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**Looking into key bacterial proteins involved in gut dysbiosis**

Zeng XY *et al*. Bacterial proteins in gut dysbiosis

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**Abstract**

The gastrointestinal microbiota plays a pivotal role in health and has been linked to many diseases. With the rapid accumulation of pyrosequencing data of the bacterial composition, the causal-effect relationship between specific dysbiosis features and diseases is now being explored. The aim of this review is to describe the key functional bacterial proteins and antigens in the context of dysbiosis related-diseases. We subjectively classify the key functional proteins into two categories: Primary key proteins and secondary key proteins. The primary key proteins mainly act by themselves and include biofilm inhibitors, toxin degraders, oncogene degraders, adipose metabolism modulators, anti-inflammatory peptides, bacteriocins*,*host cell regulators, adhesion and invasion molecules, and intestinal barrier regulators. The secondary key proteins mainly act by eliciting host immune responses and include flagellin, outer membrane proteins, and other autoantibody-related antigens. Knowledge of key bacterial proteins is limited compared to the rich microbiome data. Understanding and focusing on these key proteins will pave the way for future mechanistic level cause-effect studies of gut dysbiosis and diseases.

**Key Words:** Gut microbiota; Pyrosequencing; Bacteria; Protein; Immune; Dysbiosis

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**Core Tip:** Revealing the causal-effect relationship between specific dysbiosis features and diseases requires understanding the roles of key bacterial proteins that are involved in dysbiosis. Some bacterial proteins may affect the microbiome by their inherent functions. Others shape the microbiome mainly by eliciting host immune responses. These key proteins warrant attention in future bioinformatic analyses and mechanistic studies.

**INTRODUCTION**

The gastrointestinal microbiota is linked to numerous diseases, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), colorectal cancer, cirrhosis, and many others. Thanks to the rapid decrease in the cost of pyrosequencing, the gut microbiota, often represented by the fecal bacteria composition, is now easy to profile by 16S rDNA sequencing and shotgun metagenomic sequencing. With the accumulation of known microbiome-disease correlations in many descriptive studies, the mechanisms of known dysbiosis features in the pathogenesis of related diseases have become a new frontier to be explored. Understanding these mechanisms is a prerequisite to developing the precise intervention methods targeting the gut microbiome. Thus, it is necessary to review the key microbial proteins involved in gut dysbiosis.

The gut microbiome produces numerous products for itself and the host. The collection of small molecules produced by the gut microbiota, termed the metabolome, represents promising targets for investigation and translation. The methodology and findings of studies of the gut metabolome have been reviewed elsewhere[1,2]. In addition, the gut microbiota produces exosomes, which have been reviewed by other excellent reviews[3]. The virome[4,5], parasitome[6], helminths, and protozoa-omics[6] are also recognized by omic-approaches but with less well documented mechanisms. In this review, we will focus on the key peptides, proteins, and antigens produced by bacteria and fungi in the context of dysbiosis and diseases.

To organize the review, we categorize the bacterial proteins into two groups: (1) the primary key proteins, whose action mainly depends on their inherent properties (Table 1); and (2) the secondary key proteins, whose action mainly depends on the host response to them (Figure 1, Table 2). This classification mainly depends on the current knowledge and is relative. Often, the bacteria-host interaction is bilateral. Thus, this classification is subjective and only helps navigate the mechanisms. For each group, we organize the key proteins according to their functions to assist in navigating this field rapidly.

**PRIMARY KEY PROTEINS**

***Biofilm inhibitors***

Biofilm formation is a process of extracellular synthesis by bacteria, and it has adverse effects on the immune response of the host[7], resulting in dysbiosis[8]. Bacteria are found in the intestinal mucosa of humans and clinical observations have revealed bacterial biofilms associated with mucosal colonization in patients with IBD[7]. Many infections also involve pathogens forming biofilms, including enterohemorrhagic *Escherichia coli* (EHEC)[9]. Probiotics have been documented to produce enzymes degrading biofilms of other species. *Escherichia coli* (*E. coli*) Nissle 1917 (EcN), a probiotic capable of alleviating inflammation, can produce its own biofilm and outcompete that of other intestinal pathogens[10]. Fang *et al*[11] found that DegP, a bifunctional (protease and chaperone) periplasmic protein secreted by EcN, contributes to the inhibition of EHEC biofilms by directly interacting with the EHEC cell surface while not affecting its own biofilm. Another probiotic, *Lactobacillus rhamnosus* *GG* (LGG), could also disrupt the biofilm formation of pathogenic *E. coli* and *Salmonella*[12]. This effect is mediated by its lectin like proteins, termed Llp1 (lectin-like protein 1) and Llp2[12]. Llp2, which is more active than Llp1, showed inhibitory activity against biofilm formation by various pathogens, including clinical *Salmonella* species and uropathogenic *E. coli* (UPEC) [12]. Thus, biofilm production and inhibition might represent key bacterial events in microbiome evolution, as well as promising targets to manage dysbiosis.

***Toxin degraders***

Probioticsmay degrade pathogenic toxins and thus contribute to the homeostasis of gut microbiota. *Clostridium difficile* (*C. difficile*) mediates intestinal inflammation and mucosal damage by releasing two potent exotoxins, toxin A and toxin B[13], while the fungal probiotic *Saccharomyces boulardii* (*S. boulardii*) is known as the most efficient probiotic to prevent intestinal inflammation and mucosal damage associated with *C. difficile* infection[14]. The protective effect of *S. boulardii* is dependent on a 54 kDa protease, which digests both toxin A and its receptor binding sites[15]. Several human studies demonstrated that treatment with *S. boulardii* CNCM I-745 in dysbiosis leads to faster reestablishment of a healthy microbiome[16].

***Oncogene degraders***

Oncogene c-MYC is associated with oncogenic transcription in malignant tumor driven by chronic bacterial infections[17], and the up-regulated c-MYC also indicates a poor prognosis in some human cancers[18]. The Lon protease from UPEC shows potential for therapeutic targeting of c-MYC in cancers, the degradation of c-MYC is dependent on both direct Lon protease cleavage and Hly-dependent activation of CK1α1, and UPEC represses transcriptional MYC regulators to inhibit c-MYC expression[19]. In mice, the recombinant Lon (rLon) protease without major toxicity delayed tumor development and increased survival in MYC-dependent bladder and colon cancer models[19]. These results indicate that probiotics may block tumor proliferation by degrading the oncogene.

***Adipose metabolism modulators***

*Akkermansia muciniphila (A. muciniphila)*, one of the gut microbiota, is connected with metabolic disorders, and it reduces the energy absorption under cold conditions in the intestine epithelium[20]. P9 is an 84 kDa protein, which is secreted by *A. muciniphila*. P9 increases the glucagon-like peptide-1 (GLP-1) secretion in a calcium-dependent manner and specifically promotes interscapular brown adipose tissue (iBAT) non-shivering thermogenesis in the gut hormone-releasing L cells and HFD mice[21]. The ligand–receptor capture (LRC)-TriCEPS technology shows that the P9 interacts with intercellular adhesion molecule 2 (ICAM-2), and ICAM-2 reduces the secretion of the P9-induced GLP-1 in a dose-dependent manner[21]. Moreover, P9 induced the secretion of interleukin-6 (IL-6) in macrophages[21], and IL-6 can stimulate GLP-1 secretion by intestinal L cells[22].

***Anti-inflammatory peptides***

The mucosal immune response plays an important role in IBD pathogenesis, and perturbations of the gut microbiota are a key element[23]. Probiotics can modulate the intestinal cytokine milieu to treat IBD[24] and other diseases. Peptide B7 from the probiotic *Bifidobacterium longum* decreases CCR2 expression on all antigen presenting cells from healthy controls but not from active IBD patients[25]. Although this bioactive peptide is useless for the treatment of active IBD patients, we cannot ignore its potential to prevent inflammation flares in the quiescent phase[25]. Another probiotic, *Faecalibacterium prausnitzii* (*F. prausnitzii*), one of the most abundant species in the human gut microbiota, possesses a 15 kDa protein with anti-inflammatory properties, termed a microbial anti-inflammatory molecule (MAM)[26]. The inflammatory suppressive role of MAMs from *F. prausnitzii* may be related to their effects on the inhibition of the NF-κB pathway, several cell immune responses such as Th1, Th2, and Th17 cells, and the expression of TGF-β[27,28]. The micro integral membrane protein (MIMP) identified from *Lactobacillus plantarum* was found to decrease proinflammatory cytokines (IFN-γ, IL-17 and IL-23), increase anti-inflammatory cytokines (IL-4 and IL-10), and fortify the intestinal barrier in a dextran sulphate sodium induced colitis model[29]. Probiotics have been documented to produce enzymes hydrolyzing key proteins in the NF-κB pathway[30]. O-GlcNAcase (OGA) is rich in *Bacteroidetes* and *Firmicutes*, the major probiotics distributed in the human gut, and reduced expression of bacterial *OGA* genes has been found in ulcerative colitis (UC)[30]. Bacterial OGAs are an advanced therapeutic strategy in UC that act by hydrolyzing O-GlcNAcylated NF-κB-p65 and IKKβ to inhibit NF-κB signaling in both immune cells and intestinal epithelial cells[30].

***Bacteriocins***

Bacteriocins are ribosomally synthesized bactericidal or bacteriostatic peptides[31,32]. Bacteriocins from probiotics maintain the microbial population-level and community-level dynamics and inhibit other strains[33]. Bacteriocins are mainly divided into two classes: Posttranslationally modified class I and unmodified class II[34,35]. In a previous study, pediocin, enterocin-A, and enterocin-B were regarded as class II bacteriocins[31], and nisin belonged to class I bacteriocins[36,37]. Pediocin PA-1/AcH secreted by *Pediococcus acidilactici* (*P. acidilactici*) MM33 and nisin Z secreted by *Lactococcus lactis* (*L. lactis)* MM19, have been proven to reduce colonization of vancomycin-resistant enterococci (VRE) *in vivo*[38]. Microcin-producing EcN limits the expansion of competing *Enterobacteriaceae*, including commensal *E. coli*, adherent-invasive *E. coli*, and *Salmonella* *enterica* in the inflamed gut[39] by utilizing catecholate siderophores[40]. *Enterococcus faecium* produces two synergistic bacteriocins, enterocin-A (a pediocin-like bacteriocin) and enterocin-B. Although the inhibitory spectra of enterocins A and B have small differences, both enterocins from *Enterococcus faecium* TI36 inhibit a wide spectrum of Gram-positive bacteria but not Gram-negative bacteria[41]. With a similar inhibitory spectrum, enterocin A has lower minimum inhibitory concentration (MIC) values than enterocin B[41].

Furthermore, the bactericidal effect is drastically increased when a mixture of the two bacteriocins is used[41]. The findings of a previous study suggested that the heterodimer of bacteriocin A and B from *Enterococcus faecium* por1 had antibacterial, pathogenic biofilm degradation potential but did not result in haemolysis of human red blood cells[42]. A cancer cell growth inhibitory potential of enterocins has been demonstrated, and apoptotic makers were observed in enterocin treated cancer cells including HeLa, HT-29, and AGS cells[42]. The mechanism of their effects on cancer is that cancer cells have more microvilli on their surface, which allows the membrane of cancer cells to bind large quantities of bacteriocins[43]; thus, Nisin A from *L. lactis* changes the integrity of the cancer cell membrane and obstructs the rearrangement of phospholipids, resulting in increased ion permeability[44]. These bacteriocins enable probiotics to treat enterobacterial infections in the gut and even some cancers.

***Host cell circle regulators***

Some oral bacteria disseminate into the colon and alter the composition of the microbiota in the colon, resulting in intestinal dysbiosis and possibly leading to colorectal cancer (CRC)[8]. FadA from *Fusobacterium nucleatum* drives CRC proliferation through E-cadherin and increases the expression of transcription factors and inflammatory genes *via* activation of β-catenin signaling[45]. Some bacterial proteins provide new strategies to treat cancer. An 8 kDa protein called p8 was isolated from *Lactobacillus rhamnosus* (LR) KCTC 12202BP, which regulates the p53-p21-Cyclin B1/Cdk1 signaling pathway and causes cell growth arrest at the G2 phase in a dose-dependent manner[46]. Bacterial drug delivery systems are being applied to treat CRC. The p8 protein from *Pediococcus pentosaceus* SL4 (PP-p8) showed antiproliferative activity in a mouse CRC model[47]. Moreover, endogenous p8 expression was much more effective than exogenous recombinant- p8 expression. This makes gene therapy possible[47].

HPRP-A1 and its enantiomer HPRP-A2 are derived from ribosomal protein L1 (RpL1) of *Helicobacter pylori*[48]. These proteins can resist infection including fungi, bacteria, and parasites[49,50]. Moreover, they have anticancer potential, and both peptides lead to apoptosis *via* caspase-3-, caspase-8-, and caspase-9-dependent pathways and inhibit cancer cell growth by arresting the cell cycle at the G0/G1 phase and G2/M phase. HPRP-A1 and its enantiomer HPRP-A2 play an important role in the inhibition of gastrointestinal cancer[51–53].

***Adhesion and invasion molecules***

*Fusobacterium nucleatum* (Fn) is associated with CRC and promotes tumor formation. Fn is able to adhere to and invade intestinal endothelial cells by binding to adhesin FadA, a virulence factor from Fn[54]. FadA from *E. coli* enhances the connection between host epithelial cells and bacteria. FadA has two forms, anchored form (pre-FadA) and secreted form (mature FadA), thus the pre FadA-mFadA complex is regarded as a unique adhesin/invasin[54]. Fusobacterial lectin (Fap2) might mediate the binding of Fn to the host factor Gal-GalNAc in CRC, and Gal-GalNAc is highly expressed in human colorectal adenocarcinoma and metastases[55]. Other findings support that Fap2 of Fn not only leads to colonization but also facilitates tumor immunity evasion[55]. Fap2 directly binds to and activates TIGIT (an inhibitory receptor on human natural killer cells and different T cells), and the interaction between these two molecules inhibits the cytotoxicity of NK cells and the activities of cytotoxic T lymphocytes and T helper cells, increasing the immune evasion of tumor cells[56]. Fap2, as an apoptosis-inducing protein, also induces host lymphocyte apoptosis and destroys the host immune response, facilitating Fn survival[57]. Liu *et al*[58]identified outer membrane vesicles (OMVs) in Fn by LC/MS/MS analysis and identified several pathogenic proteins in OMVs, including FadA, Fap2, MORN2, YadA (Yersinia adhesin)-like protein, and autotransporter proteins[58]. The MORN2 domains of Fn may contribute to adhesion and active invasion[59]. Two YadA-like proteins exist in OMVs and outer membrane fractions, which reveal great adhesion ability[58]; therefore, YadA-like proteins are involved in resisting host immune defenses dependent on resisting serum killing activity and phagocytosis[60]. OMVs provide new insight into the research and development of vaccines against Fn[58].

*Bacteroidetes* is one of the most numerous Gram-negative bacteria in the mammalian gastrointestinal tract[61]. Cell envelope-associated multiprotein systems, namely, Sus (starch utilization system)-like systems[62], are abundant in *Bacteroides*. Polysaccharide utilization loci (PULs) in Sus-like systems are not only used to bind to and degrade dietary sugar[63], but they also encode a unique pathway, the *ccfA–E* genes, called commensal colonization factors (CCF systems) for species-specific saturable niche colonization[64]. Moreover, the CCF system is medicated by *B. fragilis* colonization during infection with *Citrobacter rodentium* and antibiotic treatment[64].

LGG has a very good mucus adhesive capacity compared to another *Lactobacillus strains*[65].The LGG-specific SpaCBA pili are long and thin proteinaceous protrusions on bacterial surface, which involved in three pilin monomers: SpaA , SpaB, and SpaC[66]. The SpaCBA pili mediate adhesive capacity to mucus and contribute to biofilm formation[67]. Moreover, the SpaCBA pili may also regular immune response. The spaCBA knockout LGG had twofold increased IL-8 and some pro-inflammatory markers in Caco-2 cells compared to wild-type[67].

***Intestinal barrier regulators***

Under dysbiosis, increased permeability of the intestinal epithelium leads to low-grade inflammation and metabolic dysfunctions[68]. However, according to the leaky gut hypothesis, if only the *F. prausnitzii* is present as a probiotic, it will not beneficial to the intestine health and dysbiosis-induced diseases but enter the bloodstream by passing though the gut barrier and may cause systemic consequences because of obesity and a high-fat diet (HFD)[69,70]. Moosavi *et al*[71] show that *F. prausnitzii*–derived extracellular vesicles (EVs) contain different proteins with a molecular weight of 11 to 245 kDa. Compared with *F. prausnitzii*, its EVs in the Caco-2 cell line significantly regulate the intestinal barrier permeability due to increasing the expression of the tight junction (TJ) protein encoding genes *ZO1* and *OCLN*, as well as *PPARα and PPARγ* genes and their targeted gene *ANGPTL4* at the mRNA level[71]. TJ proteins connect the adjacent epithelial cells and block the paracellular space in order to obstruct pathogens[72]. ANGPTL4 inhibits blood lipase lipoproteins in the bloodstream, which reduces the intake of free fatty acids and cholesterol into the tissues[73–76].

TcpC from EcN enhanced the intestinal barrier function by increasing the expression of the TJ proteins ZO-1, ZO-2, and claudin-14[77–79]. Moreover, the positive strains ECOR63 and ECOR57 increased the transepithelial electrical resistance (TER) in T-84 monolayers to strengthen the intestinal barrier[80]. In addition, OMVs and other soluble factors from these probiotic bacteria increase the upregulation of ZO-1 and claudin-14, but downregulation of claudin-2[81]. Raising claudin-2 levels lead to increased barrier permeability[82] and result in CD and UC[83,84]. OMVs and soluble factors, rather than TcpC, are able to strengthen the intestinal barrier[81].

**SECONDARY KEY PROTEINS**

***Flagellin***

Flagellin is a common conserved component of bacteria, and it induces both innate and specific immunity, showing a close relationship between dysbiosis and IBD[85], but flagellin of some probiotics has anti-inflammatory effects[86]. Flagellin is regarded as the major antigen in pathogenic bacteria. Flagellin binds with the pattern-recognition receptor Toll-like receptor 5 (TLR5), inducing the secretion of proinflammatory cytokines[87]. Compared with healthy controls, both Crohn’s disease (CD) and UC patients have a relative increase in the proportion of flagellin specific CD4+ T-cells. Cook *et al*[85] found a positive correlation between the relative abundance of bacteria [*Escherichia*/*Shigella* and (*Ruminococcus*) *gnavus* group] in IBD patients and high concentrations of flagellin antibodies, including anti-Fla2 IgG and anti-Fla2 IgA[88]. Specifically, CBir1 flagellin has been associated with complicated CD, and enzyme-linked immunosorbent assays proved that anti-CBir1 IgG is independently associated with CD[89]. Flagellin may provide a clinically novel approach to prevent pathogen infections, including vancomycin-resistant Enterococcus (VRE). Intestinal epithelial cells and Paneth cells secrete the antimicrobial protein (AMP) RegIIIγ to kill microorganisms and directly respond to flagellin *via* the Toll-like receptor (TLR)–myeloid differentiation factor 88–mediated pathway[90]. Flagellin of EcN stimulates intestinal epithelial cells to produce human β-defensin 2 *via* three main MAP kinase pathways, including ERK1/2, JNK, and p38[91]. Bacterial flagellin also induces negative regulation of inflammation. *Roseburia intestinalis* (*R. intestinalis*), a dominant symbiotic microbiota in the intestine, suppresses inflammation by inducing Treg cells and upregulating anti-inflammatory cytokines. However, *R. intestinalis* is significantly reduced in CD patients[86]. Flagellin in *R. intestinalis* induces the expression of lncRNA (HIF1A-AS2) in a dose- and time-dependent manner *via* p38 STAT1 activation, and HIF1A-AS2 inhibits the expression of inflammatory genes by suppressing NF-kB signaling pathway activation[92].

***Outer membrane proteins***

Some evidence linking intestinal dysbiosis with autoimmune diseases has shown that they are both associated with increased inflammation[93,94]. Bacterial outer membrane proteins are more likely to trigger an immune response, and the perinuclear antineutrophil cytoplasmic antibody (p-ANCA) present in many autoimmune diseases cross-reacts with outer membrane proteins. P-ANCA autoantibody is associated with UC. A p-ANCA monoclonal antibody detects outer membrane porins OmpC and OmpW expressed by colonic bacteria *Bacteroides caccae* and *E. coli*[95,96]. A structural relationship of the cross-reactive bacterial proteins and the p-ANCA autoantigen has been observed in IBD[95,96]. OmpC also enhances the adhesion and invasion of the CD-associated *E. coli* strain LF82 in intestinal epithelial cells through the sigma (E) regulatory pathway[97]. *Fusobacterium nucleatum* (Fn)has been found to be increased in the microbiota of diarrhea dominant IBS. The FomA protein is a major outer membrane protein of Fn[98]. The FomA of Fn is an immune adjuvant, which is a Toll-like receptor 2 (TLR2) agonist that induces upregulation of CD86 and MHC II in mice and primary B cells *in vitro* and antigen-specific antibody IgA and IgG secretion *in vivo*[99]. These characteristics enhance inflammation in the small intestine epithelium in both cell and mouse experiments[99]. Fn causes microbial dysbiosis, exacerbates visceral hypersensitivity in a colonization-independent manner, and induces the specific IgA agonist FomA[100]. Moreover, FomA has been proven to be an antigen that stimulates the secretion of symptom-associated antibodies[100].

***Other autoantibody-related antigens***

Primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH) are frequently associated with chronic IBD, including UC and CD[101]. The immune reaction in PSCs is mediated by autoantibodies, including pANCA, that recognize both β-tubulin isotype 5 (TBB-5) and the bacterial antigen cell division protein FtsZ[102]. Human TBB-5 and FtsZ share a high degree of structural homology in evolutionarily conserved epitopes[103]. Moreover, B cells respond directly to microbial constituents in PSCs and AIH[104].

*Helicobacter hepaticus* (Hh) can induce intestinal inflammation in DC-LMP1/CD40 mice[105]. These immunodeficient mice lost intestinal CD103+ DCs and IL-10+ Helios−induced Tregs (iTregs) but had increased IL17+ IFNγ+ Th17/Th1 cells and pathogenic IFNγ+ Th1 cells[106,107]. They developed fetal colitis similar to human IBD, because CD40-CD40L interactions are connected with the pathogenesis of IBD[108,109].A 60 kDa Hh-protein, GroEL, as the main antigen recognized by antibodies in an iTreg-free setting, triggers fatal colitis[110]. The bacterial GroEL and human heat shock protein 60 (Hsp60) share a high similarity and molecular mimicry[111,112], hence the antibodies cross-react with Hsp60 and GroEL, which contribute to IBD and autoimmune diseases[110].

**CONCLUSION**

Understanding the key bacterial proteins is significant to both the diagnosis and management of dysbiosis related diseases. For the incendiary proteins involved in autoimmune diseases and tumors, the presence of the specific marker in the microbiota or its specific antibodies might indicate the prognosis of diseases. The therapeutic value of targeting these markers would also be tempting. Knowing the key elements of microbiota could provide much more specific target than generally modulating the microbiota, which is super-high dimensional in taxonomy.

The current mainstream microbiome manipulation approaches are intensively investigated, including supplement of probiotics and prebiotics, and fecal microbiota transplantation (FMT). However, the probiotics should be strain-defined to gain standardized safety, dose, and effect; the adverse events associated with FMT have been found recently. The transplanted probiotics met indigenous microbiome-mediated mucosal colonization resistance in mice and even a specific colonization resistance in a person-, strain-, and region- dependent manner in humans[113]. Our recent mathematical model studies also suggested intriguing behavior of microbiome in response to probiotic supplement[114]. For FMT, the risk of unknown infections is still inevitable even after rigorous tests on the donors. The specific microbial proteins are easier to be cloned, purified, tested, optimized, and standardized, which is crucial for the pharmacology. Furthermore, the natural beneficial bacterial proteins can be artificially engineered and optimized to maximum their mechanism. This review summarizes the pathogenic and therapeutic mechanisms of some bioactive microbial proteins. This field is cutting edge, and there is a need for further studies to explore the role of the key gut microbial proteins in dysbiosis associated diseases.

**REFERENCES**

1 **Chen MX**, Wang SY, Kuo CH, Tsai IL. Metabolome analysis for investigating host-gut microbiota interactions. *J Formos Med Assoc* 2019; **118** Suppl 1: S10-S22 [PMID: 30269936 DOI: 10.1016/j.jfma.2018.09.007]

2 **De Angelis M**, Garruti G, Minervini F, Bonfrate L, Portincasa P, Gobbetti M. The Food-gut Human Axis: The Effects of Diet on Gut Microbiota and Metabolome. *Curr Med Chem* 2019; **26**: 3567-3583 [PMID: 28462705 DOI: 10.2174/0929867324666170428103848]

3 **Dagnelie MA**, Corvec S, Khammari A, Dréno B. Bacterial extracellular vesicles: A new way to decipher host-microbiota communications in inflammatory dermatoses. *Exp Dermatol* 2020; **29**: 22-28 [PMID: 31633842 DOI: 10.1111/exd.14050]

4 **Lopetuso LR**, Ianiro G, Scaldaferri F, Cammarota G, Gasbarrini A. Gut Virome and Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2016; **22**: 1708-1712 [PMID: 27206017 DOI: 10.1097/MIB.0000000000000807]

5 **Zuo T**, Lu XJ, Zhang Y, Cheung CP, Lam S, Zhang F, Tang W, Ching JYL, Zhao R, Chan PKS, Sung JJY, Yu J, Chan FKL, Cao Q, Sheng JQ, Ng SC. Gut mucosal virome alterations in ulcerative colitis. *Gut* 2019; **68**: 1169-1179 [PMID: 30842211 DOI: 10.1136/gutjnl-2018-318131]

6 **Marzano V**, Mancinelli L, Bracaglia G, Del Chierico F, Vernocchi P, Di Girolamo F, Garrone S, Tchidjou Kuekou H, D'Argenio P, Dallapiccola B, Urbani A, Putignani L. "Omic" investigations of protozoa and worms for a deeper understanding of the human gut "parasitome". *PLoS Negl Trop Dis* 2017; **11**: e0005916 [PMID: 29095820 DOI: 10.1371/journal.pntd.0005916]

7 **Srivastava A**, Gupta J, Kumar S, Kumar A. Gut biofilm forming bacteria in inflammatory bowel disease. *Microb Pathog* 2017; **112**: 5-14 [PMID: 28942174 DOI: 10.1016/j.micpath.2017.09.041]

8 **Koliarakis I**, Messaritakis I, Nikolouzakis TK, Hamilos G, Souglakos J, Tsiaoussis J. Oral Bacteria and Intestinal Dysbiosis in Colorectal Cancer. *Int J Mol Sci* 2019; **20** [PMID: 31450675 DOI: 10.3390/ijms20174146]

9 **Sharma G**, Sharma S, Sharma P, Chandola D, Dang S, Gupta S, Gabrani R. Escherichia coli biofilm: development and therapeutic strategies. *J Appl Microbiol* 2016; **121**: 309-319 [PMID: 26811181 DOI: 10.1111/jam.13078]

10 **Hancock V**, Dahl M, Klemm P. Probiotic Escherichia coli strain Nissle 1917 outcompetes intestinal pathogens during biofilm formation. *J Med Microbiol* 2010; **59**: 392-399 [PMID: 20110388 DOI: 10.1099/jmm.0.008672-0]

11 **Fang K**, Jin X, Hong SH. Probiotic Escherichia coli inhibits biofilm formation of pathogenic E. coli *via* extracellular activity of DegP. *Sci Rep* 2018; **8**: 4939 [PMID: 29563542 DOI: 10.1038/s41598-018-23180-1]

12 **Petrova MI**, Imholz NC, Verhoeven TL, Balzarini J, Van Damme EJ, Schols D, Vanderleyden J, Lebeer S. Lectin-Like Molecules of Lactobacillus rhamnosus GG Inhibit Pathogenic Escherichia coli and Salmonella Biofilm Formation. *PLoS One* 2016; **11**: e0161337 [PMID: 27537843 DOI: 10.1371/journal.pone.0161337]

13 **Pothoulakis C**, Lamont JT. Microbes and microbial toxins: paradigms for microbial-mucosal interactions II. The integrated response of the intestine to Clostridium difficile toxins. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G178-G183 [PMID: 11208538 DOI: 10.1152/ajpgi.2001.280.2.G178]

14 **McFarland LV**. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. *Am J Gastroenterol* 2006; **101**: 812-822 [PMID: 16635227 DOI: 10.1111/j.1572-0241.2006.00465.x]

15 **Castagliuolo I**, LaMont JT, Nikulasson ST, Pothoulakis C. Saccharomyces boulardii protease inhibits Clostridium difficile toxin A effects in the rat ileum. *Infect Immun* 1996; **64**: 5225-5232 [PMID: 8945570 DOI: 10.1128/IAI.64.12.5225-5232.1996]

16 **Moré MI**, Swidsinski A. Saccharomyces boulardii CNCM I-745 supports regeneration of the intestinal microbiota after diarrheic dysbiosis - a review. *Clin Exp Gastroenterol* 2015; **8**: 237-255 [PMID: 26316791 DOI: 10.2147/CEG.S85574]

17 **Wierstra I**, Alves J. Cyclin E/Cdk2, P/CAF, and E1A regulate the transactivation of the c-myc promoter by FOXM1. *Biochem Biophys Res Commun* 2008; **368**: 107-115 [PMID: 18206647 DOI: 10.1016/j.bbrc.2008.01.039]

18 **Chen H**, Liu H, Qing G. Targeting oncogenic Myc as a strategy for cancer treatment. *Signal Transduct Target Ther* 2018; **3**: 5 [PMID: 29527331 DOI: 10.1038/s41392-018-0008-7]

19 **Butler DSC**, Cafaro C, Putze J, Wan MLY, Tran TH, Ambite I, Ahmadi S, Kjellström S, Welinder C, Chao SM, Dobrindt U, Svanborg C. A bacterial protease depletes c-MYC and increases survival in mouse models of bladder and colon cancer. *Nat Biotechnol* 2021 [PMID: 33574609 DOI: 10.1038/s41587-020-00805-3]

20 **Chevalier C**, Stojanović O, Colin DJ, Suarez-Zamorano N, Tarallo V, Veyrat-Durebex C, Rigo D, Fabbiano S, Stevanović A, Hagemann S, Montet X, Seimbille Y, Zamboni N, Hapfelmeier S, Trajkovski M. Gut Microbiota Orchestrates Energy Homeostasis during Cold. *Cell* 2015; **163**: 1360-1374 [PMID: 26638070 DOI: 10.1016/j.cell.2015.11.004]

21 **Yoon HS**, Cho CH, Yun MS, Jang SJ, You HJ, Kim JH, Han D, Cha KH, Moon SH, Lee K, Kim YJ, Lee SJ, Nam TW, Ko G. Akkermansia muciniphila secretes a glucagon-like peptide-1-inducing protein that improves glucose homeostasis and ameliorates metabolic disease in mice. *Nat Microbiol* 2021; **6**: 563-573 [PMID: 33820962 DOI: 10.1038/s41564-021-00880-5]

22 **Ellingsgaard H**, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, Eppler E, Bouzakri K, Wueest S, Muller YD, Hansen AM, Reinecke M, Konrad D, Gassmann M, Reimann F, Halban PA, Gromada J, Drucker DJ, Gribble FM, Ehses JA, Donath MY. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat Med* 2011; **17**: 1481-1489 [PMID: 22037645 DOI: 10.1038/nm.2513]

23 **Mowat AM**. To respond or not to respond - a personal perspective of intestinal tolerance. *Nat Rev Immunol* 2018; **18**: 405-415 [PMID: 29491358 DOI: 10.1038/s41577-018-0002-x]

24 **Fernández-Tomé S**, Montalban-Arques A, Díaz-Guerra A, Galvan-Roman JM, Marin AC, Mora-Gutiérrez I, Ortega Moreno L, Santander C, Sánchez B, Chaparro M, Gisbert JP, Bernardo D. Peptides encrypted in the human intestinal microbial-exoproteome as novel biomarkers and immunomodulatory compounds in the gastrointestinal tract. *J Funct Foods* 2019; **52**: 459–468 [DOI: 10.1016/j.jff.2018.11.036]

25 **Fernández-Tomé S**, Marin AC, Ortega Moreno L, Baldan-Martin M, Mora-Gutiérrez I, Lanas-Gimeno A, Moreno-Monteagudo JA, Santander C, Sánchez B, Chaparro M, Gisbert JP, Bernardo D. Immunomodulatory Effect of Gut Microbiota-Derived Bioactive Peptides on Human Immune System from Healthy Controls and Patients with Inflammatory Bowel Disease. *Nutrients* 2019; **11** [PMID: 31683517 DOI: 10.3390/nu11112605]

26 **Quévrain E**, Maubert MA, Michon C, Chain F, Marquant R, Tailhades J, Miquel S, Carlier L, Bermúdez-Humarán LG, Pigneur B, Lequin O, Kharrat P, Thomas G, Rainteau D, Aubry C, Breyner N, Afonso C, Lavielle S, Grill JP, Chassaing G, Chatel JM, Trugnan G, Xavier R, Langella P, Sokol H, Seksik P. Identification of an anti-inflammatory protein from Faecalibacterium prausnitzii, a commensal bacterium deficient in Crohn's disease. *Gut* 2016; **65**: 415-425 [PMID: 26045134 DOI: 10.1136/gutjnl-2014-307649]

27 **Breyner NM**, Michon C, de Sousa CS, Vilas Boas PB, Chain F, Azevedo VA, Langella P, Chatel JM. Microbial Anti-Inflammatory Molecule (MAM) from *Faecalibacterium prausnitzii* Shows a Protective Effect on DNBS and DSS-Induced Colitis Model in Mice through Inhibition of NF-κB Pathway. *Front Microbiol* 2017; **8**: 114 [PMID: 28203226 DOI: 10.3389/fmicb.2017.00114]

28 **Quévrain E**, Maubert MA, Sokol H, Devreese B, Seksik P. The presence of the anti-inflammatory protein MAM, from Faecalibacterium prausnitzii, in the intestinal ecosystem. *Gut* 2016; **65**: 882 [PMID: 26669616 DOI: 10.1136/gutjnl-2015-311094]

29 **Yin M**, Yan X, Weng W, Yang Y, Gao R, Liu M, Pan C, Zhu Q, Li H, Wei Q, Shen T, Ma Y, Qin H. Micro Integral Membrane Protein (MIMP), a Newly Discovered Anti-Inflammatory Protein of Lactobacillus Plantarum, Enhances the Gut Barrier and Modulates Microbiota and Inflammatory Cytokines. *Cell Physiol Biochem* 2018; **45**: 474-490 [PMID: 29402771 DOI: 10.1159/000487027]

30 **He X**, Gao J, Peng L, Hu T, Wan Y, Zhou M, Zhen P, Cao H. Bacterial O-GlcNAcase genes abundance decreases in ulcerative colitis patients and its administration ameliorates colitis in mice. *Gut* 2020 [PMID: 33310751 DOI: 10.1136/gutjnl-2020-322468]

31 **Martinez FA**, Balciunas EM, Converti A, Cotter PD, de Souza Oliveira RP. Bacteriocin production by Bifidobacterium spp. A review. *Biotechnol Adv* 2013; **31**: 482-488 [PMID: 23384878 DOI: 10.1016/j.biotechadv.2013.01.010]

32 **Klaenhammer TR**. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol Rev* 1993; **12**: 39-85 [PMID: 8398217 DOI: 10.1111/j.1574-6976.1993.tb00012.x]

33 **Riley MA**, Wertz JE. Bacteriocin diversity: ecological and evolutionary perspectives. *Biochimie* 2002; **84**: 357-364 [PMID: 12423779 DOI: 10.1016/s0300-9084(02)01421-9]

34 **Collado MC**, Hernández M, Sanz Y. Production of bacteriocin-like inhibitory compounds by human fecal Bifidobacterium strains. *J Food Prot* 2005; **68**: 1034-1040 [PMID: 15895738 DOI: 10.4315/0362-028x-68.5.1034]

35 **Riley MA**, Wertz JE. Bacteriocins: evolution, ecology, and application. *Annu Rev Microbiol* 2002; **56**: 117-137 [PMID: 12142491 DOI: 10.1146/annurev.micro.56.012302.161024]

36 **Lee H**, Kim HY. Lantibiotics, class I bacteriocins from the genus Bacillus. *J Microbiol Biotechnol* 2011; **21**: 229-235 [PMID: 21464591]

37 **Sahl HG**, Bierbaum G. Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from gram-positive bacteria. *Annu Rev Microbiol* 1998; **52**: 41-79 [PMID: 9891793 DOI: 10.1146/annurev.micro.52.1.41]

38 **Millette M**, Cornut G, Dupont C, Shareck F, Archambault D, Lacroix M. Capacity of human nisin- and pediocin-producing lactic Acid bacteria to reduce intestinal colonization by vancomycin-resistant enterococci. *Appl Environ Microbiol* 2008; **74**: 1997-2003 [PMID: 18245231 DOI: 10.1128/AEM.02150-07]

39 **Sassone-Corsi M**, Nuccio SP, Liu H, Hernandez D, Vu CT, Takahashi AA, Edwards RA, Raffatellu M. Microcins mediate competition among Enterobacteriaceae in the inflamed gut. *Nature* 2016; **540**: 280-283 [PMID: 27798599 DOI: 10.1038/nature20557]

40 **Patzer SI**, Baquero MR, Bravo D, Moreno F, Hantke K. The colicin G, H and X determinants encode microcins M and H47, which might utilize the catecholate siderophore receptors FepA, Cir, Fiu and IroN. *Microbiology (Reading)* 2003; **149**: 2557-2570 [PMID: 12949180 DOI: 10.1099/mic.0.26396-0]

41 **Casaus P**, Nilsen T, Cintas LM, Nes IF, Hernández PE, Holo H. Enterocin B, a new bacteriocin from Enterococcus faecium T136 which can act synergistically with enterocin A. *Microbiology (Reading)* 1997; **143 ( Pt 7)**: 2287-2294 [PMID: 9245817 DOI: 10.1099/00221287-143-7-2287]

42 **Ankaiah D**, Palanichamy E, Antonyraj CB, Ayyanna R, Perumal V, Ahamed SIB, Arul V. Cloning, overexpression, purification of bacteriocin enterocin-B and structural analysis, interaction determination of enterocin-A, B against pathogenic bacteria and human cancer cells. *Int J Biol Macromol* 2018; **116**: 502-512 [PMID: 29729340 DOI: 10.1016/j.ijbiomac.2018.05.002]

43 **Chan SC**, Hui L, Chen HM. Enhancement of the cytolytic effect of anti-bacterial cecropin by the microvilli of cancer cells. *Anticancer Res* 1998; **18**: 4467-4474 [PMID: 9891511]

44 **Joo NE**, Ritchie K, Kamarajan P, Miao D, Kapila YL. Nisin, an apoptogenic bacteriocin and food preservative, attenuates HNSCC tumorigenesis *via* CHAC1. *Cancer Med* 2012; **1**: 295-305 [PMID: 23342279 DOI: 10.1002/cam4.35]

45 **Rubinstein MR**, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling *via* its FadA adhesin. *Cell Host Microbe* 2013; **14**: 195-206 [PMID: 23954158 DOI: 10.1016/j.chom.2013.07.012]

46 **An BC**, Hong S, Park HJ, Kim BK, Ahn JY, Ryu Y, An JH, Chung MJ. Anti-Colorectal Cancer Effects of Probiotic-Derived p8 Protein. *Genes (Basel)* 2019; **10** [PMID: 31430963 DOI: 10.3390/genes10080624]

47 **An BC**, Ryu Y, Yoon YS, Choi O, Park HJ, Kim TY, Kim SI, Kim BK, Chung MJ. Colorectal Cancer Therapy Using a *Pediococcus pentosaceus* SL4 Drug Delivery System Secreting Lactic Acid Bacteria-Derived Protein p8. *Mol Cells* 2019; **42**: 755-762 [PMID: 31707776 DOI: 10.14348/molcells.2019.0064]

48 **Pütsep K**, Brändén CI, Boman HG, Normark S. Antibacterial peptide from H. pylori. *Nature* 1999; **398**: 671-672 [PMID: 10227288 DOI: 10.1038/19439]

49 **Liu R**, Ni Y, Song J, Xu Z, Qiu J, Wang L, Zhu Y, Huang Y, Ji M, Chen Y. Research on the effect and mechanism of antimicrobial peptides HPRP-A1/A2 work against Toxoplasma gondii infection. *Parasite Immunol* 2019; **41**: e12619 [PMID: 30788848 DOI: 10.1111/pim.12619]

50 **Zhu J**, Huang Y, Chen M, Hu C, Chen Y. Functional Synergy Of Antimicrobial Peptides And Chlorhexidine Acetate Against Gram-Negative/Gram-Positive Bacteria And A Fungus In Vitro And In Vivo. *Infect Drug Resist* 2019; **12**: 3227-3239 [PMID: 31686873 DOI: 10.2147/IDR.S218778]

51 **Cho E**, Lee JK, Park E, Seo CH, Luchian T, Park Y. Antitumor activity of HPA3P through RIPK3-dependent regulated necrotic cell death in colon cancer. *Oncotarget* 2018; **9**: 7902-7917 [PMID: 29487701 DOI: 10.18632/oncotarget.24083]

52 **Hu C**, Chen X, Huang Y, Chen Y. Synergistic effect of the pro-apoptosis peptide kla-TAT and the cationic anticancer peptide HPRP-A1. *Apoptosis* 2018; **23**: 132-142 [PMID: 29397453 DOI: 10.1007/s10495-018-1443-1]

53 **Hao W**, Hu C, Huang Y, Chen Y. Coadministration of kla peptide with HPRP-A1 to enhance anticancer activity. *PLoS One* 2019; **14**: e0223738 [PMID: 31703065 DOI: 10.1371/journal.pone.0223738]

54 **Xu M**, Yamada M, Li M, Liu H, Chen SG, Han YW. FadA from Fusobacterium nucleatum utilizes both secreted and nonsecreted forms for functional oligomerization for attachment and invasion of host cells. *J Biol Chem* 2007; **282**: 25000-25009 [PMID: 17588948 DOI: 10.1074/jbc.M611567200]

55 **Abed J**, Emgård JE, Zamir G, Faroja M, Almogy G, Grenov A, Sol A, Naor R, Pikarsky E, Atlan KA, Mellul A, Chaushu S, Manson AL, Earl AM, Ou N, Brennan CA, Garrett WS, Bachrach G. Fap2 Mediates Fusobacterium nucleatum Colorectal Adenocarcinoma Enrichment by Binding to Tumor-Expressed Gal-GalNAc. *Cell Host Microbe* 2016; **20**: 215-225 [PMID: 27512904 DOI: 10.1016/j.chom.2016.07.006]

56 **Gur C**, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, Enk J, Bar-On Y, Stanietsky-Kaynan N, Coppenhagen-Glazer S, Shussman N, Almogy G, Cuapio A, Hofer E, Mevorach D, Tabib A, Ortenberg R, Markel G, Miklić K, Jonjic S, Brennan CA, Garrett WS, Bachrach G, Mandelboim O. Binding of the Fap2 protein of Fusobacterium nucleatum to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* 2015; **42**: 344-355 [PMID: 25680274 DOI: 10.1016/j.immuni.2015.01.010]

57 **Kaplan CW**, Lux R, Huynh T, Jewett A, Shi W, Haake SK. Fusobacterium nucleatum apoptosis-inducing outer membrane protein. *J Dent Res* 2005; **84**: 700-704 [PMID: 16040725 DOI: 10.1177/154405910508400803]

58 **Liu J**, Hsieh CL, Gelincik O, Devolder B, Sei S, Zhang S, Lipkin SM, Chang YF. Proteomic characterization of outer membrane vesicles from gut mucosa-derived fusobacterium nucleatum. *J Proteomics* 2019; **195**: 125-137 [PMID: 30634002 DOI: 10.1016/j.jprot.2018.12.029]

59 **Manson McGuire A**, Cochrane K, Griggs AD, Haas BJ, Abeel T, Zeng Q, Nice JB, MacDonald H, Birren BW, Berger BW, Allen-Vercoe E, Earl AM. Evolution of invasion in a diverse set of Fusobacterium species. *mBio* 2014; **5**: e01864 [PMID: 25370491 DOI: 10.1128/mBio.01864-14]

60 **Heise T**, Dersch P. Identification of a domain in Yersinia virulence factor YadA that is crucial for extracellular matrix-specific cell adhesion and uptake. *Proc Natl Acad Sci U S A* 2006; **103**: 3375-3380 [PMID: 16488979 DOI: 10.1073/pnas.0507749103]

61 **Eckburg PB**, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. *Science* 2005; **308**: 1635-1638 [PMID: 15831718 DOI: 10.1126/science.1110591]

62 **Martens EC**, Koropatkin NM, Smith TJ, Gordon JI. Complex glycan catabolism by the human gut microbiota: the Bacteroidetes Sus-like paradigm. *J Biol Chem* 2009; **284**: 24673-24677 [PMID: 19553672 DOI: 10.1074/jbc.R109.022848]

63 **Koropatkin NM**, Cameron EA, Martens EC. How glycan metabolism shapes the human gut microbiota. *Nat Rev Microbiol* 2012; **10**: 323-335 [PMID: 22491358 DOI: 10.1038/nrmicro2746]

64 **Lee SM**, Donaldson GP, Mikulski Z, Boyajian S, Ley K, Mazmanian SK. Bacterial colonization factors control specificity and stability of the gut microbiota. *Nature* 2013; **501**: 426-429 [PMID: 23955152 DOI: 10.1038/nature12447]

65 **Tuomola EM**, Ouwehand AC, Salminen SJ. The effect of probiotic bacteria on the adhesion of pathogens to human intestinal mucus. *FEMS Immunol Med Microbiol* 1999; **26**: 137-142 [PMID: 10536300 DOI: 10.1111/j.1574-695X.1999.tb01381.x]

66 **Segers ME**, Lebeer S. Towards a better understanding of Lactobacillus rhamnosus GG--host interactions. *Microb Cell Fact* 2014; **13** Suppl 1: S7 [PMID: 25186587 DOI: 10.1186/1475-2859-13-S1-S7]

67 **Lebeer S**, Claes I, Tytgat HL, Verhoeven TL, Marien E, von Ossowski I, Reunanen J, Palva A, Vos WM, Keersmaecker SC, Vanderleyden J. Functional analysis of Lactobacillus rhamnosus GG pili in relation to adhesion and immunomodulatory interactions with intestinal epithelial cells. *Appl Environ Microbiol* 2012; **78**: 185-193 [PMID: 22020518 DOI: 10.1128/AEM.06192-11]

68 **Ahmadi Badi S**, Moshiri A, Fateh A, Rahimi Jamnani F, Sarshar M, Vaziri F, Siadat SD. Microbiota-Derived Extracellular Vesicles as New Systemic Regulators. *Front Microbiol* 2017; **8**: 1610 [PMID: 28883815 DOI: 10.3389/fmicb.2017.01610]

69 **Maguire M**, Maguire G. Gut dysbiosis, leaky gut, and intestinal epithelial proliferation in neurological disorders: towards the development of a new therapeutic using amino acids, prebiotics, probiotics, and postbiotics. *Rev Neurosci* 2019; **30**: 179-201 [PMID: 30173208 DOI: 10.1515/revneuro-2018-0024]

70 **Camilleri M**. Leaky gut: mechanisms, measurement and clinical implications in humans. *Gut* 2019; **68**: 1516-1526 [PMID: 31076401 DOI: 10.1136/gutjnl-2019-318427]

71 **Moosavi SM**, Akhavan Sepahi A, Mousavi SF, Vaziri F, Siadat SD. The effect of *Faecalibacterium prausnitzii* and its extracellular vesicles on the permeability of intestinal epithelial cells and expression of PPARs and ANGPTL4 in the Caco-2 cell culture model. *J Diabetes Metab Disord* 2020; **19**: 1061-1069 [PMID: 33520823 DOI: 10.1007/s40200-020-00605-1]

72 **Ulluwishewa D**, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC. Regulation of tight junction permeability by intestinal bacteria and dietary components. *J Nutr* 2011; **141**: 769-776 [PMID: 21430248 DOI: 10.3945/jn.110.135657]

73 **Shan L**, Yu XC, Liu Z, Hu Y, Sturgis LT, Miranda ML, Liu Q. The angiopoietin-like proteins ANGPTL3 and ANGPTL4 inhibit lipoprotein lipase activity through distinct mechanisms. *J Biol Chem* 2009; **284**: 1419-1424 [PMID: 19028676 DOI: 10.1074/jbc.M808477200]

74 **Yoshida K**, Shimizugawa T, Ono M, Furukawa H. Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase. *J Lipid Res* 2002; **43**: 1770-1772 [PMID: 12401877 DOI: 10.1194/jlr.c200010-jlr200]

75 **Mandard S**, Zandbergen F, van Straten E, Wahli W, Kuipers F, Müller M, Kersten S. The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity. *J Biol Chem* 2006; **281**: 934-944 [PMID: 16272564 DOI: 10.1074/jbc.M506519200]

76 **Sukonina V**, Lookene A, Olivecrona T, Olivecrona G. Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. *Proc Natl Acad Sci U S A* 2006; **103**: 17450-17455 [PMID: 17088546 DOI: 10.1073/pnas.0604026103]

77 **Hering NA**, Richter JF, Fromm A, Wieser A, Hartmann S, Günzel D, Bücker R, Fromm M, Schulzke JD, Troeger H. TcpC protein from E. coli Nissle improves epithelial barrier function involving PKCζ and ERK1/2 signaling in HT-29/B6 cells. *Mucosal Immunol* 2014; **7**: 369-378 [PMID: 23900194 DOI: 10.1038/mi.2013.55]

78 **Ukena SN**, Singh A, Dringenberg U, Engelhardt R, Seidler U, Hansen W, Bleich A, Bruder D, Franzke A, Rogler G, Suerbaum S, Buer J, Gunzer F, Westendorf AM. Probiotic Escherichia coli Nissle 1917 inhibits leaky gut by enhancing mucosal integrity. *PLoS One* 2007; **2**: e1308 [PMID: 18074031 DOI: 10.1371/journal.pone.0001308]

79 **Zyrek AA**, Cichon C, Helms S, Enders C, Sonnenborn U, Schmidt MA. Molecular mechanisms underlying the probiotic effects of Escherichia coli Nissle 1917 involve ZO-2 and PKCzeta redistribution resulting in tight junction and epithelial barrier repair. *Cell Microbiol* 2007; **9**: 804-816 [PMID: 17087734 DOI: 10.1111/j.1462-5822.2006.00836.x]

80 **Toloza L**, Giménez R, Fábrega MJ, Alvarez CS, Aguilera L, Cañas MA, Martín-Venegas R, Badia J, Baldomà L. The secreted autotransporter toxin (Sat) does not act as a virulence factor in the probiotic Escherichia coli strain Nissle 1917. *BMC Microbiol* 2015; **15**: 250 [PMID: 26518156 DOI: 10.1186/s12866-015-0591-5]

81 **Alvarez CS**, Badia J, Bosch M, Giménez R, Baldomà L. Outer Membrane Vesicles and Soluble Factors Released by Probiotic *Escherichia coli* Nissle 1917 and Commensal ECOR63 Enhance Barrier Function by Regulating Expression of Tight Junction Proteins in Intestinal Epithelial Cells. *Front Microbiol* 2016; **7**: 1981 [PMID: 28018313 DOI: 10.3389/fmicb.2016.01981]

82 **Luettig J**, Rosenthal R, Barmeyer C, Schulzke JD. Claudin-2 as a mediator of leaky gut barrier during intestinal inflammation. *Tissue Barriers* 2015; **3**: e977176 [PMID: 25838982 DOI: 10.4161/21688370.2014.977176]

83 **Hering NA**, Fromm M, Schulzke JD. Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. *J Physiol* 2012; **590**: 1035-1044 [PMID: 22219336 DOI: 10.1113/jphysiol.2011.224568]

84 **Landy J**, Ronde E, English N, Clark SK, Hart AL, Knight SC, Ciclitira PJ, Al-Hassi HO. Tight junctions in inflammatory bowel diseases and inflammatory bowel disease associated colorectal cancer. *World J Gastroenterol* 2016; **22**: 3117-3126 [PMID: 27003989 DOI: 10.3748/wjg.v22.i11.3117]

85 **Cook L**, Lisko DJ, Wong MQ, Garcia RV, Himmel ME, Seidman EG, Bressler B, Levings MK, Steiner TS. Analysis of Flagellin-Specific Adaptive Immunity Reveals Links to Dysbiosis in Patients With Inflammatory Bowel Disease. *Cell Mol Gastroenterol Hepatol* 2020; **9**: 485-506 [PMID: 31790809 DOI: 10.1016/j.jcmgh.2019.11.012]

86 **Shen Z**, Zhu C, Quan Y, Yang J, Yuan W, Yang Z, Wu S, Luo W, Tan B, Wang X. Insights into Roseburia intestinalis which alleviates experimental colitis pathology by inducing anti-inflammatory responses. *J Gastroenterol Hepatol* 2018; **33**: 1751-1760 [PMID: 29532517 DOI: 10.1111/jgh.14144]

87 **Means TK**, Hayashi F, Smith KD, Aderem A, Luster AD. The Toll-like receptor 5 stimulus bacterial flagellin induces maturation and chemokine production in human dendritic cells. *J Immunol* 2003; **170**: 5165-5175 [PMID: 12734364 DOI: 10.4049/jimmunol.170.10.5165]

88 **Lodes MJ**, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, Fort M, Hershberg RM. Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest* 2004; **113**: 1296-1306 [PMID: 15124021 DOI: 10.1172/JCI20295]

89 **Targan SR**, Landers CJ, Yang H, Lodes MJ, Cong Y, Papadakis KA, Vasiliauskas E, Elson CO, Hershberg RM. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology* 2005; **128**: 2020-2028 [PMID: 15940634 DOI: 10.1053/j.gastro.2005.03.046]

90 **Kinnebrew MA**, Ubeda C, Zenewicz LA, Smith N, Flavell RA, Pamer EG. Bacterial flagellin stimulates Toll-like receptor 5-dependent defense against vancomycin-resistant Enterococcus infection. *J Infect Dis* 2010; **201**: 534-543 [PMID: 20064069 DOI: 10.1086/650203]

91 **Schlee M**, Wehkamp J, Altenhoefer A, Oelschlaeger TA, Stange EF, Fellermann K. Induction of human beta-defensin 2 by the probiotic Escherichia coli Nissle 1917 is mediated through flagellin. *Infect Immun* 2007; **75**: 2399-2407 [PMID: 17283097 DOI: 10.1128/IAI.01563-06]

92 **Quan Y**, Song K, Zhang Y, Zhu C, Shen Z, Wu S, Luo W, Tan B, Yang Z, Wang X. Roseburia intestinalis-derived flagellin is a negative regulator of intestinal inflammation. *Biochem Biophys Res Commun* 2018; **501**: 791-799 [PMID: 29772233 DOI: 10.1016/j.bbrc.2018.05.075]

93 **Wu HJ**, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes* 2012; **3**: 4-14 [PMID: 22356853 DOI: 10.4161/gmic.19320]

94 **de Oliveira GLV**, Leite AZ, Higuchi BS, Gonzaga MI, Mariano VS. Intestinal dysbiosis and probiotic applications in autoimmune diseases. *Immunology* 2017; **152**: 1-12 [PMID: 28556916 DOI: 10.1111/imm.12765]

95 **Cohavy O**, Bruckner D, Gordon LK, Misra R, Wei B, Eggena ME, Targan SR, Braun J. Colonic bacteria express an ulcerative colitis pANCA-related protein epitope. *Infect Immun* 2000; **68**: 1542-1548 [PMID: 10678972 DOI: 10.1128/iai.68.3.1542-1548.2000]

96 **Wei B**, Dalwadi H, Gordon LK, Landers C, Bruckner D, Targan SR, Braun J. Molecular cloning of a Bacteroides caccae TonB-linked outer membrane protein identified by an inflammatory bowel disease marker antibody. *Infect Immun* 2001; **69**: 6044-6054 [PMID: 11553542 DOI: 10.1128/IAI.69.10.6044-6054.2001]

97 **Rolhion N**, Carvalho FA, Darfeuille-Michaud A. OmpC and the sigma(E) regulatory pathway are involved in adhesion and invasion of the Crohn's disease-associated Escherichia coli strain LF82. *Mol Microbiol* 2007; **63**: 1684-1700 [PMID: 17367388 DOI: 10.1111/j.1365-2958.2007.05638.x]

98 **Bolstad AI**, Jensen HB, Bakken V. Taxonomy, biology, and periodontal aspects of Fusobacterium nucleatum. *Clin Microbiol Rev* 1996; **9**: 55-71 [PMID: 8665477 DOI: 10.1128/CMR.9.1.55-71.1996]

99 **Toussi DN**, Liu X, Massari P. The FomA porin from Fusobacterium nucleatum is a Toll-like receptor 2 agonist with immune adjuvant activity. *Clin Vaccine Immunol* 2012; **19**: 1093-1101 [PMID: 22623652 DOI: 10.1128/CVI.00236-12]

100 **Gu X**, Song LJ, Li LX, Liu T, Zhang MM, Li Z, Wang P, Li M, Zuo XL. *Fusobacterium nucleatum* Causes Microbial Dysbiosis and Exacerbates Visceral Hypersensitivity in a Colonization-Independent Manner. *Front Microbiol* 2020; **11**: 1281 [PMID: 32733392 DOI: 10.3389/fmicb.2020.01281]

101 **Broomé U**, Bergquist A. Primary sclerosing cholangitis, inflammatory bowel disease, and colon cancer. *Semin Liver Dis* 2006; **26**: 31-41 [PMID: 16496231 DOI: 10.1055/s-2006-933561]

102 **Tripathi A**, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, Knight R. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 397-411 [PMID: 29748586 DOI: 10.1038/s41575-018-0011-z]

103 **Terjung B**, Söhne J, Lechtenberg B, Gottwein J, Muennich M, Herzog V, Mähler M, Sauerbruch T, Spengler U. p-ANCAs in autoimmune liver disorders recognise human beta-tubulin isotype 5 and cross-react with microbial protein FtsZ. *Gut* 2010; **59**: 808-816 [PMID: 19951907 DOI: 10.1136/gut.2008.157818]

104 **Terjung B**, Spengler U. Atypical p-ANCA in PSC and AIH: a hint toward a "leaky gut"? *Clin Rev Allergy Immunol* 2009; **36**: 40-51 [PMID: 18626795 DOI: 10.1007/s12016-008-8088-8]

105 **Cahill RJ**, Foltz CJ, Fox JG, Dangler CA, Powrie F, Schauer DB. Inflammatory bowel disease: an immunity-mediated condition triggered by bacterial infection with Helicobacter hepaticus. *Infect Immun* 1997; **65**: 3126-3131 [PMID: 9234764 DOI: 10.1128/IAI.65.8.3126-3131.1997]

106 **Barthels C**, Ogrinc A, Steyer V, Meier S, Simon F, Wimmer M, Blutke A, Straub T, Zimber-Strobl U, Lutgens E, Marconi P, Ohnmacht C, Garzetti D, Stecher B, Brocker T. CD40-signalling abrogates induction of RORγt+ Treg cells by intestinal CD103+ DCs and causes fatal colitis. *Nat Commun* 2017; **8**: 14715 [PMID: 28276457 DOI: 10.1038/ncomms14715]

107 **Kusters P**, Seijkens T, Bürger C, Legein B, Winkels H, Gijbels M, Barthels C, Bennett R, Beckers L, Atzler D, Biessen E, Brocker T, Weber C, Gerdes N, Lutgens E. Constitutive CD40 Signaling in Dendritic Cells Limits Atherosclerosis by Provoking Inflammatory Bowel Disease and Ensuing Cholesterol Malabsorption. *Am J Pathol* 2017; **187**: 2912-2919 [PMID: 28935569 DOI: 10.1016/j.ajpath.2017.08.016]

108 **Ludwiczek O**, Kaser A, Tilg H. Plasma levels of soluble CD40 Ligand are elevated in inflammatory bowel diseases. *Int J Colorectal Dis* 2003; **18**: 142-147 [PMID: 12548417 DOI: 10.1007/s00384-002-0425-4]

109 **Danese S**, Sans M, Fiocchi C. The CD40/CD40L costimulatory pathway in inflammatory bowel disease. *Gut* 2004; **53**: 1035-1043 [PMID: 15194658 DOI: 10.1136/gut.2003.026278]

110 **Friedrich V**, Forné I, Matzek D, Ring D, Popper B, Jochum L, Spriewald S, Straub T, Imhof A, Krug A, Stecher B, Brocker T. *Helicobacter hepaticus* is required for immune targeting of bacterial heat shock protein 60 and fatal colitis in mice. *Gut Microbes* 2021; **13**: 1-20 [PMID: 33550886 DOI: 10.1080/19490976.2021.1882928]

111 **Füst G**, Uray K, Bene L, Hudecz F, Karádi I, Prohászka Z. Comparison of epitope specificity of anti-heat shock protein 60/65 IgG type antibodies in the sera of healthy subjects, patients with coronary heart disease and inflammatory bowel disease. *Cell Stress Chaperones* 2012; **17**: 215-227 [PMID: 22038196 DOI: 10.1007/s12192-011-0301-7]

112 **Bachmaier K,** Penninger JM. Chlamydia and Antigenic Mimicry. In: Oldstone M, editor. Molecular Mimicry: Infection-Inducing Autoimmune Disease. Berlin, Heidelberg: Springer Berlin Heidelberg, 2005: 153-163

113 **Zmora N**, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, Kotler E, Zur M, Regev-Lehavi D, Brik RB, Federici S, Cohen Y, Linevsky R, Rothschild D, Moor AE, Ben-Moshe S, Harmelin A, Itzkovitz S, Maharshak N, Shibolet O, Shapiro H, Pevsner-Fischer M, Sharon I, Halpern Z, Segal E, Elinav E. Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. *Cell* 2018; **174**: 1388-1405.e21 [PMID: 30193112 DOI: 10.1016/j.cell.2018.08.041]

114 **Li M**, Xu R, Li YQ. Sequential laxative-probiotic usage for treatment of irritable bowel syndrome: a novel method inspired by mathematical modelling of the microbiome. *Sci Rep* 2020; **10**: 19291 [PMID: 33168839 DOI: 10.1038/s41598-020-75225-z]

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**Figure Legends**



**Figure 1 Summary of the location or form of key bio-active microbiota proteins.** FtsZ and outer membrane proteins OmpC and OmpW were testified to stimulate perinuclear antineutrophil cytoplasmic antibody (p-ANCA). Flagellin was proved to stimulate p-ANCA, flagellin specific CD4+ T-cells, and flagellin associated IgG and IgA.

**Table 1 Summary of primary key proteins**

|  |  |  |  |
| --- | --- | --- | --- |
| **Classification** | **Name** | **Function** | **Ref.** |
| Enzyme | DegP | Inhibiting EHEC biofilms. | [11] |
| Enzyme | Llp1, Llp2 | Inhibiting biofilm formation of pathogen. | [12] |
| Enzyme | Protease of *S. boulardii* | Digesting both *C. difficile* toxin A and its receptor binding sites. | [15] |
| Enzyme | Lon protease | Degrading the oncogene c-MYC. | [19] |
| Secreted protein | P9 | Inducing the secretion of GLP-1. Inducing the secretion of IL-6 in macrophages. | [21,22] |
| Secreted protein | Peptide B7 | Reducing CCR2 expression on all APCs from health people. | [25] |
| Secreted protein | MAM | Inhibiting the NF-κB pathway and several cell immune responses. Inducing expression of TGF β. | [26–28] |
| Surface layer protein | MIMP | Inducing the secretion of anti- inflammatory cytokines and inhibiting inflammatory cytokines. Enhancing the intestinal barrier. | [29] |
| Enzyme | OGA | Hydrolysing O-GlcNAcylated NF-κB-p65 and IKKβ to inhibit NF-κB signaling. | [30] |
| Bacteriocins | PediocinPA-1/AcH nisin Z | Reducing colonization of VRE *in vivo*. | [38] |
|  | Microcin | limiting the expansion of pathogens. | [39,40] |
| Bacteriocins | Enterocins | Inhibiting a wide spectrum of Gram-positive bacteria. Inhibiting the growth of cancer cells. | [42,43] |
| Bacteriocins | Bacteriocin A, B | Degrading pathogenic biofilm and having antibacterial potential. | [36] |
| Bacteriocins | Nisin A | Changing the integrity of the cancer cell membrane. | [44] |
| Secreted protein | P8 | Inducing host cell growth arrest at the G2 phase. | [46,47] |
| Ribosomal proteins | HPRP-A1 HPRP-A2 | Resisting infection. Arresting the cancer cells cycle at the G0/G1 phase and G2/M phase. | [48-53] |
| Innermembrane protein | Pre-FadA | Binding host epithelial cells. | [54] |
| Secreted protein | m-FadA | Inducing the invasion of host cells. | [54] |
| Outer membrane protein | Fap2 | Leading to colonization of Fn. Facilitating tumor immunity evasion. Binding to and activating TIGIT. Inducing host lymphocyte apoptosis. | [55–57] |
| Secreted proteins | OMVs of Fn | Inducing the colonization of host epithelial cells. | [58-60] |
| Cell envelope-associated multiprotein systems | Sus-like systems | Inducing the colonization of host epithelial cells. | [64] |
| Pili | SpaCBA | Inducing the adhesion of mucus. | [66,67] |
| Secreted proteins | EVs | Inducing the expression of the TJ protein-encoding genes and regulating the intestinal barrier. Inducing the expression of *PPARα* and *PPARγ* genes and *ANGPTL4* gene. Inhibiting blood lipase lipoproteins in the bloodstream. | [71,72] |
| Secreted proteins | TcpC OMVs of EcN | Enhancing epithelial barrier. | [77-81] |

EcN: *Escherichia coli* Nissle 1917; EVs: extracellular vesicles; OMVs: outer membrane vesicles.

**Table 2 Summary of secondary key proteins**

|  |  |  |  |
| --- | --- | --- | --- |
| **Classification** | **Name** | **Function** | **Ref.** |
| Flagellin |  | Inducing the secretion of proinflammatory cytokines. | [87] |
| Flagellin |  | Recuiting flagellin specific CD4+ T-cells. | [85] |
| Flagellin |  | Inducing the secretion of flagellin antibodies. | [88,89] |
| Flagellin |  | Inducing the secretion of AMPs. | [90] |
| Flagellin |  | Inducing the secretion of human β-defensin 2. | [91] |
| Flagellin |  | Inducing the expression of lncRNA (HIF1A-AS2) and suppressing NF-kB signaling pathway activation. | [92] |
| Outer membrane protein | OmpCOmpW | Adhesion and invasion of the CD-associated *Escherichia coli* in intestinal epithelial cells. Cross-reactive bacterial proteins. | [95-97] |
| Outer membrane protein | FomA | Inducing upregulation of CD86, MHC II, and primary B cells. Inducing secretion of antigen-specific antibody IgA and IgG. | [99,100] |
| Bacterial division protein | FtsZ | Cross-reacting with TBB-5 and mediating the secretion of p-ANCA. | [102,103] |
| Bacterial heat shock protein | GroEL | Cross-reacting with Hsp60 and inducing antibodies. | [110,111] |

p-ANCA: perinuclear antineutrophil cytoplasmic antibody.



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