Application of molecular biology and genomics of probiotics for enteric cytoprotection

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Probiotic Bacteria and Enteric Infections

Cytoprotection by Probiotic Bacteria

Springer

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Chapter 6

Application of Molecular Biology and Genomics of Probiotics for Enteric Cytoprotection

Salomeh Moslehi-Jenabian, Dennis Sandris Nielsen and Lene Jespersen

6.1 Introduction

The intestinal microbiota plays an essential role in host nutrition, intestinal cell proliferation and differentiation, development of the immune system and acquired responses to pathogens. Alterations in the composition of the intestinal microbiota have recently been linked to various diseases, including inflammatory bowel disease, allergy and diabetes type II (Guarner and Malagelada 2003; Larsen et al. 2010; Lomax and Calder 2009). Probiotics are among the variable indigenous constituents of the gut microbiota. There are various evidences for different beneficial functions of probiotics and the mechanisms underlying these health effects include both microbe-microbe and microbe-host interactions. Nevertheless, the molecular basis of these mechanisms is still largely unknown. However, recent modern molecular biology based -omics technologies (genomics, proteomics and metabolomics), allowing simultaneous analysis of huge numbers of genes, proteins or metabolites, have revealed insights into understanding the molecular basis for these health promoting activities and increased our knowledge concerning the roles of probiotics in microbe-microbe and host-microbe interactions. The microbial genomic content reflects metabolism, physiology, biosynthetic capabilities of the microorganism, and its ability to adapt to varying conditions and environments. Hence, genome analysis of probiotics will help us to understand their metabolic processes and functionality in human health and well-being. Beside the scientific importance, it will provide a way to improve functional foods, which attracts the interest of the industry and consumers. Consequently, it is of significant concern to exploit the recent studies on the molecular details of the interaction of probiotics with the human host and other microbes. This chapter provides an overview of current progresses in molecular and genomic technologies of probiotics to elucidate the role of these microorganisms in human health and well being. Emphasis will be on the model probiotic

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bacteria *Lactobacillus* spp. and *Bifidobacterium* spp., which are phylogenetically distant relatives with different features. When relevant, references will be made to the probiotic yeast *Saccharomyces cerevisiae* var. *boulardii* (van der Aa Kühle and Jespersen 2003), which is widely used as a therapeutic agent.

6.2 Functional Genomics

Functional genomic analyses including whole genome sequencing, genome data mining and comparative genomics have been useful in understanding the influence of genetic content, organization, function and regulation on gut and probiotic functionality as well as to identify the differences and similarities between probiotics since many of the probiotic features are species and even strain dependent. Functional genomic analysis is therefore essential to understand the cellular physiology, metabolic pathways, sensing and signalling in order to clarify mechanisms underlying the probiotic functions of these microorganisms (Klaenhammer et al. 2002). In addition, genomic tools to investigate the gene regulatory networks are important in order to analyse the response of microorganisms to different environmental conditions, especially, the gut-related environmental stresses.

Various studies have investigated the molecular response of probiotics using *in vitro* models mimicking the gut and intestinal environment, for instance acid and bile stress response and tolerance. In many cases, the genes and proteins identified encompass the general stress proteins like GroEL, GroES and DnaK (Frees et al. 2003; Lim et al. 2000; Weiss and Jespersen 2010), and functions related to maintenance of the cell-envelope integrity due to the destructive effect of bile on the cell wall (Bron et al. 2006). It has been shown that these responses are controlled by different regulators that are involved in control of the general stress response (Ferreira et al. 2001, 2003). *In vitro* models are useful for investigating the response of the microorganism to a specific intestinal stress. However, investigation of the full response of a given microorganism will only be achieved using *in vivo* approaches. Therefore, some functional genomic approaches have focused on the study of genetic responses of microorganisms *in vivo* with the goal of identifying bacterial genes that are important during residence in the gut.

Three main strategies have been developed for the identification of genes that are highly expressed *in vivo*, as compared with laboratory conditions: (1) (recombination-based) *in vivo* expression technology ((R-)IVET), (2) signature-tagged mutagenesis (STM), and (3) selective capture of transcribed sequences (SCOTS). These *in vivo* gene identification strategies have been applied for investigation of important genes in bacterial pathogenesis (Mahan et al. 2000). In addition, IVET has recently been employed to identify genes potentially influencing the probiotic functionality in both *Lactobacillus reuteri* 100-23 (Walter et al. 2003) and *Lactobacillus plantarum* WCFS1 (Bron et al. 2004). This approach allows identification of promoter elements that are expressed during *in vivo* transit of probiotic cultures, and reveals the corresponding genes driven by these promoters.

6 Application of

The DNA microarray technology, by monitoring the global gene expression to elucidate the mechanisms of action (Azcarate-Peril et al. 2003). Real-time PCR, can be used to monitor the cells under different conditions (Tao et al. 2003).

Targeted insertion of reporter genes into presumptive regulatory regions have been used to identify new gene types (Azcarate-Peril et al. 2003).

Thus far, few studies have identified features that are unique to the enteric cytoprotective probiotics.

6.3 Genes Related to the Gut Environment

Tolerance of probiotics to their adaptation to the gut environment. Different probiotics have different metabolic activities in the gut environment, v

6.3.1 Genes

Genes encoding for the adaptation to intestinal stress. DnaK, DnaJ, and Hsp70 are sub-lethal adaptive proteins. CRL 639 (Lactobacillus casei) expression of stress proteins after exposure to the gut tract model (Walter et al. 2003). Inducible operons. ATPase, a multiprotein complex that expulse protons from the cell membrane. Stress induces the expression of the membrane proteins of the cell and the cytoplasmic proteins (Kullen et al. 2003).

which are phylogenetically references will be made to *rdii* (van der Aa Kühle and gent.

The DNA microarray technique is a functional genomic approach enabling monitoring the global transcriptional response at the time of sampling and can be used to elucidate the genomic expression of gut-related bacteria in the intestinal tract (Azcarate-Peril et al. 2004; Denou et al. 2007). This approach together with real-time PCR, can be used for quantitative analysis of the transcriptional response of the cells under conditions of interest, e.g., cells that are located at specific intestinal sites (Tao et al. 2006).

Targeted insertional mutagenesis is another alternative to study the gene regions that are presumed to be involved in probiotic traits, and thereby a number of gene regions have been characterized and functionally correlated to important phenotypes (Azcarate-Peril et al. 2004; Velez et al. 2007).

Thus far, functional genomic analyses have revealed a number of interesting features that are generally considered to be important for the roles of probiotics in enteric cytoprotection and health.

6.3 Genes and Molecules Involved in Adaptation of Probiotics to the Gut Niche

Tolerance of probiotics to the stress conditions of the intestinal environment and their adaptation to the gut niche play significant roles in the functionality of probiotics. Different genomic studies have demonstrated the genetic adaptation and metabolic activity of *Lactobacillus* spp. or *Bifidobacterium* spp. in the intestinal environment, which will be discussed in detail in the following sections.

6.3.1 Genes and Molecules Involved in Stress Adaptation

Genes encoding acid resistance responses are essential in tolerance of probiotics to intestinal stress. As an example induction of putative heat shock proteins, i.e., DnaK, DnaJ, GrpE, GroES and GroEL, in acid adapted cells (exposure of cells to sub-lethal adaptive acid conditions) has been shown in *Lactobacillus acidophilus* CRL 639 (Lorca et al. 2002). Recently, a transcriptomic study has shown the expression of stress related genes GroEL, DnaK and ClpP in *L. acidophilus* NCFM after exposure to gastric juice following passage through an *in vitro* gastrointestinal tract model (Weiss and Jespersen 2010). In *L. acidophilus*, the *atp* operon is an acid inducible operon containing 8 genes encoding the various subunits of the F_1F_0 -ATPase, a multimeric enzyme either synthesizing ATP using protons or conversely expulse protons out of the cell with the energy provided by ATP hydrolysis. Acidic stress induces expression of the *atp* operon accompanied by an increase in the activity of the membrane-bound enzyme, which results in active expulsion of protons out of the cell and maintenance of cytoplasmic pH under acidic environmental conditions (Kullen and Klaenhammer 1999). Further studies have shown the presence of

four loci contributing to acid resistance in the *L. acidophilus* NCFM genome. The role of the four loci in acid tolerance was proved by insertional mutagenesis in these regions, which resulted in acid sensitive derivatives (Azcarate-Peril et al. 2004). A two-component regulatory system has been found in *L. acidophilus* NCFM playing a role in acid resistance (Azcarate-Peril et al. 2005). Insertional mutagenesis of this two-component regulatory system resulted in an acid sensitive mutant. Whole-genome microarray analysis of the mutant showed that expression of 80 genes including two oligopeptide-transport systems, other components of the proteolytic enzyme system, and a *luxS* homolog was affected by the mutation. The gene *luxS* is involved in AI-2 mediated interspecies quorum sensing (cell-to-cell communication) among bacteria (Federle and Bassler 2003). A transcriptomic study has shown that the *luxS* gene is induced by acidic stress in *L. acidophilus* NCFM and *Lactobacillus rhamnosus* GG and plays a role in the acid stress response in these probiotics. It was observed that in both species, the *luxS* gene was transiently up-regulated after acidic shock (pH 4.0). Acid adaptation of cells attenuated the transcription of the *luxS* gene. Thus, this gene might be important in not only the survival of *Lactobacillus* spp. during the passage through the gastrointestinal tract, but also in the cell-to-cell communication among bacteria in the intestinal microbiota (Moslehi-Jenabian et al. 2009). Genome wide expression analysis experiments using microarrays have revealed that in *L. reuteri* ATCC 55730, the *clpL* chaperone gene (encoding an ATPase with chaperone activity) was involved in the early response to severe acidic shock. This was validated by mutation in *clpL* and the mutant was significantly more sensitive to acidic stress compared to the wild type (Wall et al. 2007).

Genes involved in the tolerance to bile salts are also important for survival of probiotics after passage through the gastrointestinal tract. DNA micro-array analysis of the global transcriptional response of *L. plantarum* WCFS1 against bile revealed 12 bile-responsive gene clusters. Seven of the identified bile-responsive genes and gene clusters encoded typical stress-related functions, including glutathione reductase and glutamate decarboxylase, involved in oxidative and acid stress defence, respectively. Besides, 14 bile-responsive genes and gene clusters were detected that encoded proteins located in the cell envelope, including the *dlt* operon and the F_1F_0 ATPase. The induction of a high number of genes encoding cell envelope functions show the significant effect of bile salts on the integrity and/or functionality of the cytoplasmic membrane and cell wall (Bron et al. 2006). Genes encoding bile salt hydrolases (*bsh*) have been identified in intestinal *Lactobacillus* spp., i.e., *L. acidophilus* NCFM (McAuliffe et al. 2005), *Lactobacillus johnsonii* 100-100 (Elkins et al. 2001) and *L. plantarum* WCFS1 (Lambert et al. 2008a), which shows the ecological adaptation of these species to the intestine and the importance of this trait for *Lactobacillus* spp. in order to colonize the lower gastrointestinal tract.

As for *Lactobacillus* spp., *Bifidobacterium* spp. have developed a system that attempt to maintain their cytoplasmic pH near neutral under acidic stress. In this respect, the proton-translocating ATPase (F_1F_0 -ATPase) plays an important role and is encoded by the *atp* operon including nine genes. This multi-subunit enzyme is essential for growth of *Bifidobacterium* spp. under acidic conditions (Ventura et al. 2004). It has been shown that bile induces expression of the F_1F_0 -ATPase

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and increases the membrane-bound H^+ -ATPase activity in *Bifidobacterium anima-*
lis. Comparison of *B. animalis* IPLA 4549 and a mutant with acquired resistance
to bile (*B. animalis* 4549dOx) has shown that the bile-resistance mutant was able
to tolerate bile by increasing the intracellular ATP reserve and by inducing proton
pumping by the F_1F_0 -ATPase (Sanchez et al. 2006). Genes encoding bile salt hydro-
lases (*bsh*) have been detected in *Bifidobacterium longum* BB536 (Shuhaimi et al.
2001), *Bifidobacterium bifidum* ATCC 11863 (Kim et al. 2004), *Bifidobacterium*
adolescentis ATCC 15705 (Kim et al. 2005) and a bile tolerant strain of *B. animalis*
subsp. *lactis* KL612 (Kim and Lee 2008). In a recent study, two putative multidrug
resistance (MDR) transporter genes, i.e. the BL0920 gene from *B. longum* subsp.
longum NCC2705 and its homolog, Bbr0838 gene, from *Bifidobacterium breve*
UCC2003, were induced after exposure to sub-inhibitory concentrations of bile.
The expression of the BL0920 gene in *Escherichia coli* conferred resistance to bile,
which was probably mediated by active efflux from the cells. This study represents
the first identified bifidobacterial bile efflux pump (Gueimonde et al. 2009). Mo-
lecular analysis of *B. longum* NCC2705 cells grown in the intestinal tract of mice
revealed that different genes and proteins are expressed in the cells for adaptation
of *B. longum* to intestinal stress. Among these, EF-Tu (related to the retention or
attachment), bile salt hydrolase and stress proteins which protect *B. longum* against
the action of bile salts and other destructive components of the gastrointestinal tract
have been identified. In addition, it has been found that intestinal growth triggered
phosphorylation of LuxS protein (the active form of LuxS) that possibly play a key
role in the regulation of quorum sensing between microorganisms of intestinal mi-
crobiota (Yuan et al. 2008).

6.3.2 Genes and Molecules Involved in Nutritional Adaptation

The complete sequencing of several *Lactobacillus* spp. genomes has revealed a
considerable degree of auxotrophy for amino acids and other cellular components.
To compensate for these auxotrophies, *Lactobacillus* spp. have been shown to en-
code multiple genes for transport and uptake of macromolecules and metabolism of
complex carbohydrates (Pfeiler and Klaenhammer 2007). Due to their auxotrophy,
Lactobacillus spp. will predominantly be present in the ileum, which is a nutri-
tional richer environment than e.g. the colon. Comparing the genome sequence of
intestinal isolates of *Lactobacillus* spp. with food isolates indicates a strong degree
of niche adaptation. As an example, *Lactobacillus helveticus* DPC 4571, a cheese
starter culture, has additional genes for fatty acid biosynthesis and specific amino-
acid metabolism, but remarkably fewer cell-surface proteins and phosphoenolpyru-
vate phosphotransferase systems for sugar utilization compared to *L. acidophilus*
NCFM, which is a closely related species well adapted to the intestine. In addition,
no functional mucus-binding proteins or transporters for complex carbohydrates are
encoded by the *L. helveticus* DPC 4571 genome, indicating adaptation to the milk
environment. Whereas *L. acidophilus* that is adapted to the gut ecological niche,

contains functional gene sets such as mucus-binding and cell surface proteins and enzyme complexes that are absent from *L. helveticus* DPC 4571 (Altermann et al. 2005; Callanan et al. 2008), emphasizing the importance of these gene sets for gut adaptation and probiotic functionality. The genes encoding the mucus-binding or cell surface proteins found in the genome of intestinal *Lactobacillus* spp. are predicted to produce secreted proteins such as the S-layer proteins, which are maintained at the cell envelope via either covalent interactions affected by the sortase enzyme or electrostatic interactions, and interact with human intestinal compounds such as extracellular matrix proteins and mucus (Åvall-Jääskeläinen and Palva 2005). These extracellular proteins are essential not only in the interaction of probiotics with host cells or tissues, but also in degradation of complex extracellular carbon sources and have a prominent role in the adaptation to environmental changes and intestinal persistence (Boekhorst et al. 2006; Buck et al. 2005). Analysis of the predicted extracellular proteins of *L. plantarum* WCFS1 has revealed that at least 12 proteins are predicted to be directly involved in adherence to host components like collagen and mucin, and about 30 extracellular enzymes, mainly hydrolases and transglycosylases, predicted to be involved in substrate degradation by *L. plantarum* WCFS1 to maintain the growth in different environmental niches (Boekhorst et al. 2006).

In vivo studies using an IVET strategy based on the *in vivo* selection of an antibiotic-resistant phenotype have shown induction of 3 *in vivo* induced genes that are highly expressed in *L. reuteri* 100-23 during intestinal colonisation in *Lactobacillus*-free mice (Walter et al. 2003). In another study using a recombinase-based-IVET approach in *L. plantarum* WCFS1, 72 different genes were induced during passage through the gastrointestinal tract of conventional mice. Most of these genes were related to carbon and amino-acid metabolism and stress response (Bron et al. 2004). The homologues of many of these genes have been found in intestinal pathogens and associated with survival and adaptation to the gut environment. Whole genome transcriptional profiling of *L. plantarum* during colonization in the cecum of germ-free mice showed up-regulation of genes involved in carbohydrate transport and metabolism, compared with *in vitro* growth conditions. Indeed, the mouse diet had an essential impact on the *in situ* transcriptome of *L. plantarum* WCFS1 (Marco et al. 2009). Similar studies have shown transcription of metabolic genes in *Lactobacillus casei* DN-114 001 (Oozeer et al. 2005) and in *L. johnsonii* NCC533 (Denou et al. 2007, 2008) as adaptation to the environmental conditions in the murine intestine. In the latter species the expression of different sets of genes was observed to depend on its location in the mouse intestine (Denou et al. 2007, 2008). Some *Lactobacillus* spp. can utilize fructo-oligosaccharides which are known as prebiotics (non-digestible oligosaccharides which stimulate growth and/or metabolic activity of probiotics in the host intestine) and thereby interact metabolically with host and other microbes. *L. acidophilus* NCFM metabolise fructo-oligosaccharides by inducing the transcription of a specific transport and degradation system (Barrangou et al. 2003). Similarly, *L. plantarum* WCFS1 have a specific gene expression pattern when exposed to fructo-oligosaccharides, even though it is only able to degrade the short chains of these compounds (Saulnier et al. 2007).

Contrary to *Lactob* therefore well adapted some growth substrate erty gives them an ec *rium* genomics has de nucleotides, vitamins, lize complex carbohyc sugar degradation path 2007b) and preliminar bifidobacterial genes in in the human infant gu to hydrolyse different t for fructo-oligosacchar gosaccharides have be 2008; Ryan et al. 2005

6.4 Genes and N with Enterol

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6.4.1 Genes and Interaction.

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Contrary to *Lactobacillus* spp., *Bifidobacterium* spp. are autotrophic and are therefore well adapted to growth in an environment with low concentrations of some growth substrates such as the human colon (Ventura et al. 2007a). This property gives them an ecological advantage in the intestinal ecosystem. *Bifidobacterium* genomics has demonstrated their relative broad autotrophy for amino acids, nucleotides, vitamins, and cofactors and has verified their ability to degrade and utilize complex carbohydrates (Schell et al. 2002). Gene clusters coding for complex sugar degradation pathways are abundant in bifidobacterial genomes (Ventura et al. 2007b) and preliminary intestinal transcriptomic studies have shown expression of bifidobacterial genes including oligosaccharide metabolism and vitamin production in the human infant gut (Klaassens et al. 2009). *Bifidobacterium* spp. are also able to hydrolyse different types of fructo-oligosaccharides (prebiotics) and the operons for fructo-oligosaccharide metabolism, specific transporters and hydrolases for oligosaccharides have been identified in the bifidobacterial genome (Gonzalez et al. 2008; Ryan et al. 2005).

6.4 Genes and Molecules Involved in Interaction of Probiotics with Enteropathogens and Gut Microbial Symbionts

Interaction of probiotics with enteropathogens in the intestinal tract involves different mechanisms, including nutrient-based interactions, competition for specific adhesion sites (competitive exclusion) and production of antimicrobial compounds.

6.4.1 Genes and Molecules Involved in Nutrient-Based Interactions

Nutrient-based interactions between probiotic bacteria and other members of the gut microbiota has been proved using germ-free mice models colonized by *Bacteroides thetaiotaomicron* ATCC 29148 (a prominent component of the adult human gut microbiota), *B. longum* NCC2705 and *L. casei* DN-114 001 or combinations of these microorganisms. Whole genome transcriptional profiling of all bacterial species as well as the intestinal epithelium showed that presence of *B. longum* triggered an expansion in the diversity of polysaccharides targeted for degradation by *B. thetaiotaomicron* (e.g., mannose- and xylose-containing glycans), and induced host genes involved in innate immunity. Presence of *L. casei* in this model resulted in an expanded capacity of *B. thetaiotaomicron* to metabolize polysaccharides and increased expression of genes for inorganic ion transport and metabolism, the same results as those observed by *B. longum*. This model showed how a resident symbiont and a probiotic species adapt their substrate utilization in response to each other (Sonnenburg et al. 2006). Indeed, it has been proposed that depletion of iron by *Bifidobacterium* spp. which is an essential nutrient for many intestinal pathogens

(but not for *Lactobacillus* spp.) could be an important factor in the protective effect of *Bifidobacterium* spp. against pathogens in the gut (Kot and Bezkorovainy 1993).

6.4.2 Genes and Molecules Involved in Competitive Exclusion

One of the beneficial roles of probiotics is competition with enteropathogens to adhere to intestinal mucus or competitive exclusion. Therefore, the capacity of probiotic bacteria to adhere to the intestinal mucosa is an important factor for competitive exclusion. Different molecular methods including comparative genomics have revealed a number of genes involved in the adhesion of probiotic *Lactobacillus* spp. to the intestinal tract, such as genes encoding mucus-binding proteins (Altermann et al. 2005; Buck et al. 2005), surface layer proteins (Buck et al. 2005; van Pijkeren et al. 2006), fibronectin-binding proteins (Altermann et al. 2005; Buck et al. 2005), fimbriae (Pridmore et al. 2004), EPS clusters (Altermann et al. 2005; Pridmore et al. 2004), mucus-binding pilli (Kankainen et al. 2009) and mannose-specific adhesion proteins (Pretzer et al. 2005).

Multiple copies of genes encoding mucus-binding proteins have been found in different *Lactobacillus* spp. The predicted mucus-binding proteins are unusually large proteins representing the largest open reading frames (ORFs) in the genome, with relatively low amino acid identity offering considerable sequence variability within surface proteins which are supposed to have important roles in mucus binding (Altermann et al. 2005; Pridmore et al. 2004). Inactivation of genes encoding a mucus-binding protein, a fibronectin-binding protein and a surface layer protein in *L. acidophilus* had a great impact on adherence to intestinal Caco-2 epithelial cells. The adhesion ability was reduced significantly in the mucus-binding protein mutant (65%), the fibronectin-binding protein mutant (76%), and the surface layer protein mutant (84%). However, the decreased adhesion ability in the latter mutant was due to the loss of multiple surface proteins that may be embedded in the S-layer. This study showed that in *L. acidophilus* NCFM multiple cell surface proteins individually have a role in the ability of organism to attach to intestinal cells (Buck et al. 2005). Recently, a transcriptomic study using an *in vitro* gastrointestinal tract model has shown up-regulation of the genes encoding mucin binding protein and fibronectin-binding protein in *L. acidophilus* NCFM after exposure to duodenal juice and bile (Weiss and Jespersen 2010). The important role of mucus-binding pilli in the adhesion ability has been proved in *L. rhamnosus* GG. Comparative genomics of this probiotic bacterium with a starter culture strain *L. rhamnosus* LC705 (exhibiting reduced binding to mucus) revealed one genomic island in *L. rhamnosus* GG which was not present in the other strain and contained 3 pilli encoding genes (spaCBA). Molecular analysis showed that the spaC gene is involved in the adherence of strain *L. rhamnosus* GG to human intestinal mucus and presence of this gene is crucial for the interaction between *Lactobacillus* spp. and host tissues offering a likely explanation of the longer persistence of *L. rhamnosus* GG in the intestinal tract compared to other *L. rhamnosus* strains (Kankainen et al. 2009). Furthermore,

a mannose-specific adhesion surface protein has been identified. In a model, a *msa* knock-out strain with intestinal epithelial cells, *L. plantarum* 299v induced a protein with mannose-specific adhesion observed in the *msa* mutant. Host responses in the intestine are a likely mechanism for mannose specific adhesion observed in different probiotic strains and is the target of *S. cerevisiae* var. *boulardii* cell wall (Moslehi-Jenabian et al. 2009). Adherence and translocation of cell wall to bind enteropathogens is shown to bind enteropathogens (Gedek 1999).

The genome sequenced glycoprotein-binding protein that could be involved in the adhesion of bacteria to galactose chains as masses of 36 kDa and been shown that severe growth media which inhibit factor antigen II to galactose-binding protein.

6.4.3 Genes and Molecules Involved in Antimicrobial Activity

Probiotics are able to inhibit the growth of enteropathogens (antimicrobial peptides) and produce stable peptides with antimicrobial activity (et al. 2005). Numerous studies by probiotics with antimicrobial activity (Todorov and Dicks 2000) was not proved that the effect against pathogens is due to the production of probiotics and enteropathogens which has the ability to inhibit the growth of mouse model due to the

ant factor in the protective effect (Kot and Bezkorovainy 1993).

Competitive Exclusion

etition with enteropathogens to. Therefore, the capacity of pro-an important factor for competi- ing comparative genomics have 1 of probiotic *Lactobacillus* spp. us-binding proteins (Altermann ; (Buck et al. 2005; van Pijkeren n et al. 2005; Buck et al. 2005), nann et al. 2005; Pridmore et al. and mannose-specific adhesion

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a mannose-specific adhesin gene (*msa*) which encodes a sortase-dependent cell surface protein has been found in *L. plantarum* WCFS1 (Pretzer et al. 2005). In a pig model, a *msa* knock-out mutant of *L. plantarum* 299v exhibited decreased association with intestinal epithelia and increased jejunal fluid absorption. The wild-type *L. plantarum* 299v induced expression of the gene encoding pancreatitis-associated protein, a protein with proposed bactericidal properties but this feature was not observed in the *msa* mutant that suggests a role for the *msa* gene in the induction of host responses in the pig intestine (Gross et al. 2008). Adhesion to mannose residues is a likely mechanism behind various bacterium-host interactions. Presence of mannose specific adhesin genes and mannose-specific binding properties has been observed in different pathogens such as *E. coli* and *Salmonella enterica* serovar Typhimurium and is the basis for competitive exclusion by the potent probiotic yeast *S. cerevisiae* var. *boulardii* that have mannose containing polysaccharides in the cell wall (Moslehi-Jenabian et al. 2010). *S. cerevisiae* var. *boulardii* prevents bacterial adherence and translocation in the intestinal epithelial cells, due to the capacity of cell wall to bind enteropathogens. The *S. cerevisiae* var. *boulardii* cell wall has been shown to bind enterohaemorrhagic *E. coli* and *S. enterica* serovar Typhimurium (Gedek 1999).

The genome sequence of *B. longum* and other *Bifidobacterium* spp. contain predicted glycoprotein-binding fimbriae and mucus and fibronectin-binding proteins that could be involved in the bacterial adhesion to the intestinal tract (Klaassens et al. 2009; Schell et al. 2002). *B. adolescentis* BB-119 binds to type V collagen at galactose chains as target site via its two cell surface proteins with molecular masses of 36 kDa and 52 kDa and lectin-like activity (Mukai et al. 1997). It has been shown that several species of *Bifidobacterium* produce a compound in the growth media which inhibits binding of enterotoxigenic *E. coli*-expressing colonization factor antigen II to ganglioside GM1 (asialo GM1 or GA1), a common bacterium-binding structure (Fujiwara et al. 1997).

6.4.3 Genes and Molecules Involved in Production of Antimicrobial Compounds

Probiotics are able to interact with enteropathogens by production of bacteriocins (antimicrobial peptides). Bacteriocins are a heterogeneous family of small, heat stable peptides with antimicrobial activity against closely related bacteria (Cotter et al. 2005). Numerous studies have shown the production of various bacteriocins by probiotics with antimicrobial effect against enteropathogens (Corr et al. 2007; Todorov and Dicks 2004; Zamfir et al. 2007). However, in most of these studies, it was not proved that the bacteriocin production was the main reason for inhibitory effect against pathogens by the probiotics. Nevertheless, bacteriocin-based interaction of probiotics and enteropathogens have been proved for *L. salivarius* UCC118 which has the ability to eliminate *Listeria monocytogenes* EGDe and LO28 from a mouse model due to the production of the broad spectrum bacteriocin Abp118 (also

known as salivaricin) (Corr et al. 2007). It was observed that a bacteriocin-negative derivative of *L. salivarius* UCC118 was not able to protect mice against listerial infection. On the other hand, *L. salivarius* UCC118 could not protect the mice against infection with a *L. monocytogenes* derivative expressing the bacteriocin-immunity protein (Corr et al. 2007). This study demonstrates precisely the importance of bacteriocin production by probiotics for the protection against enteropathogens. In addition to bacteriocins, production of lactic acid and H_2O_2 has also been shown to be important measures used by *Lactobacillus* spp. against enteropathogens (De Keersmaecker et al. 2006; Pridmore et al. 2008).

In addition to inhibiting enteropathogens by production of antimicrobial compounds, it has been shown that the probiotic yeast *S. cerevisiae* var. *boulardii* produces two proteins of 54 and 120 kDa being responsible for degradation or neutralisation of bacterial toxins. The 54 kDa protein is a serine protease that decreases the enterotoxic and cytotoxic activities of *Clostridium difficile* by proteolysis of *C. difficile* toxin A and inhibits binding of the toxin to its brush border membrane receptor. *In vivo* studies have shown that oral administration of *S. cerevisiae* var. *boulardii* or its supernatant decreases toxin A-induced intestinal secretion and permeability due to activity of this enzyme (Castagliuolo et al. 1996, 1999; Pothoulakis et al. 1993). The 120 kDa protein has no proteolytic activity but competes specifically with the chloride secretion stimulated by the toxins of *Vibrio cholera* by reducing the cyclic adenosine monophosphate (cAMP) in the intestinal cells (Czerucka et al. 1994; Czerucka and Rampal 1999). Both *S. cerevisiae* var. *boulardii* and *S. cerevisiae* W303 have the ability to protect Fisher rats against cholera toxin (Brandão et al. 1998). *S. cerevisiae* var. *boulardii* also synthesizes a protein phosphatase that dephosphorylates endotoxins such as lipopolysaccharides of *E. coli* 055B5 and inactivates its cytotoxic effects (Buts et al. 2006).

6.5 Genes and Molecules Involved in Interaction of Probiotics with Host

Probiotic-host interactions that benefit the host can be investigated by genome mining and molecular analysis of the bacterial proteins or macromolecules, which might be involved. Probiotics interact with host and confer beneficial effects by means of different mechanisms including metabolic interactions, modulation of mucosal barrier function and modulation of the innate and adaptive immune system.

6.5.1 Genes and Molecules Involved in Metabolic Interactions

Probiotics interact metabolically with the host by modifying the nutritive function of the epithelium. For example, expression of the *ldh* gene encoding lactate hydrogenase by *Lactobacillus* spp. after entrance to the gastrointestinal tract and

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production of lactate by these bacteria, that later could be converted to butyric acid by the activity of some of the colon bacteria such as *Eubacterium hallii* (Duncan et al. 2004; Marco et al. 2007; Oozeer et al. 2005). The production of butyrate is important due to its beneficial effect on the gut epithelium. Butyrate is an important source of energy for the colonic mucosal cells, and it has been suggested to be essential for the maintenance of the colonic epithelium (Hamer et al. 2008).

Bile salt hydrolase activity and metabolism of bile salts is another positive effect of probiotics on the host positively influencing host physiology due to its role in biological processes in the host, i.e. in serum cholesterol lowering. Bile salts have antimicrobial and emulsification properties and probiotics by metabolizing these compounds might affect the amount of fat absorbed by the body. Bile salt deconjugation is the obligatory first reaction in further oxidation and dehydroxylation steps of bile salts by intestinal bacteria, and it includes the production of secondary bile salts, which negatively affects the host by being involved in formation of gallstones and colon cancer. On the other hand, bile salt deconjugation plays a role in mucin production and excretion in the intestinal lumen, and this could affect the nutritional environment encountered by the intestinal microbiota (Lambert et al. 2008b). Besides, bile acids act as local signalling molecules that regulate innate immunity (Inagaki et al. 2006), while re-absorbed bile salts act as signalling molecules involved in regulation of systemic endocrine functions (Watanabe et al. 2006).

6.5.2 Genes and Molecules Involved in Modulation of Mucosal Barrier Function

Probiotics preserve the barrier function by different mechanisms such as induction of mucin secretion (Mack et al. 2003), up-regulation of cytoprotective heat shock proteins (Petrof et al. 2004; Tao et al. 2006), enhancement of tight-junction functions (Klingberg et al. 2005; Seth et al. 2008) and modulation of epithelial cell apoptosis (Yan et al. 2007). Some of the signalling pathways involved in these mechanisms have been identified; however, the probiotic effector molecules and the genes encoding them are mostly unidentified.

Induction of mucin secretion is one of the mechanisms by which probiotics strengthen the intestinal barrier functionality. This mechanism is dependent on direct adhesion of probiotics to the epithelial cells as it has been shown by losses in the ability of mucin induction followed by spontaneous mutation in the *adh* gene (involved in adhesion) in *L. plantarum* 299v (Mack et al. 2003).

Increase in the level of inflammatory cytokines and apoptosis of intestinal epithelial cell lead to disruption of epithelial integrity. It has been indicated that *L. rhamnosus* GG prevents cytokine-induced apoptosis in human and mouse intestinal epithelial cells by regulating signalling pathways, i.e., by activation of the anti-apoptotic Akt/protein kinase B and inhibition of activation of the pro-apoptotic p38/mitogen-activated protein kinase by tumor necrosis factor-alpha (TNF- α), interleukin-1 alpha (IL-1 α), or gamma-interferon (IFN- γ) (Yan and Polk 2002). Two

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secreted proteins (p75 and p40) have been found in the spent culture of this probiotic bacterium, which activate Akt that has inhibitory effects on cytokine-induced apoptosis and loss of intestinal epithelial cells. Thereby these two proteins promote cell growth in human and mouse colon epithelial cells and cultured mouse colon explants (Yan et al. 2007). Intestinal epithelial tight junction is structured by distribution of different specific proteins such as occludin, zonula occludens (ZO-1, ZO-2, and ZO-3), claudins, E-cadherin, beta-catenin and junctional adhesion molecules (Anderson and VanItallie 1995). Hydrogen peroxide induces the re-distribution of these proteins and cause disruption of tight junctions. Secretory proteins of *L. rhamnosus* GG have been shown to protect intestinal epithelial tight junctions and the barrier function from hydrogen peroxide-induced damages by preserving the distribution of occludin, zonula occludens (ZO-1), E-cadherin, and beta-catenin in the intercellular junctions by a protein kinase C (PKC)- and mitogen-activated protein (MAP) kinase-dependent mechanism (Seth et al. 2008). In addition, an acid and heat stable low-molecular-weight peptide has been found in the spent culture of *L. rhamnosus* GG that induce expression of heat shock proteins (Hsp25 and Hsp72) in intestinal epithelial cells in a time- and concentration-dependent manner (Tao et al. 2006). DNA microarray experiments showed that Hsp72 is one of the genes most highly up-regulated in response to exposure to *L. rhamnosus* GG spent culture. Real-time PCR and electrophoretic mobility shift assays indicated that the *L. rhamnosus* GG spent culture modulates the activity of certain signalling pathways in intestinal epithelial cells by activating MAP kinases. In addition, functional studies suggested that treatment of gut epithelial cells with *L. rhamnosus* GG spent culture protects them from oxidative stress, possibly by preserving cytoskeletal integrity. Inhibition of nuclear factor-kappaB (NF- κ B) and induction of heat shock proteins in colonic epithelial cells through proteasome inhibition has also been observed after exposure of the epithelial cells to spent culture of the probiotic mixture VSL#3 (*L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. longum*, *Bifidobacterium infantis*, *B. breve* and *Streptococcus salivarius* subsp. *thermophilus*) (Petrof et al. 2004). Investigation of individual strains of VSL#3 showed that spent culture of *B. infantis* had the highest effect on increasing the TER compared with spent cultures of other probiotic strains in the mixture. *B. infantis* spent culture decreased claudin-2, and increased ZO-1 and occludin expression in T84 cells, which was mediated by changes in MAP kinases. Besides, *B. infantis* spent culture inhibited reduction of TER induced by TNF- α and IFN- γ and re-distribution of tight junction proteins. In addition, oral administration of spent culture reduced colonic permeability in mice (Ewaschuk et al. 2008). These results may account for the anti-inflammatory and cytoprotective effects reported for probiotics and the mechanism of microbial-epithelial interaction. However, more research is needed to identify the unknown factor(s) in spent culture of various probiotics, which exert the protective effects on intestinal epithelial cells mediated by multiple signalling pathways.

Anti-inflammatory effects and lowering the proinflammatory response has also been shown for *S. cerevisiae* var. *boulardii* upon exposure to enteropathogens (Chen et al. 2006; van der Aa Kühle et al. 2005). Production of products with anti-inflammatory effect has also been shown by *S. cerevisiae* var. *boulardii*. This yeast pro-

duces a soluble factor (<10 kDa) that inhibits the interaction with *C. difficile*-toxin A, and activation of extracellular matrix metalloproteinases in both human colonocytes and *S. cerevisiae* var. *boulardii* products, which blocks NF- κ B activation in intestinal epithelial cells and mono-

6.5.3 Genes and Molecules Involved in Adaptive Immunity

Modulation of the innate and adaptive immunity by probiotics on human health is mostly ascribed to dendritic cell (DC) function (Seth et al. 2005). Numerous studies have shown that probiotics have immunomodulatory effects. However, only a few probiotics have been studied in this context. One of the probiotics studied is *B. longum* NCC 539. This probiotic is a serine protease inhibitor of the serpin family have the inhibition of human neutrophil function.

The ability of probiotic *L. casei* to modulate regulatory T-cell varies among different strains. The C-type lectin receptor Dectin-1 (CD168) is a 3-grabbing nonintegrin, which is involved in the interaction (Smits et al. 2005). It has been shown that SlpA in *L. acidophilus* NCTC 11637 is a ligand for DC-SIGN. The protein SlpA is a ligand that is functionally involved in the interaction (Konstantinov et al. 2008).

Teichoic acids, and especially lipoteichoic acid, are immunostimulatory compounds (Kühn et al. 2001). Transcriptional regulation of the cecum of germ-free mice is affected by cell surface-related functions under growth conditions. Genes involved in the regulation of D-alanyl substitution levels of teichoic acid biosynthesis have been proposed that *L. plantarum* minimize the levels of D-alanine (Kühn et al. 2009). The importance of *Lactobacillus* spp. in their

spends culture of this probiotic effects on cytokine-induced these two proteins promote and cultured mouse colonization is structured by distribution of occludens (ZO-1, ZO-2) and adhesion molecules induces the re-distribution of tight junction proteins of *L. rhamnosus*. In addition, an acid and in the spent culture of *L. rhamnosus* (Hsp25 and Hsp72) in dependent manner (Tao et al. 2002) is one of the genes most identified in the spent culture of *L. rhamnosus* GG. It has been indicated that the *L. rhamnosus* GG signalling pathways in addition, functional studies of *L. rhamnosus* GG spent culture have shown that the re-distribution of cytoskeletal integrity, and of heat shock proteins in addition has also been observed after the spent culture of probiotic mixture VSL#3 (*L. p. bulgaricus*, *B. longum*, *L. acidophilus* subsp. *thermophilus*). Proteins of VSL#3 showed that the re-distribution of tight junctions easing the TER compared with the control culture. *B. infantis* spent culture also showed an increase in expression in T84 cells, and *B. infantis* spent culture also showed an increase in expression and re-distribution of tight junctions in culture reduced colonic inflammation. This may account for the anti-inflammatory effects of probiotics and the mechanism which is needed to identify the proteins which exert the protective signalling pathways. Inflammatory response has also been observed to enteropathogens (Chen et al. 2006). Products with anti-inflammatory effects of *S. boulardii*. This yeast pro-

6.5.3 Genes and Molecules Involved in Modulation of the Innate and Adaptive Immune System

Modulation of the innate and adaptive immune system is another beneficial effect of probiotics on human health. Modulation of the immune system by *Lactobacillus* spp. is mostly ascribed to priming immunoregulatory responses via modulation of dendritic cell (DC) function and induction of regulatory T cells (Rook and Brunet 2005). Numerous studies have shown the immunomodulating effects of probiotics. However, only a few probiotic effector molecules have been identified in this context. One of the probiotic effector molecules which has been identified based on genomics of *B. longum* NCC2705 is a gene encoding a homologue of the eukaryotic-type serine protease inhibitor (serpin) (Schell et al. 2002). Some of the members of the serpin family have the capacity to suppress inflammatory responses through inhibition of human neutrophil elastase (Ivanov et al. 2006).

The ability of probiotic *Lactobacillus* spp. to prime DCs to drive development of regulatory T-cell varies among different species and depends on their recognition of the C-type lectin receptor DC-SIGN (DC-specific intercellular adhesion molecule 3-grabbing nonintegrin), which has an important role in regulatory T-cell stimulation (Smits et al. 2005). It has been shown that mutation in a surface layer protein (SlpA) in *L. acidophilus* NCFM resulted in significant reduction in binding capacity to DC-SIGN. The protein SlpA is the first identified probiotic bacterial DC-SIGN ligand that is functionally involved in the modulation of DC and T cell functions (Konstantinov et al. 2008).

Teichoic acids, and especially lipoteichoic acids (LTA), are one of the major immunostimulatory components of pathogenic Gram-positive bacteria (Morath et al. 2001). Transcriptional profiling of *L. plantarum* WCFS1 during colonization of the cecum of germ-free mice have shown that a set of bacterial genes encoding cell surface-related functions were differentially regulated compared with *in vitro* growth conditions. Genes encoding the biosynthetic pathway responsible for addition of D-alanyl substituent to LTA were down regulated, while the expression levels of teichoic acid biosynthetic genes remained virtually unchanged and it has been proposed that *L. plantarum* WCFS1 modifies its gene expression *in vivo* and minimize the levels of D-alanylated LTA present on the cell surface (Marco et al. 2009). The importance of teichoic acid composition on the cell wall of probiotic *Lactobacillus* spp. in their immunomodulatory effect has been studied using a mu-

tant of *L. plantarum* NCIMB8826 (*dlt*) which was modified in the teichoic acid biosynthesis pathway and presented much less D-Ala in its teichoic acids compared to the wild type strain. This mutation positively influenced the Toll-like receptor 2 (TLR-2)-dependent immunomodulatory properties of *L. plantarum* NCIMB8826 (Grangette et al. 2005). A considerably lower secretion of proinflammatory cytokines by peripheral blood mononuclear cells and monocytes in parallel with a significant increase in IL-10 production was observed after stimulation with the mutant as compared to the parental strain. In addition, the mutant was significantly more protective in a murine colitis model compared to its wild type counterpart. These studies demonstrated that composition of teichoic acids has a great impact on the immunomodulatory effect of *L. plantarum* and this probiotic modifies its gene expression *in vivo* in a way that will increase its immunomodulatory effect. However, it should be kept in mind that *dlt* mutants of other strains of *Lactobacillus* spp. have shown other behaviour. As an example *dlt* mutations in *L. rhamnosus* GG resulted in unaltered immunomodulation and the mutant showed lower survival under intestinal conditions (Velez et al. 2007). Similar results were found for a *L. reuteri* 100-23 *dlt* mutant (Walter et al. 2007). These studies confirm that the immunomodulatory effects of probiotic *Lactobacillus* spp. are strongly species and even strain dependent.

Investigation of the immunomodulatory effect of probiotic preparation VSL#3 has shown that VSL#3 is a strong inducer of IL-10 by DCs from blood and intestinal tissue, and prevents generation of Th1 cells. However, individual strains within VSL#3 presented different immunomodulatory effects on DCs and bifidobacteria strains (*B. longum*, *B. infantis* and *B. breve*) offered the highest anti-inflammatory effects. Interaction of cell wall components of these *Bifidobacterium* spp. with human intestinal lamina propria mononuclear cells, whole blood, or an enriched blood dendritic cell population showed that *Bifidobacterium* spp. up-regulate IL-10 production by DCs and decrease IFN- γ production by T cells (Hart et al. 2004). In a similar study using a murine macrophage-like cell line, *B. adolescentis* and *B. longum* induced higher secretion of a proinflammatory cytokine IL-12 and TNF- α , compared to *B. bifidum*, *B. breve*, and *B. infantis*, whereas *B. adolescentis* did not stimulate the production of anti-inflammatory IL-10 as the other tested bacteria, showing that this bacterium is less capable of down-regulating the inflammatory response in macrophage-like cell line (He et al. 2002). However, the signalling pathways and genes involved in these immunomodulatory effects have not yet been identified.

6.6 Conclusion and Future Perspectives

Recent functional genomic analyses and molecular studies have identified some of the genes and molecules offering the health benefits of probiotics. These studies have proved the role of both stress response genes and genes involved in adaptation to new ecological environments being important for the functionality of the probiot-

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modified in the teichoic acid in its teichoic acids compared to the Toll-like receptor 2 of *L. plantarum* NCIMB8826. The production of proinflammatory cytokines in parallel with a decrease in monocytes after stimulation with the mutant was significantly different from its wild type counterpart. Teichoic acids have a great impact on this probiotic modifies its immunomodulatory effect. Other strains of *Lactobacillus* with mutations in *L. rhamnosus* mutant showed lower survival. These results were found for a *L.* studies confirm that the immunomodulatory effects are strongly species and

probiotic preparation VSL#3. DCs from blood and intestinal tissue, individual strains within the gut on DCs and bifidobacteria have the highest anti-inflammatory effect. *Bifidobacterium* spp. with human blood, or an enriched blood culture of *B.* spp. up-regulate IL-10 production in dendritic cells (Hart et al. 2004). In mice, *B. adolescentis* and *B. longum* induce cytokine IL-12 and TNF- α , whereas *B. adolescentis* did not induce TNF- α as the other tested bacteria, thus modulating the inflammatory response. However, the signalling pathways and effects have not yet been

identified. Some of these studies have identified some of the mechanisms involved in adaptation and functionality of the probiotic

agents. In addition, different genes have been found to be involved in adhesion of probiotics to intestinal epithelial cells and production of antimicrobial products, which might be crucial for competitive exclusion and interaction with enteropathogens. More specifically, different genes have been recognized to be involved in mucin secretion, in regulation of the different signalling pathways resulting in anti-inflammatory effects, and in strengthening the epithelial tight junctions, which have protective effects on intestinal epithelial functionality. However, only a limited number of genes have been identified in this regard and additional studies are necessary to uncover all genes involved and to clarify the specific mechanisms at the molecular level. Several studies have detected some genes that are involved in modulating the immune response toward development of T-regulatory cells, but the exact mechanisms and genes are still missing. Altogether, genomics and molecular studies have been proved to be useful to unravel the genes and molecules involved in probiotic functionality and to recognize the regions in the genome that might be interesting for further investigation to identify the exact mechanisms involved in the beneficial effects of probiotics on human health. In this context, effective *in vitro* and *in vivo* models combined with omic approaches and assisted by mathematical models that help exploiting the complex information obtained will facilitate identification of the precise mechanisms by which probiotic microorganisms confer their health benefits. However, considering the biodiversity of these microorganisms in the gut microbiota and the fact that their mode of action is species and even strain dependent, we are still in the beginning of this field of research. Therefore, it is crucial to understand the human gut microbiome to get a comprehensive view of the genetic diversity of the gut residents and the variations in their molecular characteristics and host interactions. This will allow us to find more efficient probiotics and to develop new functional food products and therapeutic agents with improved quality.

References

- Altermann E, Russell WM, Azcarate-Peril MA, Barrangou R, Buck BL, McAuliffe O, Southern N, Dobson A, Duong T, Callanan M, Lick S, Hamrick A, Cano R, Klaenhammer TR (2005) Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. *Proc Natl Acad Sci U S A* 102(11):3906–3912
- Anderson JM, VanItallie CM (1995) Tight junctions and the molecular-basis for regulation of paracellular permeability. *Am J Physiol-Gastrointest Liver Physiol* 32(4):G467–G475
- Åvall-Jääskeläinen S, Palva A (2005) *Lactobacillus* surface layers and their applications. *FEMS Microbiol Rev* 29(3):511–529
- Azcarate-Peril MA, Altermann E, Hoover-Fitzula RL, Cano RJ, Klaenhammer TR (2004) Identification and inactivation of genetic loci involved with *Lactobacillus acidophilus* acid tolerance. *Appl Environ Microbiol* 70(9):5315–5322
- Azcarate-Peril MA, McAuliffe O, Altermann E, Lick S, Russell WM, Klaenhammer TR (2005) Microarray analysis of a two-component regulatory system involved in acid resistance and proteolytic activity in *Lactobacillus acidophilus*. *Appl Environ Microbiol* 71(10):5794–5804
- Barrangou R, Altermann E, Hutkins R, Cano R, Klaenhammer TR (2003) Functional and comparative genomic analyses of an operon involved in fructooligosaccharide utilization by *Lactobacillus acidophilus*. *Proc Natl Acad Sci U S A* 100(15):8957–8962

- Boekhorst J, Wels M, Kleerebezem M, Siezen RJ (2006) The predicted secretome of *Lactobacillus plantarum* WCFS1 sheds light on interactions with its environment. *Microbiology* 152:3175–3183
- Brandão RL, Castro IM, Bambirra EA, Amaral SC, Fietto LG, Tropia MJ, Neves MJ, Dos Santos RG, Gomes NC, Nicoli JR (1998) Intracellular signal triggered by cholera toxin in *Saccharomyces boulardii* and *Saccharomyces cerevisiae*. *Appl Environ Microbiol* 64(2):564–568
- Bron PA, Grangette C, Mercenier A, de Vos WM, Kleerebezem M (2004) Identification of *Lactobacillus plantarum* genes that are induced in the gastrointestinal tract of mice. *J Bacteriol* 186(17):5721–5729
- Bron PA, Molenaar D, Vos WM, Kleerebezem M (2006) DNA micro-array-based identification of bile-responsive genes in *Lactobacillus plantarum*. *J Appl Microbiol* 100(4):728–738
- Buck BL, Altermann E, Svingerud T, Klaenhammer TR (2005) Functional analysis of putative adhesion factors in *Lactobacillus acidophilus* NCFM. *Appl Environ Microbiol* 71(12):8344–8351
- Buts JP, Dekeyser N, Stilmant C, Delem E, Smets F, Sokal E (2006) *Saccharomyces boulardii* produces in rat small intestine a novel protein phosphatase that inhibits *Escherichia coli* endotoxin by dephosphorylation. *Pediatr Res* 60(1):24–29
- Callanan M, Kaleta P, O'Callaghan J, O'Sullivan O, Jordan K, McAuliffe O, Sangrador-Vegas A, Slattery L, Fitzgerald GF, Beresford T, Ross RP (2008) Genome sequence of *Lactobacillus helveticus*, an organism distinguished by selective gene loss and insertion sequence element expansion. *J Bacteriol* 190(2):727–735
- Castagliuolo I, LaMont JT, Nikulasson ST, Pothoulakis C (1996) *Saccharomyces boulardii* protease inhibits *Clostridium difficile* toxin A effects in the rat ileum. *Infect Immun* 64(12):5225–5232
- Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C (1999) *Saccharomyces boulardii* protease inhibits the effects of *Clostridium difficile* toxins A and B in human colonic mucosa. *Infect Immun* 67(1):302–307
- Chen X, Kokkotou EG, Mustafa N, Bhaskar KR, Sougioultzis S, O'Brien M, Pothoulakis C, Kelly CP (2006) *Saccharomyces boulardii* inhibits ERK1/2 mitogen-activated protein kinase activation both *in vitro* and *in vivo* and protects against *Clostridium difficile* toxin A-induced enteritis. *J Biol Chem* 281(34):24449–24454
- Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CGM (2007) Bacteriocin production as a mechanism for the antif Infective activity of *Lactobacillus salivarius* UCC118. *Proc Natl Acad Sci U S A* 104(18):7617–7621
- Cotter PD, Hill C, Ross RP (2005) Bacteriocins: developing innate immunity for food. *Nat Rev Microbiol* 3(10):777–788
- Czerucka D, Rampal P (1999) Effect of *Saccharomyces boulardii* on cAMP- and Ca²⁺-dependent Cl⁻ secretion in T84 cells. *Dig Dis Sci* 44(11):2359–2368
- Czerucka D, Roux I, Rampal P (1994) *Saccharomyces boulardii* inhibits secretagogue-mediated adenosine 3',5'-cyclic monophosphate induction in intestinal cells. *Gastroenterology* 106(1):65–72
- De Keersmaecker SCJ, Verhoeven TLA, Desair J, Marchal K, Vanderleyden J, Nagy I (2006) Strong antimicrobial activity of *Lactobacillus rhamnosus* GG against *Salmonella typhimurium* is due to accumulation of lactic acid. *FEMS Microbiol Lett* 259(1):89–96
- Denou E, Berger B, Barretto C, Panoff JM, Arigoni F, Brussow H (2007) Gene expression of commensal *Lactobacillus johnsonii* strain NCC533 during *in vitro* growth and in the murine gut. *J Bacteriol* 189(22):8109–8119
- Denou E, Pridmore RD, Berger B, Panoff JM, Arigoni F, Brussow H (2008) Identification of genes associated with the long-gut-persistence phenotype of the probiotic *Lactobacillus johnsonii* strain NCC533 using a combination of genomics and transcriptome analysis. *J Bacteriol* 190(9):3161–3168
- Duncan SH, Louis P, Flint HJ (2004) Lactate-utilizing bacteria, isolated from human faeces, that produce butyrate as a major fermentation product. *Appl Environ Microbiol* 70(10):5810–5817

Elkins CA, Moslehi-Jenabian S (2008) Salt transport in the gut. *Physiol* 147:3

Ewaschuk JB, Jellison JA, Kluge AG (2008) The role of the gut microbiota in host health. *Microbiol* 154:1299

Ferreira A, O'Brien M (2008) Stress resistance in the gut. *Microbiol* 154:1299

Ferreira A, Su (2008) Survival of the fittest. *Microbiol* 154:1299

Frees D, Vogen (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Fujiwara S, Hatakeyama S (2008) Fluids of the gut. *Microbiol* 154:1299

Gedek BR (1998) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Gonzalez R, K (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Grangette C, P (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Gross G, van (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Guarner F, Ma (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Gueimonde M (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Hamer HM, J (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Hart AL, Lan (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Knight SC (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

He F, Morita (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Iminen S (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Inagaki T, Mc (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

JA, Repa (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Ivanov D, En (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Arigoni F (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Kankainen M (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Vesterlund (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

T, Laukka (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

Hatakka K (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

T, Auvine (2008) *Escherichia coli* O157:H7. *Microbiol* 154:1299

- predicted secretome of *Lactobacillus* environment. Microbiology 152:3175–3185
- i, Tropia MJ, Neves MJ, Dos Santos J (2004) Identification of *Lactobacillus* strains that inhibit growth of *Escherichia coli* O157 in the small intestine of mice. J Bacteriol 186:1511–1517
- micro-array-based identification of *Lactobacillus* strains. Microbiol 100(4):728–738
- 05) Functional analysis of putative *Lactobacillus* strains. Appl Environ Microbiol 71(12):8344–8349
- 2006) *Saccharomyces boulardii* protease inhibits *Escherichia coli* endotoxin activity. J Infect Dis 193:1005–1010
- K, McAuliffe O, Sangrador-Vegas A (2004) Genome sequence of *Lactobacillus* strains and insertion sequence element analysis. J Bacteriol 186:1511–1517
- 96) *Saccharomyces boulardii* protease inhibits *Escherichia coli* endotoxin activity. Infect Immun 64(12):5225–5230
- akis C (1999) *Saccharomyces boulardii* toxins A and B in human colonic mucosa. J Infect Dis 179:1005–1010
- S, O'Brien M, Pothoulakis C, Kelly J (2004) Gen-activated protein kinase activation of *Escherichia coli* toxin A-induced enteritis. J Infect Dis 189:1005–1010
- (2007) Bacteriocin production as a virulence factor of *UCC118*. Proc Natl Acad Sci U S A 104:1005–1010
- innate immunity for food. Nat Rev Microbiol 5:1005–1010
- dii* on cAMP- and Ca²⁺-dependent signaling. Gastroenterology 126:1005–1010
- lardii* inhibits secretagogue-mediated intestinal cells. Gastroenterology 126:1005–1010
- K, Vanderleyden J, Nagy I (2006) G-protein-coupled receptor signaling against *Salmonella typhimurium*. J Infect Dis 193:1005–1010
- H (2007) Gene expression of commensal bacteria in the murine gut. J Infect Dis 193:1005–1010
- ussow H (2008) Identification of the probiotic *Lactobacillus johnsonii* transcriptome analysis. J Bacteriol 186:1511–1517
- is isolated from human faeces, that inhibits *Escherichia coli* O157. J Bacteriol 186:1511–1517
- Elkins CA, Moser SA, Savage DC (2001) Genes encoding bile salt hydrolases and conjugated bile salt transporters in *Lactobacillus johnsonii* 100-100 and other *Lactobacillus* species. Microbiology 147:3403–3412
- Ewaschuk JB, Diaz H, Meddings L, Diederichs B, Dmytrash A, Backer J, Langen MLV, Madsen KL (2008) Secreted bioactive factors from *Bifidobacterium infantis* enhance epithelial cell barrier function. Am J Physiol-Gastrointest Liver Physiol 295(5):G1025–G1034
- Federle MJ, Bassler BL (2003) Interspecies communication in bacteria. J Clin Invest 112(9):1291–1299
- Ferreira A, O'Byrne CP, Boor KJ (2001) Role of sigma(B) in heat, ethanol, acid, and oxidative stress resistance and during carbon starvation in *Listeria monocytogenes*. Appl Environ Microbiol 67(10):4454–4457
- Ferreira A, Sue D, O'Byrne CP, Boor KJ (2003) Role of *Listeria monocytogenes* sigma(B) in survival of lethal acidic conditions and in the acquired acid tolerance response. Appl Environ Microbiol 69(5):2692–2698
- Frees D, Vogensen FK, Ingmer H (2003) Identification of proteins induced at low pH in *Lactococcus lactis*. Int J Food Microbiol 87(3):293–300
- Fujiwara S, Hashiba H, Hirota T, Forstner JF (1997) Proteinaceous factor(s) in culture supernatant fluids of bifidobacteria which prevents the binding of enterotoxigenic *Escherichia coli* to ganglioside GM1. Appl Environ Microbiol 63(2):506–512
- Gedek BR (1999) Adherence of *Escherichia coli* serogroup O 157 and the *Salmonella* Typhimurium mutant DT 104 to the surface of *Saccharomyces boulardii*. Mycoses 42(4):261–264
- Gonzalez R, Klaassens ES, Malinen E, de Vos WM, Vaughan EE (2008) Differential transcriptional response of *Bifidobacterium longum* to human milk, formula milk, and galactooligosaccharide. Appl Environ Microbiol 74(15):4686–4694
- Grangette C, Nutten S, Palumbo E, Morath S, Hermann C, Dewulf J, Pot B, Hartung T, Hols P, Mercenier A (2005) Enhanced anti-inflammatory capacity of a *Lactobacillus plantarum* mutant synthesizing modified teichoic acids. Proc Natl Acad Sci U S A 102(29):10321–10326
- Gross G, van der Meulen J, Snel J, van der Meer R, Kleerebezem M, Niewold TA, Hulst MM, Smits MA (2008) Mannose-specific interaction of *Lactobacillus plantarum* with porcine jejunal epithelium. FEMS Immunol Med Microbiol 54(2):215–223
- Guarner F, Malagelada JR (2003) Gut flora in health and disease. Lancet 361(9356):512–519
- Gueimonde M, Garrigues C, van Sinderen D, de los Reyes-Gavilan CG, Margolles A (2009) Bile-inducible efflux transporter from *Bifidobacterium longum* NCC(2705) conferring bile resistance. Appl Environ Microbiol 75(10):3153–3160
- Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer RJ (2008) Review article: the role of butyrate on colonic function. Aliment Pharmacol Thera 27(2):104–119
- Hart AL, Lammers K, Brigidi P, Vitali B, Rizzello F, Gionchetti P, Campieri M, Kamm MA, Knight SC, Stagg AJ (2004) Modulation of human dendritic cell phenotype and function by probiotic bacteria. Gut 53(11):1602–1609
- He F, Morita H, Ouwehand AC, Hosoda M, Hiramatsu M, Kurisaki J, Isolauri E, Benno Y, Salminen S (2002) Stimulation of the secretion of pro-inflammatory cytokines by *Bifidobacterium* strains. Microbiol Immunol 46(11):781–785
- Inagaki T, Moschetta A, Lee YK, Peng L, Zhao GX, Downes M, Yu RT, Shelton JM, Richardson JA, Repa JJ, Mangelsdorf DJ, Kliewer SA (2006) Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc Natl Acad Sci U S A 103(10):3920–3925
- Ivanov D, Emonet C, Foata F, Affolter M, Delley M, Fisseha M, Blum-Sperisen S, Kochhar S, Arigoni F (2006) A serpin from the gut bacterium *Bifidobacterium longum* inhibits eukaryotic elastase-like serine proteases. J Biol Chem 281(25):17246–17252
- Kankainen M, Paulin L, Tynkkynen S, von Ossowski I, Reunanen J, Partanen P, Satokari R, Vesterlund S, Hendrickx APA, Lebeer S, De Keersmaecker SCJ, Vanderleyden J, Hamalainen T, Laukkanen S, Salovuori N, Ritari J, Alatalo E, Korpela R, Mattila-Sandholm T, Lassig A, Hatakka K, Kinnunen KT, Karjalainen H, Saxelin M, Laakso K, Surakka A, Palva A, Salusjarvi T, Auvinen P, de Vos WM (2009) Comparative genomic analysis of *Lactobacillus rhamnosus* strains. J Bacteriol 186:1511–1517

- sus* GG reveals pili containing a human-mucus binding protein. *Proc Natl Acad Sci U S A* 106(40):17193–17198
- Kim GB, Lee BH (2008) Genetic analysis of a bile salt hydrolase in *Bifidobacterium animalis* subsp *lactis* KL612. *J Appl Microbiol* 105(3):778–790
- Kim GB, Miyamoto CM, Meighen EA, Lee BH (2004) Cloning and characterization of the bile salt hydrolase genes (*bsh*) from *Bifidobacterium bifidum* strains. *Appl Environ Microbiol* 70(9):5603–5612
- Kim GB, Brochet M, Lee BH (2005) Cloning and characterization of a bile salt hydrolase (*bsh*) from *Bifidobacterium adolescentis*. *Biotechnol Lett* 27(12):817–822
- Klaassens ES, Boesten RJ, Haarman M, Knol J, Schuren FH, Vaughan EE, de Vos WM (2009) Mixed-species genomic microarray analysis of faecal samples reveals differential transcriptional responses of *Bifidobacteria* in breast- and formula-fed infants. *Appl Environ Microbiol* 75(9):2668–2676
- Klaenhammer T, Altermann E, Arigoni F, Bolotin A, Breidt F, Broadbent J, Cano R, Chaillou S, Deutscher J, Gasson M, van de GM, Guzzo J, Hartke A, Hawkins T, Hols P, Hutkins R, Kleerebezem M, Kok J, Kuipers O, Lubbers M, Maguin E, McKay L, Mills D, Nauta A, Overbeek R, Pel H, Pridmore D, Saier M, van SD, Sorokin A, Steele J, O'Sullivan D, de VW, Weimer B, Zagorec M, Siezen R (2002) Discovering lactic acid bacteria by genomics. *Antonie Van Leeuwenhoek* 82(1–4):29–58
- Klingberg TD, Pedersen MH, Cencic A, Budde BB (2005) Application of measurements of transepithelial electrical resistance of intestinal epithelial cell monolayers to evaluate probiotic activity. *Appl Environ Microbiol* 71(11):7528–7530
- Konstantinov SR, Smidt H, de Vos WM, Bruijns SCM, Singh SK, Valence F, Molle D, Lortal S, Altermann E, Klaenhammer TR, van Kooyk Y (2008) S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. *Proc Natl Acad Sci U S A* 105(49):19474–19479
- Kot E, Bezkorovainy A (1993) Effects of Mg^{2+} and Ca^{2+} on Fe^{2+} uptake by *Bifidobacterium thermophilum*. *Int J Biochem* 25(7):1029–1033
- Kullen MJ, Klaenhammer TR (1999) Identification of the pH-inducible, proton-translocating F_1F_0 -ATPase (*atpBEFHAGDC*) operon of *Lactobacillus acidophilus* by differential display: gene structure, cloning and characterization. *Mol Microbiol* 33(6):1152–1161
- Lambert JM, Bongers RS, de Vos WM, Kleerebezem M (2008a) Functional analysis of four bile salt hydrolase and penicillin acylase family members in *Lactobacillus plantarum* WCFS1. *Appl Environ Microbiol* 74(15):4719–4726
- Lambert JM, Siezen RJ, de Vos WM, Kleerebezem M (2008b) Improved annotation of conjugated bile acid hydrolase superfamily members in Gram-positive bacteria. *Microbiology* 154:2492–2500
- Larsen N, Vogensen FK, van den Berg FWJ, Nielsen DS, Andreasen AS, Pedersen BK, bu Al-Soud W, Sorensen SJ, Hansen LH, Jakobsen M (2010) Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *Plos One* 5(2):e9085
- Lim EM, Ehrlich SD, Maguin E (2000) Identification of stress-inducible proteins in *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Electrophoresis* 21(12):2557–2561
- Lomax AR, Calder PC (2009) Probiotics, immune function, infection and inflammation: a review of the evidence from studies conducted in humans. *Curr Pharm Des* 15(13):1428–1518
- Lorca GL, De Valdez GF, Ljungh A (2002) Characterization of the protein-synthesis dependent adaptive acid tolerance response in *Lactobacillus acidophilus*. *J Mol Microbiol Biotechnol* 4(6):525–532
- Mack DR, Ahrne S, Hyde L, Wei S, Hollingsworth MA (2003) Extracellular MUC3 mucin secretion follows adherence of *Lactobacillus* strains to intestinal epithelial cells *in vitro*. *Gut* 52(6):827–833
- Mahan MJ, Heithoff DM, Sinsheimer RL, Low DA (2000) Assessment of bacterial pathogenesis by analysis of gene expression in the host. *Ann Rev Genet* 34:139–164
- Marco ML, Bongers RS, de Vos WM, Kleerebezem M (2007) Spatial and temporal expression of *Lactobacillus plantarum* genes in the gastrointestinal tracts of mice. *Appl Environ Microbiol* 73(1):124–132

Marco ML, Pe
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ties in *Lact*
Morath S, Gey
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Oozeer R, Fure
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15(12):546
Pothoulakis C,
charomyces
ileum. *Gast*
Pretzer G, Snel
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of the man
Pridmore RD,
Altermann
(2004) The
NCC 533. I
Pridmore RD,
tobacillus j
283(2):210
Rook GAW, Bi
Ryan SM, Fitz
of a novel b
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lis is involv
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99(22):144
Seth A, Yan F,
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Smits HH, Enj
Yazdanbaki

- protein. *Proc Natl Acad Sci U S A*
- rolase in *Bifidobacterium animalis*
- ing and characterization of the bile
n strains. *Appl Environ Microbiol*
- zation of a bile salt hydrolase (bsh)
)817–822
- I, Vaughan EE, de Vos WM (2009)
mples reveals differential transcrip-
fed infants. *Appl Environ Microbiol*
- F, Broadbent J, Cano R, Chaillou S,
awkins T, Hols P, Hutkins R, Kleere-
Kay L, Mills D, Nauta A, Overbeek
le J, O'Sullivan D, de VW, Weimer
bacteria by genomics. *Antonie Van*
- pplication of measurements of tran-
ll monolayers to evaluate probiotic
- gh SK, Valence F, Molle D, Lortal
) S layer protein A of *Lactobacillus*
T cell functions. *Proc Natl Acad Sci*
- Fe²⁺ uptake by *Bifidobacterium ther-*
- pH-inducible, proton-translocating
acidophilus by differential display:
ol 33(6):1152–1161
- 08a) Functional analysis of four bile
1 *Lactobacillus plantarum* WCFS1.
- nproved annotation of conjugated bile
eria. *Microbiology* 154:2492–2500
- reasen AS, Pedersen BK, bu Al-Soud
robiota in human adults with type 2
9085
- s-inducible proteins in *Lactobacillus*
57–2561
- nfection and inflammation: a review
harm Des 15(13):1428–1518
- 1 of the protein-synthesis dependent
philus. *J Mol Microbiol Biotechnol*
- 003) Extracellular MUC3 mucin se-
testinal epithelial cells *in vitro*. *Gut*
- Assessment of bacterial pathogenesis
t 34:139–164
-) Spatial and temporal expression of
cts of mice. *Appl Environ Microbiol*
- Marco ML, Peters THF, Bongers RS, Molenaar D, van Hemert S, Sonnenburg JL, Gordon JJ, Kleerebezem M (2009) Lifestyle of *Lactobacillus plantarum* in the mouse caecum. *Environ Microbiol* 11(10):2747–2757
- McAuliffe O, Cano RJ, Klaenhammer TR (2005) Genetic analysis of two bile salt hydrolase activities in *Lactobacillus acidophilus* NCFM. *Appl Environ Microbiol* 71(8):4925–4929
- Morath S, Geyer A, Hartung T (2001) Structure-function relationship of cytokine induction by lipoteichoic acid from *Staphylococcus aureus*. *J Exp Med* 193(3):393–397
- Moslehi-Jenabian S, Gori K, Jespersen L (2009) AI-2 signalling is induced by acidic shock in probiotic strains of *Lactobacillus* spp. *Int J Food Microbiol* 135(3):295–302
- Moslehi-Jenabian S, Lindegaard L, Jespersen L (2010) Beneficial effects of probiotic and food borne yeasts on human health. *Nutrients* 2(4):449–473
- Mukai T, Toba T, Ohori H (1997) Collagen binding of *Bifidobacterium adolescentis*. *Curr Microbiol* 34(5):326–331
- Oozeer R, Furet JP, Goupil-Feuillerat N, Anba J, Mengaud J, Corthier G (2005) Differential activities of four *Lactobacillus casei* promoters during bacterial transit through the gastrointestinal tracts of human-microbiota-associated mice. *Appl Environ Microbiol* 71(3):1356–1363
- Petrof EO, Kojima K, Ropeleski MJ, Musch MW, Tao Y, De Simone C, Chang EB (2004) Probiotics inhibit nuclear factor-kappa B and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. *Gastroenterology* 127(5):1474–1487
- Pfeiler EA, Klaenhammer TR (2007) The genomics of lactic acid bacteria. *Trends Microbiol* 15(12):546–553
- Pothoulakis C, Kelly CP, Joshi MA, Gao N, O'Keane CJ, Castagliuolo I, LaMont JT (1993) *Saccharomyces boulardii* inhibits *Clostridium difficile* toxin A binding and enterotoxicity in rat ileum. *Gastroenterology* 104(4):1108–1115
- Pretzer G, Snel J, Molenaar D, Wiersma A, Bron PA, Lambert J, de Vos WM, van der Meer R, Smits MA, Kleerebezem M (2005) Biodiversity-based identification and functional characterization of the mannose-specific adhesin of *Lactobacillus plantarum*. *J Bacteriol* 187(17):6128–6136
- Pridmore RD, Berger B, Desiere F, Vilanova D, Barretto C, Pittet AC, Zwahlen MC, Rouvet M, Altermann E, Barrangou R, Mollet B, Mercenier A, Klaenhammer T, Arigoni F, Schell MA (2004) The genome sequence of the probiotic intestinal bacterium *Lactobacillus johnsonii* NCC 533. *Proc Natl Acad Sci U S A* 101(8):2512–2517
- Pridmore RD, Pittet AC, Praplan F, Cavadini C (2008) Hydrogen peroxide production by *Lactobacillus johnsonii* NCC 533 and its role in anti-*Salmonella* activity. *FEMS Microbiol Lett* 283(2):210–215
- Rook GAW, Brunet LR (2005) Microbes, immunoregulation, and the gut. *Gut* 54(3):317–320
- Ryan SM, Fitzgerald GF, van Sinderen D (2005) Transcriptional regulation and characterization of a novel beta-fructofuranosidase-encoding gene from *Bifidobacterium breve* UCC2003. *Appl Environ Microbiol* 71(7):3475–3482
- Sanchez B, Reyes-Gavilan CGD, Margolles A (2006) The F₁F₀-ATPase of *Bifidobacterium animalis* is involved in bile tolerance. *Environ Microbiol* 8(10):1825–1833
- Saulnier DAA, Molenaar D, de Vos WA, Gibson GR, Kolida S (2007) Identification of prebiotic fructooligosaccharide metabolism in *Lactobacillus plantarum* WCFS1 through microarrays. *Appl Environ Microbiol* 73(6):1753–1765
- Schell MA, Karmirantzou M, Snel B, Vilanova D, Berger B, Pessi G, Zwahlen MC, Desiere F, Bork P, Delley M, Pridmore RD, Arigoni F (2002) The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proc Natl Acad Sci U S A* 99(22):14422–14427
- Seth A, Yan F, Polk DB, Rao RK (2008) Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol* 294(4):G1060–G1069
- Shuhaimi M, Ali AM, Saleh NM, Yazid AM (2001) Cloning and sequence analysis of bile salt hydrolase (bsh) gene from *Bifidobacterium longum*. *Biotechnol Lett* 23(21):1775–1780
- Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, van Capel TMM, Zaat BAJ, Yazdanbakhsh M, Wierenga EA, van Kooyk Y, Kapsenberg ML (2005) Selective probiotic

- bacteria induce IL-10-producing regulatory T cells *in vitro* by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J Allergy Clin Immunol* 115(6):1260–1267
- Sonnenburg JL, Chen CTL, Gordon JI (2006) Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. *Plos Biol* 4(12):2213–2226
- Sougioultzis S, Simeonidis S, Bhaskar KR, Chen XH, Anton PM, Keates S, Pothoulakis C, Kelly CP (2006) *Saccharomyces boulardii* produces a soluble anti-inflammatory factor that inhibits NF-kappa B-mediated IL-8 gene expression. *Biochem Biophys Res Commun* 343(1):69–76
- Tao Y, Drabik KA, Waypa TS, Musch MW, Alverdy JC, Schneewind O, Chang EB, Petrof EO (2006) Soluble factors from *Lactobacillus* GG activate MAPKs and induce cytoprotective heat shock proteins in intestinal epithelial cells. *Am J Physiol-Cell Physiol* 290(4):C1018–C1030
- Todorov SD, Dicks LMT (2004) Effect of medium components on bacteriocin production by *Lactobacillus pentosus* ST151BR, a strain isolated from beer produced by the fermentation of maize, barley and soy flour. *World J Microbiol Biotechnol* 20(6):643–650
- van der Aa Kühle A, Jespersen L (2003) The taxonomic position of *Saccharomyces boulardii* as evaluated by sequence analysis of the D1/D2 domain of 26S rDNA, the ITS1-5.8S rDNA-ITS2 region and the mitochondrial cytochrome-c oxidase II gene. *Syst Appl Microbiol* 26(4):564–571
- van der Aa Kühle A, Skovgaard K, Jespersen L (2005) *In vitro* screening of probiotic properties of *Saccharomyces cerevisiae* var. *boulardii* and food-borne *Saccharomyces cerevisiae* strains. *Int J Food Microbiol* 101(1):29–39
- van Pijkeren JP, Canchaya C, Ryan KA, Li Y, Claesson MJ, Sheil B, Steidler L, O'Mahony L, Fitzgerald GF, van Sinderen D, O'Toole PW (2006) Comparative and functional analysis of sortase-dependent proteins in the predicted secretome of *Lactobacillus salivarius* UCC118. *Appl Environ Microbiol* 72(6):4143–4153
- Velez MP, Verhoeven TLA, Draing C, Von Aulock S, Pfitzenmaier M, Geyer A, Lambrichts I, Grangette C, Pot B, Vanderleyden J, De Keersmaecker SCJ (2007) Functional analysis of D-alanylation of lipoteichoic acid in the probiotic strain *Lactobacillus rhamnosus* GG. *Appl Environ Microbiol* 73(11):3595–3604
- Ventura M, Canchaya C, van Sinderen D, Fitzgerald GF, Zink R (2004) *Bifidobacterium lactis* DSM 10140: identification of the *atp* (*atpBEFHAGDC*) operon and analysis of its genetic structure, characteristics, and phylogeny. *Appl Environ Microbiol* 70(5):3110–3121
- Ventura M, Canchaya C, Fitzgerald GF, Gupta RS, van Sinderen D (2007a) Genomics as a means to understand bacterial phylogeny and ecological adaptation: the case of bifidobacteria. *Antonie Van Leeuwenhoek* 91(4):351–372
- Ventura M, Canchaya C, Tauch A, Chandra G, Fitzgerald GF, Chater KF, van Sinderen D (2007b) Genomics of *actinobacteria*: tracing the evolutionary history of an ancient phylum. *Microbiol Mol Biol Rev* 71(3):495–548
- Wall T, Bath M, Britton RA, Jonsson H, Versalovic J, Roos S (2007) The early response to acid shock in *Lactobacillus reuteri* involves the ClpL chaperone and a putative cell wall-altering esterase. *Appl Environ Microbiol* 73(12):3924–3935
- Walter J, Heng NC, Hammes WP, Loach DM, Tannock GW, Hertel C (2003) Identification of *Lactobacillus reuteri* genes specifically induced in the mouse gastrointestinal tract. *Appl Environ Microbiol* 69(4):2044–2051
- Walter J, Loach DM, Alqumber M, Rockel C, Hermann C, Pfitzenmaier M, Tannock GW (2007) D-alanyl ester depletion of teichoic acids in *Lactobacillus reuteri* 100-23 results in impaired colonization of the mouse gastrointestinal tract. *Environ Microbiol* 9(7):1750–1760
- Watanabe M, Houten SM, Mataka C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, Schoonjans K, Bianco AC, Auwerx J (2006) Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 439(7075):484–489
- Weiss GM, Jespersen L (2010) Transcriptional analysis of genes associated with stress and adhesion in *Lactobacillus acidophilus* NCFM during the passage through an *in vitro* gastrointestinal tract model. *J Mol Microbiol Biotechnol* 18(4):206–214
- Yan F, Polk DB (2002) Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. *J Biol Chem* 277(52):50959–50965

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- vitro* by modulating dendritic cell function molecule 3-grabbing nonintegrin. *J Biol Chem* 278(12):2213–2226
- and metabolic studies of the impact of anti-inflammatory factor that inhibits MAPKs and induce cytoprotective heat shock protein 70. *Cell Physiol Biochem* 118(4):2213–2226
- Biophys Res Commun 343(1):69–76
- Schneewind O, Chang EB, Petrof EO (2004) MAPKs and induce cytoprotective heat shock protein 70. *Cell Physiol Biochem* 118(4):2213–2226
- ments on bacteriocin production by *Lactobacillus* beer produced by the fermentation of *hmo* 20(6):643–650
- position of *Saccharomyces boulardii* as a probiotic. *Syst Appl Microbiol* 26(4):564–571
- vitro* screening of probiotic properties of *Saccharomyces cerevisiae* strains. *Int J Food Microbiol* 118(2):118–125
- MJ, Sheil B, Steidler L, O'Mahony L (2004) Comparative and functional analysis of *Lactobacillus salivarius* UCC118. *Appl Environ Microbiol* 70(5):3110–3121
- Pfitzenmaier M, Geyer A, Lambrichts I, van SCJ (2007) Functional analysis of *D-lactobacillus rhamnosus* GG. *Appl Environ Microbiol* 73(12):3845–3855
- Zink R (2004) *Bifidobacterium lactis* (Bifidobacterium) operon and analysis of its genetic organization. *Microbiol* 70(5):3110–3121
- Sunderen D (2007a) Genomics as a means to study the case of bifidobacteria. *Antonie van Leeuwenhoek* 91(1):1–10
- GF, Chater KF, van Sinderen D (2007b) The history of an ancient phylura. *Microbiol* 73(12):3845–3855
- os S (2007) The early response to acid stress and a putative cell wall-altering enzyme. *Microbiol* 73(12):3845–3855
- V, Hertel C (2003) Identification of *Lactobacillus* use gastrointestinal tract. *Appl Environ Microbiol* 69(12):5511–5521
- Pfitzenmaier M, Tannock GW (2007) *Lactobacillus reuteri* 100-23 results in impaired intestinal barrier function. *Microbiol* 73(12):3845–3855
- im BW, Sato H, Messaddeq N, Harney JW, Werx J (2006) Bile acids induce energy metabolism and activation. *Nature* 439(7075):484–489
- genes associated with stress and adhesion through an *in vitro* gastrointestinal tract. *Appl Environ Microbiol* 72(12):3511–3521
- ine-induced apoptosis in intestinal epithelial cells. *Appl Environ Microbiol* 73(12):3845–3855
- Yan F, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB (2007) Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology* 132(2):562–575
- Yuan J, Wang B, Sun ZK, Bo X, Yuan X, He X, Zhao HQ, Du XY, Wang F, Jiang Z, Zhang L, Jia LL, Wang YF, Wei KH, Wang J, Zhang XM, Sun YS, Huang LY, Zeng M (2008) Analysis of host-inducing proteome changes in *Bifidobacterium longum* NCC2705 grown *in vivo*. *J Proteome Res* 7(1):375–385
- Zamfir M, Brezeanu A, de Vuyst L (2007) Bactericidal effect of acidophilin 801, a bacteriocin produced by *Lactobacillus acidophilus* IBB 801. *Roman Biotechnol Lett* 12(6):3521–3531