in the genomes of the *B. longum* phylogenetic group. Serpins belong to a large class of protease inhibitors involved in inhibitory mechanism and in some physiological regulation processes (76). Recently, it has been demonstrated that the serpin protein encoded by *B. longum* NCC2705 is an efficient inhibitor of host's neutrophil and pancreatic elastases, and other granule proteases (77; 78), which are produced upon intestinal inflammation. Neutrophil elastase inhibitor produced by the strain may have a positive effect in order to reduce the tissue damage caused by the excessive activity of human neutrophil elastase under inflammatory situations and it could represent an example of interaction between an autochthonous component of the intestinal microbiota and its human host (76).

12. FUNCTIONAL GENOMICS AND BIFIDOBACTERIA

The next logical step following bacterial genome sequencing is the study of the functional products of gene

expression, which is also known as functional genomics. In this context, in order to capture the immediate, ongoing and genome-wide response of organisms to their environment, transcriptomics by micro array analysis is the method of choice. A clear example of microarray analysis in *B. longum* subsp. *longum* NCC2705 has studies the mechanism underlying preferential use of lactose over glucose as a carbon source (3). This is achieved by down regulation of the expression of the putative glucose transporter gene in a lactose-dependent manner, thereby shifting the balance of uptake and metabolism between glucose and lactose (22). Moreover, the transcriptome of *B. longum* subsp. *longum* NCC2705 has been investigated also in relation to stress response using microarrays and several protein coding sequences potentially involved in oxidative as well as heat stress defence mechanisms were identified in this manner (79). Such studies confirmed previous transcriptional analysis of heat stress response in *B. breve* UCC2003 (80) and may be used to devise novel ways of protecting the bifidobacterial cultures during manufacture and storage, as well as during gastrointestinal transit.

Transcriptomics has also been applied to investigate the molecular bases of the interactions occurring between bifidobacteria and other autochthonous components of the human gut microbiota (81). In this context the combination of *in silico* reconstructions of microbial metabolism based on transcriptional profiles and whole genome transcriptional profiling of intestinal mucosa from germ-free and colonized mice has provided valuable information on how resident GIT bacteria and probiotic microorganisms influence each other and the host. All these studies were conducted by the colonization of germ-free mice with one of the recognized dominant components of the human GIT microbiota, i.e., *Bacteroides thetaiotaomicron*, and *B. longum* subsp. *longum*, followed by whole genome transcriptional profiling of each species in their GIT environment. Such analyses have demonstrated that the presence of bifidobacteria elicits an expansion in the diversity of polysaccharides targeted for breakdown by *B. thetaiotaomicron*, such as mannose and xylose-containing glycans (81). Moreover, it was shown to induce the expression of host genes involved in innate immunity. In contrast, when such experiments were performed using *Bifidobacterium animalis* DN-173010 (Danone Vitapole, Jouy-en-Josas, France), the impact of this strain on the transcriptional profile of *B. thetaiotaomicron* was very modest (81). These findings may raise questions about the real impact of many of the so far considered probiotic bifidobacteria on the composition and functioning of the human gut microbiota. So the knowledge of the complete genetic makeup through probiogenomics efforts as well as the elucidation of the molecular mechanisms by which probiotic bacteria interact with their host and with the other microbial components of the human gut microbiota appears to be indispensable for the development of the next probiotic generations.

13. ACKNOWLEDGEMENTS

This work was financially supported by Science Foundation Ireland through the Alimentary Pharmabiotic Centre (DvS), by the Italian Award for Outstanding Young Researcher scheme “Incentivazione alla mobilita’ di studiosi stranieri e italiani residenti all’estero” and to the Marie Curie Reintegration Grant (MERG-CT-2005-03080) and by Parmalat spa, Italy to MV.

14. REFERENCES

1. T. Miyake, K. Watanabe, T. Watanabe and H. Oyaizu: Phylogenetic analysis of the genus *Bifidobacterium* and related genera based on 16S rDNA sequences. *Microbiol Immunol* 42, 661-667 (1998)

2. B. Gao and R. S. Gupta: Signature proteins that are distinctive characteristics of *Actinobacteria* and their subgrups. *Antonie van Leeuwenhoek* 90, 69-91 (2006)

3. M. Ventura, C. Canchaya, A. Tauch, G. Chandra, G. F. Fitzgerald, K. F. Chater, and D. van Sinderen: Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. *Microbiol Mol Biol Rev* 71, 495-548 (2007)

4. H. Tissier : Recherchers sur la flora intestinale normale et pathologique du nourisson. Thesis, University of Paris, Paris, France (1900)

5. S. P. Stackebrandt- The prokaryotes: an evolving electronic resource for the microbiological community. *Springer-Verlag*, New York, NY. (2000)

6. M. Ventura, C. Canchaya, A. Del Casale, F. Dellaglio, E. Neviani, G.F. Fitzgerald, and D. van Sinderen. Analysis of bifidobacterial evolution using a multilocus approch. *Int. J Syst Evol Microbiol* 56, 2783-92 (2006)

7. R. Lamendella, J. W. Santo Domingo, C. Kelty and D. B. Oerther: Bifidobacteria in feces and environmental waters. *Appl Environ Microbiol* 74, 575-84 (2008)

8. G. R. Gibson, E. R. Beatty, X Wang and J.H. Cummings: Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125, 1401-1412 (1995)

9. C. Palmer, E. M. Bik, D. B. Digiulio, D. A. Relman, and P. O. Brown: Development of the Human Infant Intestinal Microbiota. *PLoS Biol* 5, e177 (2007)

10. J.I. Gordon, T.S. Stappenbeck, and L.V. Hooper. Commensal bacteria make a difference. *Trends Microbiol* 11, 150-151 (2003)

11. E. G. Zoetendal, E. E. Vaughan and W. M. de Vos: A microbial world within us. *Mol Microbiol* 59, 1639-1650 (2006)

12. P. B. Eckburg, E. M. Bik, C. N. Bernstein, E. Purdom, L. Dethlefsen, M. Sargent, S. R. Gill, K. E. Nelson and D. A. Relman: Diversity of the human intestinal microbial flora. *Science* 308, 1635-1638 (2005)

13. R. E. Ley, P. J. Turnbaugh, S. Klein and J. I. Gordon: Microbial ecology: human gut microbes associated with obesity. *Nature* 444, 1022-1023 (2006)

14. M.J. Hopkins, H.J. Englyst, S. Macfarlane, E. Furrie. Degradation of crosslinked and non-cross-linked arabinoxylans by the intestinal microbiota in children. *Appl Environ Microbiol* 69, 6354-6360 (2003)

15. T. Matsuki, K. Watanabe, R. Tanaka, M. Fukuda and H. Oyaizu: Distribution of bifidobacterial species in human intestinal microflora examined with 16S rRNA gene targeted species specific primers. *Appl Environ Microbiol* 65, 4506-4512 (1999)

16. S. H. Duncan, A. Belenguer, G. Holtrop, A.M. Johnstone, H.J. Flint, and G.E. Lobley**.** Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Appl Environ Microbiol* 73, 1073-1078 (2007)

17. J. L Sonnenburg, J. Xu, D.D Leip, C. H. Chen, B. P. Westover, J. Weatherford, J. D. Buhler, J. I. Gordon Glycan foraging *in vivo* by an intestine-adapted bacterial symbiont. *Science* 307, 1955-9 (2005)

18. M. Ventura, D. van Sinderen, G.F. Fitzgerald, R. Zink. Insights into the taxonomy, genetics and physiology of bifidobacteria. *Antonie van Leeuwenhoek* 86, 205-223 (2004)

19. B. Henrissat: A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem J* 280, 309-16 (1991)

20. B. A. Degnan and G. T. Macfarlane: Transport and metabolism of glucose and arabinose in *Bifidobacterium breve*. *Arch Microbiol* 160, 144-151 (1993)

21. T.B Kim, S. H. Song, S. C. Kang and D. K. O. Quantitative comparison of lactose and glucose utilization in *Bifidobacterium longu*m cultures. *Biotechnol Prog* 19, 672-675 (2003)

22. S. Parche, M Beleut, E. Rezzonico, D. Jacobs, F. Arigoni, F. Titgemeyer and I. Jankovic: Lactose-over-glucose preference in *Bifidobacterium longum* NCC2705: glcP, encoding a glucose transporter, is subject to lactose repression. *J Bacteriol* 188, 1260-5 (2006)

23. P.K. Gopal, P.A. Sullivan, and J.B. Smart. Utilization of galacto-oligosaccharides as selective substrates for growth by lactic acid bacteria including *Bifidobacterium lactis* DR10 and *Lactobacillus rhamnosus* DR20. *Int Dairy J* 11, 19-25 (2001)

24. M. A. Schell, M. Karmirantzou, B. Snel, and other authors: The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract *Proc Natl Acad Sci USA* 99, 14422-14427 (2002)

25. A. Maze, M. O'Connel-Motherway, G.F. Fitzgerald, and D. van Sinderen. Identification and characterization of a putative fructose PTS of *Bifidobacterium breve* UCC2003. *Appl Environ Microbiol* 73, 545-553 (2006)

26. S. W. Hinz, M. I. Pastink, L. A. van den Broek, J. P. Vincken and A. G. Voragen: *Bifidobacterium longum* endogalactanase liberates galactotriose from type I galactans. *Appl Environ Microbiol* 71, 5501-5510 (2005)

27. A. C. Ouwehand, S. Salminen, E. Isolauri: Probiotics: an overview of beneficial effects. *Antonie Van Leeuwenhoek* 82, 279-89 (2002)

28. L. J. Fooks and G. R. Gibson: Probiotics as modulators of the gut flora.*Br J Nutr* 88, 39-49 (2002)

29. D.J. Lee, R.A. Drongowski, A.G. Coran, and C.M. Harmon. Evaluation of probiotic treatment in a neonatal animal model. *Pediatr Surg Int* 16, 237-42 (2000)

30. A. L. Servin: Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiol Rev* 28, 405-440 (2004)

31. E. Isolauri: Probiotics and gut inflammation. *Curr Opin Gastroenterol* 15, 534-537 (1999)

32. M.M. van den Berg, M.A. Benninga, and C. Di Lorenzo. Epidemiology of childhood constipation: a systematic review. *Am J Gastroenterol* 101, 2401-9 (2006)

33. M. Matsumoto, and Y. Benno. Anti-inflammatory metabolite production in the gut from the consumption of probiotic yogurt containing *Bifidobacterium animalis* subsp. *lactis* LKM512. *Biosci Biotechnol Biochem* 70, 1287-1292 (2006)

34. M. Medici, C.G. Vinderola, R. Weill, and G. Perdigón. Effect of fermented milk containing probiotic bacteria in the prevention of an enteroinvasive *Escherichia coli* infection in mice. *J Dairy Res* 72, 243-249 (2005)

35. L.E. Niers, M.O. Hoekstra, H.M. Timmerman, N.O. van Uden, P.M. de Graaf, H.H. Smits, J.L. Kimpen, and G.T. Rijkers. Selection of probiotic bacteria for prevention of allergic diseases: immunomodulation of neonatal dendritic cells. *Clin Exp Immunol* 149, 344-352 (2007)

36. D. Strachan. Damp housing and ill health. *BMJ* 299,325 (1989)

37. T. Pessi, Y. Sütas, M. Hurme, and E. Isolauri. Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. *Clin Exp Allergy* 30, 1804-1808 (2000)

38. J.M. Saavedra, Bauman, I. Oung, J.A. Perman, and R.H. Yolken. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet* 344, 1046-1049 (1994)

39. J.P. Chouraqui, L.D. van Egroo, and M.C. Fichot. Acidified milk formula supplemented with *Bifidobacterium*

lactis: impact on infant diarrhea in residential care settings. *J Pediatr Gastroenterol Nutr* 38, 288-292 (2004)

40. S. Plummer, M.A. Weaver, J.C. Harris, P. Dee, and J. Hunter. *Clostridium difficile* pilot study: effects of probiotic supplementation on the incidence of *C. difficile* diarrhoea. *Int Microbiol* 7, 59-62 (2004)

41. T. He, M.G. Priebe, Y. Zhong, C. Huang, H.J. Harmsen, G.C. Raangs, J.M. Antoine, G.W. Welling, and R.J. Vonk. Effects of yogurt and bifidobacteria supplementation on the colonic microbiota in lactose-intolerant subjects. *J Appl Microbiol* 104, 595-604 (2008)

42. M. Roberfroid, and J. Slavin. Nondigestible oligosaccharides. *Crit Rev Food Sci Nutr.* 40, 461-80 (2000)

43. R.D. Rolfe. The role of probiotic cultures in the control of gastrointestinal health. *J Nutr* 130 (2S Suppl), 396S-402S (2000)

44. L. Kopp-Hoolihan. Prophylactic and therapeutic uses of probiotics: a review. *J Am Diet Assoc* 101, 229-238 (2001)

45. M. Saxelin, S. Tynkkynen, T. Mattila-Sandholm, and W.M. de Vos. Probiotic and other functional microbes: from markets to mechanisms. *Curr Opin Biotechnol* 16, 204-211 (2005)

46. F. Guarner. Prebiotics in inflammatory bowel diseases. *Br J Nut.* Suppl 1, S85-9 (2007)

47. B.R. Goldin. Health benefits of probiotics. *Br J Nutr* 80, S203-7 (1998)

48. C. Picard, J. Fioramonti, A. Francois, T. Robinson, F. Neant, and C. Matuchansky. Review article: bifidobacteria as probiotic agents -- physiological effects and clinical benefits. *Aliment Pharmacol The.* 22, 495-512 (2005)

49. A. Klijn, A. Mercenier, and F. Arigoni: Lessons from the genomes of bifidobacteria *FEMS Microbiol Rev* 29, 491-509 (2005)

50. S.M, Ryan, G.F. Fitzgerald, and D. van Sinderen. Transcriptional regulation and characterization of a novel beta-fructofuranosidase-encoding gene from *Bifidobacterium breve* UCC2003. *Appl Environ Microbiol* 71, 3475-3482 (2005)

51. M. A. Ehrmann, M. Korakli, and R. F. Vogel.. Identification of the gene for β-fructofuranosidase of *Bifidobacterium lactis* DSM10140 (T) and characterization of the enzyme expressed in *Escherichia coli*. *Curr. Microbiol* 46, 391-397 (2003)

52. C. Janer, L.M. Rohr, C. Pelaez, M. Laloi, V. Cleusix, T. Requena, and L. Meile. Hydrolysis of oligofructoses by the recombinant beta-fructofuranosidase from *Bifidobacterium lactis*. *Syst Appl Microbiol* 27, 279-285 (2004)

53. T. Tochikura, K. Sakai, T. Fujiyoshi, T. Tachiki, and H. Kumagai. P-Nitrophenyl glycoside hydrolyzing activities in bifidobacteria and characterization of β-D-galactosidase of *Bifidobacterium longum* 401. *Agri. Biol Chem* 50, 2279-2286 (1986)

54. P.L. Moller, F. Jorgensen, O.C. Hansen, S.M. Madsen, and P. Stougaard. Intra-and extracellular β-galactosidases from *Bifidobacterium bifidum* and *B. infantis*. Molecular cloning, heterologous expression, and comparative characterization. *Appl Environ Microbiol* 67, 2276-2283 (2001)

55. M.N. Hung, Z. Xia, N.T. Hu and B.H. Lee. Molecular and biochemical analysis of β-galactosidases gene from *Bifidobacterium infantis* HL96. *Appl Environ Microbiol* 67, 4256-4263 (2001)

56. F. Jorgensen, O.C. Hansen, and P. Stougaard. High-efficiency synthesis of oligosaccharides with a truncated β-galactosidases from *Bifidobacterium bifidum*. *Appl Microbio. Biotechnol* 57, 647-652 (2001)

57. K.M. van Laere, T. Abee, H.A. Schols, G. Beldman, and A.G. Voragen. Characterization of a novel β-galactosidases from *Bifidobacterium adolescentis* DSM 20083 active towards transgalactooligosaccharides. *Appl Environ Microbiol* 66, 1379-1384 (2000)

58. A. Margolles, C.G. de los Reyes-Gavilán. Purification and functional characterization of a novel alpha-L-arabinofuranosidase from *Bifidobacterium longum* B667. *Appl Environ Microbiol* 69, 5096-103 (2003)

59. H. Y. Shin, S.Y. Park, J.H. Sung, and D.H. Kim. Purification and characterization of α-L-arabinopyranosidase and α-L-arabinosidase from *Bifidobacterium breve* K110, a human intestinal anaerobic bacterium metabolizing ginsenoside Rb2 and Rc. *Appl Environ Microbiol* 69, 7116-7123 (2003)

60. Y. Minami, K. Yazawa, Z. Tamura, T. Tanaka, and T. Yamamoto. Selectivity of utilization of galacto-oligosaccharides by bifidobacteria. *Chem Pharm Bull* 31, 1688-1691 (1983)

61. M. Xiao, K. Tanaka, X.M. Qian, K. Yamamot, and H. Kumagai. High-yield production and characterization of α-galactosidase from *Bifidobacterium breve* grown on raffinose. *Biotechnol Let.* 22, 747-751 (2000)

62. M.S. Garro, G.S. de Giori, G.F. de Valdez, and G. Oliver. α-D-Galactosidase from *Bifidobacterium longum*. *Lett Appl Microbiol* 19, 16-19 (1994)

63. S. Leder, W. Hartmeier, S.P. Marx. α-D-Galactosidase of *Bifidobacterium adolescentis DSM20083 Curr Microbiol* 38, 101-106 (1999)

64. K.M. van Laere, R. Hartemink, G. Beldman, S. Pitson, C. Dijkema, H.A. Schols, A.G. Voragen. Hydrolase and transgalactosylation activity of *Bifidobacterium adolescentis* α-galactosidase. *Appl Microbiol Biotechnol* 52, 681-688 (1999)

65. K. Sakai, T. Tachiki, H. Kumagai, and T. Tochikura. Hydrolysis of α-D-galactosyl oligosaccharides in soymilk by α- galactosidase of *Bifidobacterium breve* 203. *Agric Biol Chem* 51, 315-322.

66. F. Crociani, A. Alessandrini, M.M. Mucci, and B. Biavati. Degradation of complex carbohydrates by *Bifidobacterium* spp. *Int J Food Microbiol* 24, 199-210 (1994)

67. G.E. Ji, H.K. Han, S.W. Yun, and S.L. Rhim. Isolation of amylolytic *Bifidobacterium* sp. Int-57 and characterization of amylase. *J Microbiol Biotechnol* 2, 85-91 (1992)

68. S.K. Lee, Y.B. Kim, and G.E. Ji. Purification of amylase secreted from *Bifidobacterium adolescentis. J Appl Microbiol* 83, 267-272.

69. S.M. Ryan, G.F. Fitzgerald, and D. van Sinderen. Screening and identification of starch, amylopectin and pullulan-degrading activities in bifidobacterial strains. *Appl Environ Microbiol* 72, 5289-96 (2006)

70. L.C. Hoskins, M. Agustines, W.B. McKee, E.T. Boulding, M. Kriaris, and G. Niedermeyer. Mucin degradation in human colon ecosystems. Isolation and properties of fecal strains that degrade ABH blood group antigens and oligosaccharides from mucin glycoproteins. *J Clin Invest* 75, 944-953 (1985)

71. L. Slovakova, D. Duskova, and M. Marounek Fermentation of pectin and glucose, and activity of pectin-degrading enzymes in the rabbit caecal bacterium *Bifidobacterium pseudolongum*. *Lett Appl Microbiol* 35, 126-130 (2002)

72. C. Liepke, K. Adermann, M. Raida, H.J. Magert, W.G. Forssmann, and H.D. Zucht. Human milk provides peptides highly stimulating the growth of bifidobacteria. *Eur J Biochem* 269, 712-718. (2002)

73. M. Ventura, M. O'Connell-Motherway, S. Leahy, J.A. Moreno-Munoz, G.F. Fitzgerald, and D. van Sinderen. From bacterial genome to functionality; case bifidobacteria. *Int J Food Microbio.* 120, 2-12 (2007)

74. L.E. MacConaill, G.F. Fitzgerald, and D. van Sinderen. Investigation of protein export in *Bifidobacterium breve* UCC2003. *Appl Environ Microbio.* 69, 6994-7001 (2003)

75. P. Ruas-Madiedo, M. Gueimonde, A. Margolles, C.G. de los Reyes-Gavilán, and S. Salminen. Exopolysaccharides produced by probiotic strains modify the adhesion of probiotics and enteropathogens to human intestinal mucus. *J Food Prot* 69, 2011-2015 (2006)

76. D. Ivanov, C. Emonet, F. Foata, M. Affolter, M. Delley, M. Fisseha, S. Blum-Sperisen, S. Kochhar, and F. Arigoni. A Serpin from the Gut Bacterium *Bifidobacterium longum* Inhibits Eukaryotic Elastase-like Serine Proteases. *J Biol Chem* 281, 17246-17252 (2006)

77. N.D. Burg, and M.H. Pillinger. The neutrophil: function and regulation in innate and humoral immunity. *Clin Immunol.* 99, 7-17 (2001)

78. E.P. Reeves, H. Lu, H.L. Jacobs, C.G. Messina, S. Bolsover, G. Gabella, E.O. Potma, A. Warley, J. Roes, and A.W. Segal. Killing activity of neutrophils is mediated through activation of proteases by K+ flux. *Nature* 21, 291-297 (2002)

79. E. Rezzonico, S. Lariani, C. Barretto, G. Cuanoud, G. Giliberti, M. Delley, F. Arigoni and G. Pessi: Global transcriptome analysis of the heat shock response of *Bifidobacterium longum. FEMS Microbiol Lett* 271, 136-145 (2007)

80. M. Ventura, C. Canchaya, Z. Zhang, G. F. Fitzgerald and D. van Sinderen: How high G+C Gram-positive bacteria and in particular bifidobacteria cope with heat stress: protein players and regulators. *FEMS Microbiol Rev* 30, 734-759 (2006)

81. J. L. Sonnenburg, C. T. Chen and J. I. Gordon: Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. *PLoS Biol* 4, e413 (2006)

82. P. Alvarez-Martin, A.B. Florez and B. Mayo: Screening for plasmids among human bifidobacteria species: sequencing and analysis of pBC1 from *Bifidobacterium catenulatum* L48. *Plasmid* 57, 165-174 (2006)

83. J. H. Lee and D. J. O'Sullivan: Sequence analysis of two cryptic plasmids from *Bifidobacterium longum* DJO10A and construction of a shuttle cloning vector. *Appl Environ Microbiol* 72, 527-535 (2006)

84. Z. Yildirim, D. K. Winters and M. G. Johnson: Purification, aminoacid sequence and mode of action of bifidocin B produced by *Bifidobacterium bifidum* NCFB 1454. *J Appl Microbiol* 86, 45-54 (1999)

85. C. Lay, L. Rigottier-Gois, K. Holmstrom, M. Rajilic, E.E. Vaughan, W.M. de Vos, M.D. Collin, R. Thiel, P. Namsolleck, M. Blaut and J. Dore: Colonic microbiota signatures across five northern European countries. *Appl Environ Microbiol* 71, 4153-4155 (2005)

85. L.A.M. Van den Broek, R. Lloyd, G. Beldman, J.C. Verdoes and other authors: Cloning and characterization of arabinoxylan arabinofuranohydrolase-D3 (AXHd3) from *Bifidobacterium adolescentis* DSM20083. *Appl Microbiol Biotechnol* 67, 641-747 (2005)

86. P. Ruas-Madiedo, M Gueimonde, M Fernández-García, C.G. de los Reyes-Gavilán, A Margolles: Mucin

degradation by *Bifidobacterium* strains isolated from the human intestinal microbiota. *Appl Environ Microbiol* 74, 1936-40 (2008)

87. J. H. Lee, V. N. Karamychev, S. A. Kozyavkin, D. Mills, A. R. Pavlov, N. V. Pavlova, N. N. Polouchine, P. M. Richardson, V. V. Shakhova, A. I. Slesarev, B. Weimer, D. J. O'Sullivan: Comparative genomic analysis of the gut bacterium *Bifidobacterium longum* reveals loci susceptible to deletion during pure culture growth. *BMC Genomics* in press (2008)

Key Words: Bifidobacteria, Genomics, Probiotic Bacteria, Prebiotic Features, Review

Send correspondence to: Marco Ventura, Department of Genetics, Biology of Microorganisms, Anthropology and Evolution University of Parma, Italy, Via Usberti 11a, 43100 Parma, Italy, Tel: 339-521-905666, Fax: 339-521-905604, E-mail: marco.ventura@unipr.it

http://www.bioscience.org/current/vol14.htm