Mini-Review

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Extracellular proteins secreted by probiotic bacteria as mediators of effects that promote mucosa–bacteria interactions

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During the last few years, a substantial body of scientific evidence has accumulated suggesting that certain surface-associated and extracellular components produced by probiotic bacteria could be responsible for some of their mechanisms of action. These bacterial components would be able to directly interact with the host mucosal cells; they include exopolysaccharides, bacteriocins, lipoteichoic acids and surface-associated and extracellular proteins. Extracellular proteins include proteins that are actively transported to the bacterial surroundings through the cytoplasmic membrane, as well as those that are simply shed from the bacterial surface. Compared to the other bacterial components, the interactive ability of extracellular proteins/peptides has been less extensively studied. In this review, current findings supporting an interaction between extracellular proteins/peptides produced by probiotic bacteria (strains of the genera *Bifidobacterium*, *Lactobacillus* and *Escherichia*) and host mucosal cells are discussed. Research needs and future trends are also considered.

Proteins secreted by probiotic bacteria

The human gastrointestinal tract (GIT) is a vast surface inhabited by a complex and diverse community of micro-organisms. After thousands of years of co-evolution, the human body is completely tolerant to most of them, especially to bacteria (Gill *et al.*, 2006; Stringer, 2003; Turnbaugh *et al.*, 2007). However, some of these micro-organisms possess pathogenic potential and take advantage of certain gastrointestinal targets, colonizing the GIT and causing different intestinal disorders. Indeed, the commensal microbiota is quantitatively and qualitatively unbalanced in a wide range of intestinal and autoimmune disorders (Willing *et al.*, 2009). In this regard, probiotic bacteria could positively affect the balance between pathogenic and non-pathogenic bacteria (Araya *et al.*, 2002; Borody, 2000). Probiotics are defined as live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host (Araya *et al.*, 2002). Probiotics exert several beneficial effects on human health, including interaction with the immune system, production

of antimicrobial substances, enhancement of the mucosal barrier function and competition with enteropathogens for adhesion sites (Boesten & de Vos, 2008).

Nowadays, the unravelling of the molecular mechanisms underlying these beneficial effects is an attractive field for gut microbiologists. Among the different extracellular compounds responsible for these processes, proteins secreted and released into the environment by probiotic bacteria (extracellular proteins) might mediate certain interactions, since they would be able to interact directly with mucosal cells, such as epithelial and immune cells (Sánchez *et al.*, 2008a). Evidence of a direct interaction between bacterial extracellular proteins and the human immune system is mainly available for commensal and pathogenic species. In this context, the existence of antibodies directed against certain proteins secreted by the commensal microbiota in the framework of certain autoimmune diseases is known. Some examples of these proteins are porin OmpC from *Escherichia coli*, porin OmpW from *Bacteroides caccae*, protein I2 from *Pseudomonas fluorescens* and flagellin from *Clostridium coccoides* (Adams *et al.*, 2008; Furrie *et al.*, 2004; Iltnan *et al.*, 2006; Müller *et al.*, 2008). Theoretically, it is possible that certain extracellular proteins secreted by probiotic bacteria might also reach the gut mucosa, acting as molecular effectors responsible for downstream responses in mucosal cells.

Abbreviations: DC, dendritic cell; DC-SIGN, DC-specific ICAM-3-grabbing non-integrin; ERK, extracellular regulated kinase; GALT, gut-associated lymphoid tissue; GIT, gastrointestinal tract; GSK-3, glycogen synthase kinase-3; hBD2, human β -defensin 2; HSP, heat-shock protein; JNK, c-Jun terminal kinase; MAPK, mitogen-activated protein kinase; PI-3K, phosphatidylinositol 3-kinase; TER, transepithelial resistance; TJP, tight-junction protein; TLR, Toll-like receptor.

Extracellular proteins can be divided into two groups. The first group is composed of proteins that contain a signal peptide, which is located in the N-terminal part of the sequence to direct the protein to the protein export machinery. The second group includes surface-associated proteins that are simply shed from the bacteria due to the normal turnover of the cell wall (Anokhina *et al.*, 2006b; Turner *et al.*, 2004; Driessen & Nouwen, 2008; Sánchez *et al.*, 2009b). Secretion systems are highly conserved among eubacteria, given the high degree of homology among the genes coding for their monomeric components (van Wely *et al.*, 2001). These systems are particularly well known in Gram-negative bacteria, in which at least seven different secretion systems have been described, including those corresponding to macromolecules (Types I–VI and the twin-arginine translocation system) (Natale *et al.*, 2008; Sibbald & van Dijk, 2009). In probiotic bacteria, most of which are Gram-positive, the majority of proteins are secreted through two molecular systems: the general secretory pathway and the specific protein export systems (Natale *et al.*, 2008; van Wely *et al.*, 2001). The first is characterized by the presence of a signal peptide carrying a particular sortase cleavage motif, the best known being the Ala-X-Ala (signal peptide type I) and the Leu-Ala-Gly-Cys (signal peptide type II or lipobox sequence) (von Heijne & Abrahmsen, 1989; Willing *et al.*, 2009). In this system, proteins are synthesized as pre-proteins and transported in an unfolded state through a hydrophilic channel formed by the Sec proteins (von Heijne, 1989). The second group gathers specific export systems, in which proteins are secreted in a folded/oligomeric state, including ABC transporters, and the twin-arginine translocation system (Berks, 1996; Binet *et al.*, 1997; Jongbloed *et al.*, 2006). Finally, several glycolytic, housekeeping and ribosomal proteins are often found on the surface of bacteria (Jeffery, 2003). It is still unknown how these intracellular proteins can span the cytoplasmic membrane and reach the cell surface, although it is assumed that, once surface-exposed, these proteins could develop other functions, serving, for example, as adhesins (Candela *et al.*, 2009; Castaldo *et al.*, 2009; Hurmalainen *et al.*, 2007; Kinoshita *et al.*, 2008a, b; Ramiah *et al.*, 2008). Because of their property of having different functions, depending on the subcellular localization, these proteins are termed ‘moonlighting’ proteins (Jeffery, 2003). To date, putative extracellular proteins of probiotic bacteria have mainly been identified by bioinformatic means (usually through the identification of certain domains, such as signal peptide or cell wall anchoring motifs, by sequence homology) (Barinov *et al.*, 2009), and only a few of them have been experimentally identified and partially characterized (Sánchez *et al.*, 2008b; van Pijkeren *et al.*, 2006).

The issue of surface-associated proteins, among other protein groups supporting probiotic action in probiotic bacteria, has recently been extensively reviewed by Lebeer and co-workers (Lebeer *et al.*, 2008, 2010), by Kleerebezem *et al.* (2010) and by Buist *et al.* (2006). Another recent

report has focused on the adhesins present in one of the major probiotic groups, the genus *Lactobacillus* (Vélez *et al.*, 2007). Often, surface-associated proteins are identified by bioinformatic means (Båth *et al.*, 2005), with a few exceptions (Beck *et al.*, 2009). Furthermore, the presence of a specific genomic island coding for a pilus in *Lactobacillus rhamnosus* GG, in which a mucin-binding protein has been identified, has recently been reported (Kankainen *et al.*, 2009). There are clearly considerable difficulties in studying the specific effects of extracellular proteins in human models (i.e. clinical studies).

The aim of this review is to gather together the current information on the interaction between extracellular proteins produced by probiotic bacteria and the human gut mucosa. This group of proteins may trigger downstream responses in the host mucosa, potentially explaining some of the molecular mechanisms of action of probiotics. Current knowledge about the signalling mechanisms and the genetic/physiological changes induced by secreted proteins in mucosal cells and animal models is discussed. We will not deal with other cell-surface molecules with potential roles in the interaction with the human host, such as exopolysaccharides (Lebeer *et al.*, 2007a), indoles (Bansal *et al.*, 2010) or lipoteichoic acids (Anokhina *et al.*, 2006a).

Why study extracellular proteins secreted by probiotic bacteria?

Extracellular proteins from probiotic bacteria could diffuse through the mucus layer that covers the intestinal mucosa, enabling interaction with epithelial and immune cells. In some cases, the signal is transmitted to the nucleus via different pathways, which generally involve the consecutive action of several kinases, such as mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI-3K) and glycogen synthase kinase-3 (GSK-3) (Hoarau *et al.*, 2008). The activation of these cascades finally triggers changes in genetic expression and produces physiological changes in the cell. As we will discuss in this review, probiotic extracellular proteins regulate certain signalling pathways and cellular responses, including secretion of different effector molecules such as chemokines, cytokines or antibacterial peptides (defensins), mucus secretion, production of pseudopods, induction of changes in the surface properties, rearrangement of the tight junctions and modulation of the immune function and the response of the gut-associated lymphoid tissue (GALT) cells. These proteins are potential candidates for understanding the interaction between probiotic bacteria and the human host. The study of extracellular proteins may provide novel strategies for the clinical application of probiotic bacteria and may allow understanding of their mechanism of action. As previously stated, current knowledge about the interaction between extracellular proteins and the GALT mainly derives from data involving commensal bacterial groups. In this context, it has been observed that some

proteins secreted by the commensal microbiota might be responsible for the anomalous immune response observed in the framework of inflammatory bowel disease (Adams *et al.*, 2008; Ivison & Steiner, 2008; Schoepfer *et al.*, 2008).

There is a considerable amount of scientific evidence relating peptides secreted by probiotic bacteria to host-health promoting effects (Table 1). However, we currently lack molecular information about the identity of such peptides. Further research is needed to identify these peptides and to establish their precise molecular mechanism of action.

Receptors involved in the recognition of extracellular proteins

Molecules naturally present on the mucosal surfaces of the gut can be targets for extracellular proteins. For instance, it is known that S-layer protein from *Lactobacillus crispatus* is able to interact, directly, with the collagen molecules on the surface of epithelial cells (Antikainen *et al.*, 2002). This ability could be responsible for the competitive exclusion of enteropathogens, including *E. coli* O157:H7 (Chen *et al.*, 2007). Some studies have revealed that the interaction of the commensal microbiota and the host cells drives the correct development of the naïve intestinal mucosa, for example triggering and modulating the intestinal angiogenesis (Stappenbeck *et al.*, 2002). In fact, it is known that the commensal microbiota and the host cells have a continuous exchange of molecular information, called cross-talk (Hooper & Gordon, 2001; Hooper *et al.*, 2001). Very little is known about the cellular receptors responsible for the recognition of extracellular proteins secreted by probiotic bacteria, in contrast to receptors recognizing other bacterial products (Asong *et al.*, 2009). One example is some bacterial flagellins, recognized by Toll-like receptor 5 (TLR-5) and by the ICE protease-activating factor (IPAF) (Gewirtz, 2006; Ren *et al.*, 2006). Interestingly, recent data support the involvement of IPAF in the recognition of other bacterial molecules (Abdelaziz *et al.*, 2010). Recently, it has been suggested that the C-type lectin receptor (CLR) DC-specific ICAM-3-grabbing non-integrin (DC-SIGN) may be involved in the recognition of certain extracellular components of probiotic bacteria (Konstantinov *et al.*, 2008). CLRs are normally present on the surface of immune cells, such as dendritic cells (DCs) and macrophages, and recognize carbohydrate patterns that could be present, for instance, in extracellular glycoproteins (Benz & Schmidt, 2002). Also, the existence of an intestinal receptor for glycolipids of lactobacilli has been reported (Iwamori *et al.*, 2009). Finally, it has been suggested that some TLRs may recognize more than one type of molecule; for example TLR-2 is able to recognize different glycolipids and lipoproteins (Yan *et al.*, 2007).

Future studies should shed light on the involvement of other receptors, which could be specific for certain extracellular proteins.

Extracellular proteins secreted by probiotic bifidobacteria: enhancement of the mucosal barrier and immunomodulation

Members of the genus *Bifidobacterium* are among the first colonizers of the human GIT (Favier *et al.*, 2003). The beneficial effects of bifidobacteria on human health have largely been assessed during the last few years; moreover, decreases in the number of bifidobacteria in the colon have been associated with several diseases, including inflammatory bowel disease, irritable bowel syndrome, autoimmune disorders such as rheumatoid arthritis or lupus, and infections by enteropathogens (Gueimonde *et al.*, 2007; López *et al.*, 2010; Salminen *et al.*, 2005, 2009; Turroni *et al.*, 2009). Bifidobacterial strains are included in the formulation of functional foods for human nutrition, notably in dairy products and infant milks. Fig. 1 shows the extracellular proteins/peptides with a known interaction with mucosal cells.

Enhancement of the mucosal barrier

Some extracellular proteins produced by bifidobacteria are known to be responsible for the enhancement of the mucosal barrier and to modulate the GALT immune function in animal and cellular models. Among the extracellular proteins secreted by bifidobacteria, serine protease inhibitor (serpin) from *Bifidobacterium longum* subsp. *longum* NCC2705 was the first extracellular protein shown to interact directly with the host factors (Ivanov *et al.*, 2006). Extracellular serpin is also produced by other bifidobacterial species, including *Bifidobacterium breve*, *Bifidobacterium dentium* and *B. longum* subsp. *infantis*. It has been shown that serpin efficiently inhibits pancreatic and neutrophil elastases (Ivanov *et al.*, 2006). Neutrophils are recruited in the intestinal mucosa from the blood vessels by means of the secretion of inflammatory cytokines, and therefore are involved in inflammatory episodes. Bifidobacterial serpin, acting on enzymes directly involved in the inflammatory response, might thus mediate some of the anti-inflammatory effects of bifidobacteria (Ivanov *et al.*, 2006).

Another well-known probiotic strain, *Bifidobacterium animalis* subsp. *lactis* BB-12, produces a pentapeptide (CHWPR) in the stationary phase of growth; CHWPR has been shown to upregulate the *c-myc* and *il-6* genes in the cell line HL-60 (Mitsuma *et al.*, 2008). This pentapeptide crosses the cytoplasmic membrane and binds to the nuclear receptor ROR- γ . The complex binds to the promoter region of the genes and activates their transcription. The effect of CHWPR on intestinal cells might have a great impact on GIT physiology, since IL-6 is a dual anti- and pro-inflammatory cytokine, and since *c-myc* deregulation is observed in several human cancers.

Tight junctions in epithelial cells are key components for the regulation of the mucosal barrier function, selectively affecting the movement of solutes and water through the epithelium. In a recent study, uncharacterized extracellular proteins secreted by *B. longum* subsp. *infantis*, isolated from a

Table 1. Probiotic extracellular proteins/peptides with a known role in the interaction of potential probiotic strains with mucosal cells

Protein	Micro-organism	Role	Reference
Serpin (AAN23973)	<i>B. longum</i> subsp. <i>longum</i> NCC2705	Inhibition of pancreatic and neutrophil elastases	Ivanov <i>et al.</i> (2006)
CHWPR peptide	<i>B. animalis</i> subsp. <i>lactis</i> BB-12	Upregulation of <i>c-myc</i> and <i>il-6</i> genes	Mitsuma <i>et al.</i> (2008)
Unidentified secreted proteins	<i>B. longum</i> subsp. <i>infantis</i>	Increase of the mucosal barrier function; attenuation of inflammation and colonic permeability in IL-10-deficient mice	Ewaschuk <i>et al.</i> (2008)
Unidentified secreted proteins	<i>B. breve</i> C50	Prolonged survival and maturation of DCs; increased IL-10 and IL-12 production by DCs	Hoarau <i>et al.</i> (2008)
Unidentified secreted proteins	<i>L. acidophilus</i> PZ 1138, <i>L. fermentum</i> PZ 1162, <i>L. paracasei</i> subsp. <i>paracasei</i> LMG P-17806	Induction of hBD2 production in epithelial cells	Schlee <i>et al.</i> (2008)
Peptides NPSRQERR and PDENK	<i>L. rhamnosus</i> GG	Antimicrobial activity	Lu <i>et al.</i> (2009)
Unidentified secreted proteins	<i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. casei</i> and <i>L. delbrueckii</i> subsp. <i>bulgaricus</i>	Induction of mucin secretion	Caballero-Franco <i>et al.</i> (2007)
Unidentified secreted proteins	<i>L. rhamnosus</i> GG	Increase of the production of HSP25 and HSP72 in YAMC cells	Tao <i>et al.</i> (2006)
Unidentified secreted proteins	<i>L. acidophilus</i> and <i>L. rhamnosus</i>	Increase of the chloride/hydroxyl exchange activity in Caco-2 cells	Borthakur <i>et al.</i> (2007)
p40 (homologous to gil116493594)	<i>L. rhamnosus</i> GG	Growth promotion	Yan <i>et al.</i> (2007)
p75 (homologous to gil116493849)	<i>L. rhamnosus</i> GG	Reduction of the injuries caused by TNF- α ; attenuation of the TER decrease induced by hydrogen peroxide	Seth <i>et al.</i> (2008)
Supernatant containing P40 and p75?	<i>L. rhamnosus</i> GG	Decrease of IL-8 production in epithelial cells	Choi <i>et al.</i> (2008)
SlpA (YP_193101.1)	<i>L. acidophilus</i> NCFM	Induction of IL-10 production in DCs; DC immunomodulation	Konstantinov <i>et al.</i> (2008)
Unidentified secreted proteins	<i>E. coli</i> Nissle 1917	Inhibition of pathogen adhesion and colonization	Altenhoefer <i>et al.</i> (2004); Lasaro <i>et al.</i> (2009)
Flagellin	<i>E. coli</i> Nissle 1917	Increase of hBD2 and IL-8 production	Schlee <i>et al.</i> (2007)

Fig. 1. Some of the effects mediated by extracellular proteins secreted by different strains of the genus *Bifidobacterium*. The proteins are represented by blue dots. (a) The pentapeptide CHWPR is able to increase *c-myc* and *il-6* expression through a mechanism involving the nuclear receptor ROR γ . (b) Proteinaceous compounds can bind to TLR-2 present on the surface of dendritic cell pseudopods, increasing their survival and the production of interleukins 10 and 12, as well as affecting their maturation. This is done by a mechanism involving several kinases, including ERK, p38, PI-3K or GSK-3. (c) Proteinaceous compounds are able to increase the production of TJPs, therefore increasing transepithelial resistance (TER), through a signalling pathway involving ERK. (d) An extracellular serin protease inhibitor (serpin) produced by *B. dentium*, *B. breve* and *B. longum* subsp. *longum* (and probably other bifidobacterial species) is a suicide inhibitor of neutrophil elastase, which is involved in acute inflammatory episodes within the gut.

known probiotic cocktail, increased the production of zonula occludens-1 and occludin, two tight-junction proteins (TJPs), in epithelial cells. In addition, these increased transepithelial resistance (TER). Both events produced a reduction of colon permeability in a murine model, thus helping to maintain the mucosal barrier function (Ewaschuk *et al.*, 2008).

Immunomodulation

Extracellular proteins secreted by *B. longum* subsp. *infantis* attenuated both inflammation and colonic permeability in

IL-10-deficient mice, which lack the ability to produce sufficient quantities of the anti-inflammatory cytokine IL-10. The mechanism of action of these extracellular proteins is mediated by the modulation of two cytoplasmic mitogen-activated protein kinases (MAPKs): an increase in the levels of extracellular regulated kinase (ERK) together with a decrease in p38 MAPK (p38) (Ewaschuk *et al.*, 2008).

Finally, extracellular proteins secreted by *B. breve* C50 were shown to interact with the TLR-2 present on the surface of immature human DCs, inducing different functional and physiological changes through different pathways. Prolonged DC survival was mediated by the PI-3K pathway, DC maturation by the p38 and PI-3K pathways, increase in IL-10 production by the MAPK (p38, ERK) and PI-3K pathways, and finally an increase in IL-12 production was mediated by means of the p38 and GSK-3 pathways (Hoarau *et al.*, 2008). Further research is needed to elucidate the precise role of those proteins.

Cellular responses to extracellular proteins produced by probiotic lactobacilli

Lactobacilli are important commensal bacteria in the human GIT. Several *Lactobacillus* species are used in the food industry for the production of a panoply of fermented products. Again, certain strains are considered as probiotics. *L. rhamnosus* GG (ATCC 53103) is one of the probiotic strains that has been most closely studied, and in addition has one of the most extensive safety assessment records (Lebeer *et al.*, 2007b). Fig. 2 shows the main cellular pathways modulated in response to extracellular proteins secreted by probiotic lactobacilli. The action of certain extracellular proteins might explain some of the beneficial effects exerted by certain probiotic lactobacilli.

Enhancement of the mucosal barrier and maintenance of GIT homeostasis

Extracellular proteins secreted by probiotic lactobacilli have been shown to help maintain the mucosal barrier, mainly through MAPK-dependent mechanisms (Schlee *et al.*, 2008). The signalling mechanisms of the proteins are better characterized in lactobacilli than in bifidobacteria. Uncharacterized extracellular proteinaceous compounds secreted by *Lactobacillus acidophilus* PZ 1138, *Lactobacillus fermentum* PZ 1162 and *Lactobacillus paracasei* subsp. *paracasei* LMG P-17806 have been shown to induce production of the antimicrobial peptide human β -defensin 2 (hBD2) in epithelial cells. The signal of these extracellular proteins was shown to be transduced to the nucleus through the MAPKs ERK, p38 and c-Jun terminal kinase (JNK), where hBD2 synthesis was increased through the modulation of nuclear factor κ B (NF- κ B) and activator protein 1 (AP-1), ending finally in an increase of IL-8 production (Schlee *et al.*, 2008). In addition, two peptides present in *L. rhamnosus* GG conditioned media, NPSRQERR and

Fig. 2. Schematic representation of the molecular mechanisms modulated by extracellular proteins produced by lactobacilli. The proteins are represented by brown dots. Specific details of the signal transduction mechanisms are given in the main text. (a) Proteinaceous compounds increase *muc-2* expression in epithelial cells, resulting in an increase of mucin secretion. (b) S-layer protein A (SlpA) from *L. acidophilus* NCFM binds DC-SIGN, whose signalling increases IL-10 production by epithelial cells, whereas S-layer protein B (SlpB) increases the production of IL-12p70, TNF- α and IL-1 β . (c) Extracellular proteins increase the production of HSP25 and HSP72, and affect electrolyte transport through PI-3K signalling (d). (e) Proteins p40 and p75, secreted by *L. rhamnosus* GG, promote enterocyte growth, decrease the incidence of apoptotic processes, and enhance transepithelial resistance through the increase of TJP synthesis. (f) Proteins secreted by lactobacilli increase hBD2 and IL-8 production.

PDENK, were shown to possess antimicrobial activity against *E. coli* EAEC 042, *Salmonella enterica* serovar Typhimurium and *Staphylococcus aureus* (Lu *et al.*, 2009).

Extracellular proteinaceous compounds secreted by the lactobacilli contained in a probiotic formula (*L. plantarum*, *L. acidophilus*, *L. casei* and *L. delbrueckii* subsp. *bulgaricus*) induced mucin secretion through *muc2* gene expression in murine colonic epithelial cells, although the possible signal transduction pathways involved are not yet known (Caballero-Franco *et al.*, 2007). Further, extracellular proteins present in growth media conditioned by *L. rhamnosus* GG increased the production of the heat-shock proteins HSP25 and HSP72 in young adult mouse colon (YAMC) cells (Tao *et al.*, 2006). On the one hand, HSP overproduction was due to the transcriptional regulation mediated by heat-shock factor 1 (HSF-1); on the other

hand, the MAPKs p38 and JNK were also shown to be involved in the signalling pathway leading to HSP increase.

Extracellular proteins p40 and p75, both secreted by *L. rhamnosus* GG, are perhaps the best studied extracellular proteins. p40 is homologous to an uncharacterized surface antigen of *Lactobacillus casei* ATCC 334 (gil116493594), whereas p75 is homologous to a cell wall-associated hydrolase of the same bacterium (gil116493849) (Yan *et al.*, 2007). Yan and co-workers have shown that both p40 and p75 are efficient growth promoters, as they induced the proliferation of YAMC cells by activating protein kinase B (AKT) through a PI-3K-dependent pathway. In the same study, these two proteins were also able to reduce the colon injuries induced by tumour necrosis factor alpha (TNF- α) in murine colon tissue explants (Yan *et al.*, 2007). Moreover, the two proteins completely inhibited TNF- α -induced

apoptosis in the KSRI^{-/-} MCE cell line, which undergoes apoptosis following TNF- α treatment (Yan *et al.*, 2007). Finally p40 and p75 were capable of attenuating the TER decrease induced by hydrogen peroxide, also preventing the rearrangement of several TJPs (occludin, zonula occludens-1, E-cadherin and β -catenin) through a protein kinase C- and MAPK-dependent signalling pathway (Seth *et al.*, 2008). p40 and p75 thus appear to be important secreted proteins for GIT homeostasis, involved in both cell proliferation and apoptosis, and in the maintenance of the mucosal barrier.

To sum up, extracellular proteins secreted by probiotic lactobacilli have been shown to promote the stabilization and enhancement of the mucosal barrier function by increasing the production of human defensins, the secretion of mucus and the concentration of HSPs in epithelial cells, and by stabilizing the arrangement and concentration of TJPs.

Electrolyte absorption

Certain studies have shown that probiotic lactobacilli are promising agents for the treatment of diarrhoea (Arvola *et al.*, 1999; Pant *et al.*, 1996). In this context, it has recently been shown that unidentified extracellular proteinaceous compounds secreted by *L. acidophilus* and *L. rhamnosus* increased the chloride/hydroxyl exchange activity (Cl⁻/OH⁻) in Caco-2 cells through the PI-3K signal transduction pathway. This triggered an increase in Cl⁻ absorption, which was also related to an overproduction of the apical anion-exchange transporter SLC26A3 on the cell surface (Borthakur *et al.*, 2007). As suggested by the authors, this increased electrolyte absorption in epithelial cells might be an alternative treatment for people suffering from diarrhoea or other intestinal disorders in which electrolyte absorption is perturbed.

Immunomodulation and modulation of cytokine production

Extracellular proteins secreted by probiotic lactobacilli can modulate the activity of immune cells. S-layer protein A (SlpA) released from *L. acidophilus* NCFM cells has been shown to induce IL-10 production in DCs (Konstantinov *et al.*, 2008). DCs matured in the presence of purified SlpA expressed lower levels of the maturation marker CD86 with respect to controls, and did not induce T-cell proliferation, suggesting that this protein might somehow induce changes in the immune functions of DCs. The effects of SlpA on DCs were shown to be produced through a direct interaction of SlpA with the surface lectin DC-SIGN (Konstantinov *et al.*, 2008). This result suggests a direct role for SlpA as an anti-inflammatory protein, given that a SlpA knockout strain, which overproduced another S-layer protein (SlpB), preferentially induced a pro-inflammatory response through an increase in production of IL-12p70, TNF- α and IL-1 β . Unfortunately, no information about the components involved in the downstream signal transduction pathway was reported.

Flagellin shed from *E. coli* Nissle 1917 cells

E. coli is an abundant and important micro-organism within the human intestinal microbiota, and is well adapted to this environment. Non-pathogenic and pathogenic *E. coli* strains differ in the presence of virulence factors, normally acquired by horizontal gene transfer, and encoded on mobile genetic elements or in genome islands (Grozdanov *et al.*, 2004). *E. coli* strain Nissle 1917 (O6:K5:H1) is a probiotic strain isolated from a soldier who survived a severe outbreak of diarrhoea during World War I. It has been successfully used for the treatment of various intestinal disorders (Grozdanov *et al.*, 2004). The main molecular mechanisms modulated in the host by extracellular proteins of *E. coli* Nissle 1917 are summarized in Fig. 3.

Enhancement of the mucosal barrier

E. coli Nissle 1917 secretes proteinaceous molecules capable of blocking the adhesion and colonization of the intestinal cell line INT407 by *Salmonella* sp., *Yersinia enterocolitica*, *Shigella flexneri*, *Legionella pneumophila* and *Listeria monocytogenes* (Altenhoefer *et al.*, 2004; Lasaro *et al.*, 2009). Flagellin secreted by this strain promotes hBD2 secretion in Caco-2 cells. This is achieved through a direct interaction with TLR-5, which

Fig. 3. Flagellin shed from *E. coli* Nissle 1917 cells induces hBD2 and IL-8 production. The molecular mechanism of action involves signalling through the kinases ERK, JNK and p38, and the nuclear effectors NF- κ B and AP-1.

activates several MAPKs (ERK1/2, JNK and p38) responsible for the signal transduction to the nucleus. The increase in hBD2 was abolished by mutation of the NF- κ B and AP-1 binding sites of the hBD2-encoding gene (Schlee *et al.*, 2007).

Immunomodulation

Flagellin released from *E. coli* Nissle 1917 increased the production of the pro-inflammatory cytokine IL-8 in Caco-2 cells (Schlee *et al.*, 2007). The response to *E. coli* Nissle 1917 flagellin is particularly atypical, since flagellated microorganisms are often enteropathogens, such as *Salmonella* sp. It has been suggested that, in the case of this particular *E. coli* probiotic strain, the stimulation induced by its flagellin would not reach the threshold levels necessary for the induction of a pro-inflammatory response, producing instead an activation of the immune system through the production of IL-8 and human defensins (Kruis *et al.*, 2004).

Future perspectives

In the last few years, various papers have reported on the role of extracellular proteins secreted by probiotic bacteria (Sánchez *et al.*, 2008a, b, 2009a, c, d; Turner *et al.*, 1997, 2004). As has been shown in this review, probiotic extracellular proteins could be linked to some of the beneficial effects ascribed to the corresponding strains, although current information is now restricted to *in vitro* and animal studies. To date, our knowledge of the identity of these proteins is very limited; although several studies have reported the interaction between extracellular proteinaceous compounds and human cells, few have been identified and characterized so far.

Further research is needed to elucidate the precise molecular mechanism of action of each of these proteins in both epithelial and immune cells, notably in DCs. This will contribute to the understanding of how probiotics exert beneficial effects on the human host. This knowledge may lead to treatments to reverse some of the processes involved in the initiation, or perpetuation, of various gastrointestinal disorders, such as inflammatory bowel diseases, allergies and autoimmune diseases.

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