Limosilactobacillus fermentum, a Beneficial Inhabitant of the Intestinal Tract

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UEES

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Prologue

Probiotics refer to microorganisms that, when consumed in adequate quantities, confer health benefits to the host. Their effects extend beyond merely outcompeting harmful microbes; they also bolster the intestinal barrier and regulate immune responses. Lactic acid bacteria, particularly *Limosilactobacillus fermentum*, are commonly associated with probiotic properties and have been utilized in food fermentation and industrial processes. *L. fermentum* stands out for its ability to produce antimicrobial peptides, adhere to cells, and activate immune receptors. Indeed, different strains of these bacteria can influence the expression of specific genes in human cells. Through interactions with immune cells, they modulate crucial pathways in both innate and adaptive immune responses, particularly relevant in conditions such as colitis or inflammatory bowel disease. This manuscript provides an overview of the key characteristics of these probiotic bacteria, including the molecular mechanisms underlying their effects and their applications in both animal models and humans.

1. Introduction

The food we ingest undergoes a complex journey through the digestive tract, spanning several meters and encompassing over 20 specialized cells. Within this tract, these cells transform raw materials into essential nutrients, energy, and waste products. The gastrointestinal tract, extending from the mouth to the anus, houses vital organs crucial for digestion, including the esophagus, stomach, and intestines. Additionally, a diverse array of microorganisms, collectively referred to as gut microbiota, inhabit this system and are crucial for maintaining homeostasis. The tract is anatomically divided into upper and lower portions. The upper portion encompasses the mouth, pharynx, esophagus, stomach, and a segment of the small intestine, while the lower portion comprises the remainder parts of the small intestine and the large intestine. Within the small intestine, materials from the stomach are combined with digestive enzymes, bile, and pancreatic juices. It is here that fatty acids, amino acids, sugars, and other digestion byproducts are absorbed from the intestinal lumen.

The large intestine, also known as the colon, is responsible for absorbing water and ions. It encompasses the cecum and the appendix, followed by the ascending, transverse, and descending colon. The descending colon transitions into the sigmoid colon, which functions to store waste until elimination. The terminal segment of the large intestine is the rectum, where feces are temporarily stored. The anus serves as the final opening of the digestive tract, regulated by voluntary muscle control. Histologically, the gastrointestinal tract comprises four distinct layers: the mucosa, submucosa, muscular layer, and adventitia or serosa. The serosa is a smooth membrane that secretes fluids

facilitating muscle movement, while the adventitia consists of multiple layers of connective tissue. Peristalsis, the coordinated contraction of the muscular layer, propels food through the digestive tract by creating rhythmic waves of movement.

The submucosa is a layer of connective tissue, blood vessels, lymphatics, and nerves that extends into the mucosa, exerting influence over peristaltic activity and regulating factors including blood flow, water, and electrolyte secretion. Surrounding the lumen is the mucosa, which comprises three distinct layers: the epithelium, lamina propria, and muscularis mucosa. At the luminal interface lies the epithelium, composed of various cell types. Enterocytes, specialized intestinal cells, are responsible for absorbing ions, sugars, vitamins, peptides, lipids, and amino acids, while goblet cells secrete mucus, forming a protective barrier. Additionally, some cells within this layer produce hormones or antimicrobial proteins, while others regulate interactions between the host and microorganisms.

Various types of junctions facilitate connections between cells within the epithelium. Gap junctions, situated near the basal membrane, consist of protein complexes enabling the passage of ions, small molecules, and electrical impulses. Desmosomes and zonula adherens, comprised of transmembrane proteins, interact with cytoskeletal filaments, providing structural support. Tight junctions, found in apical membranes, are formed by multiple proteins and create a selective barrier between cells. Despite their tightness, tight junctions can undergo conformational changes, permitting the movement of solutes between cells. Enterocytes' apical surface is covered by microvilli, protected by a pericellular matrix of glycolipids and glycoproteins. Hydrolases within this layer can break down macromolecules, moving their monomers across the epithelium via specialized transporters. Moreover, water and electrolytes can traverse the barrier, with paracellular absorption modulated by junction complexes.

The intestinal barrier serves a dual purpose: it acts as a shield against potential pathogens while also contributing to intestinal immunity. Within the gut, an extensive immune system comprising lymphocytes, phagocytes, and antigen-presenting cells offers protection against invasion. Additionally, the acidic environment of the stomach poses a barrier to microorganisms, further reinforced by antibodies embedded within the mucus layer, which neutralize harmful intruders. The composition of the human microbiome is established at birth and evolves through interactions with the host. Microorganisms can be categorized based on these interactions as pathogenic, commensal, or mutualistic. Beneficial microbes play a vital role in metabolizing various compounds, including vitamins, sterols, and short-chain fatty acids (SCFA). Distinct regions of the digestive tract harbor different microbial populations; for example, anaerobic bacteria predominantly inhabit the colon, whereas aerobic bacteria are more prevalent in the cecum. Disruptions to the native microbiota, triggered by factors such as parasites, stress, or antibiotic usage, can lead to pathological disorders.

Pathogenic bacteria are known to incite inflammatory conditions, contributing to the onset of severe diseases such as cancer and obesity. Furthermore, a reduced diversity of gut microflora has been associated with inflammation. The host must interact with a multitude of microorganisms, either tolerating their presence or mounting an immune response against them. Epithelial cells, macrophages, and dendritic cells serve as sensors, expressing receptors that recognize specific molecular patterns produced by microbes. Activation of these receptors triggers the expression of inflammatory factors, facilitating the clearance of pathogens. However, when these receptors encounter commensal or mutualistic organisms, they do not elicit inflammatory responses; instead, they promote the synthesis of anti-inflammatory proteins. The proliferation of bacteria that trigger proinflammatory responses, along with a diminished number of beneficial bacteria, disrupts the functional balance of the microbiota. It has been proposed that supplementation with appropriate bacteria could reverse these conditions. Indeed, there are instances where the administration of probiotics has restored intestinal homeostasis.

Probiotics, as defined by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), are products containing live microorganisms expected to confer benefits to the host. The majority of probiotic microorganisms are bacteria, classified as prokaryotes due to their lack of a defined nucleus. These unicellular organisms are widespread, existing in various environments and playing vital roles in nutrient cycling and organic matter decomposition. Industrially, bacteria are utilized in the production of fermented goods and in treatments for sewage and oil spills. Bacteria establish diverse relationships with animals and plants. Humans, for instance, harbor approximately 10^14 bacteria, primarily in the gut and on the skin, comprising pathogenic, commensal, and beneficial strains. Bacterial cells exhibit a variety of shapes, including spherical, rod-shaped, spiral, or curved. They can exist singly, in pairs, clusters, chains, or form large multicellular structures. Bacteria employ quorum sensing, a process enabling cells to aggregate upon detecting chemical signals. These aggregates are often encapsulated within an extracellular matrix, known as a biofilm, composed of lipids, proteins, and polysaccharides.

The cytoplasm serves as the center for cellular metabolism, containing all the necessary building blocks. It is encased by a phospholipid membrane and further protected by a peptidoglycan wall. The characteristics of this wall have enabled the classification of bacteria through Gram staining. Gram-positive bacteria possess a thick peptidoglycan layer, while gram-negative bacteria feature a thinner layer, accompanied by an additional outer membrane composed of lipoproteins and oligosaccharides. Motility in bacteria is facilitated by protein complexes known as flagella, which enable movement through their environment. Additionally, attachment to various surfaces is achieved through filament proteins called fimbriae, aiding in colonization and biofilm formation. Bacteria possess a slightly larger structure known as the pilus, which is utilized for the transfer of genetic material between cells, facilitating horizontal gene transfer. Surrounding bacteria is a pericellular matrix called the glycocalyx, which shields cells from phagocytosis and is important in biofilm formation and adhesion. Bacteria can form intricate

associations with their hosts. Pathogenic bacteria are those that cause harm or severe diseases, such as *Clostridium tetani* or *Mycobacterium tuberculosis*. In contrast, commensal bacteria constitute the normal flora, although when present in different compartments, they can lead to infections. Additionally, certain bacteria can positively impact the host by contributing to gut immunity, synthesizing vitamins, metabolizing sugars into organic acids, fermenting complex carbohydrates, and inhibiting the growth of harmful microorganisms.

The benefits attributed to probiotics are closely tied to their structural characteristics and their role within the ecosystem. First, probiotic bacteria have the ability to synthesize molecules with antibacterial properties, such as peptides and organic acids. Second, these cells are capable of adhering to epithelial cells, triggering the activation of receptors that enhance the integrity of the intestinal barrier. These interactions also serve to prevent the attachment of potential pathogens. Finally, probiotics can initiate signaling pathways through receptors associated with the expression of cytokines, thereby modulating the immune response. Commonly recognized probiotics include lactic acid bacteria (LAB) and bifidobacteria, with other species of *Enterococcus*, *Lactococcus*, and *Saccharomyces* also acknowledged. Lactic acid bacteria, particularly those of the *Lactobacillus* genus, play a significant role in maintaining intestinal homeostasis. *Lactobacillus*, in particular, stands out as the most extensively studied genus, with over 200 described species.

Lactobacillales, also referred to as lactic acid bacteria, are not only present in decomposing materials like milk or plant residues but also inhabit the animal microbiome. These gram-positive bacteria carry out carbohydrate fermentation, leading to the production of organic acids and ATP generation. Two distinct mechanisms are recognized in this process. One pathway involves the exclusive production of lactic acid, while the other includes the synthesis of additional molecules such as acetic acid. Bacteria that exclusively produce lactic acid are termed homofermentative, whereas those producing other compounds are known as heterofermentative. Approximately 13 genera have been categorized within this family, including *Lactobacillus*, *Streptococcus*, and *Enterococcus*. These bacteria possess various

characteristics that make them ideal as key ingredients in functional foods. For instance, they produce exopolysaccharides known for their nutritional qualities and antibacterial compounds like bacteriocins, which are utilized for food preservation. Additionally, lactic acid bacteria (LAB) are commonly employed in the production of everyday items such as cheese, yogurt, beer, and wine. However, some of these bacteria, such as *Streptococcus mutans*, contribute to tooth decay by creating an acidic environment that leads to enamel demineralization. Particularly within the genus *Lactobacillus*, certain species are prevalent residents of the gastrointestinal tract. As a result, they must be capable of withstanding harsh environments characterized by low pH levels or high concentrations of bile salts. These bacteria can form biofilms, enabling them to survive in such conditions. Consequently, some lactic acid bacteria have developed mutualistic relationships with their hosts, providing potential protection against pathogenic invaders while obtaining shelter and nutrients. Recent advances in genetic and proteomic studies have led to the reclassification of some species into other genera. For example, *Lactobacillus fermentum* has been reclassified as *Limosilactobacillus fermentum*, reflecting the production of exopolysaccharides characteristic of this species.

L. fermentum are gram-positive bacteria with the ability to adhere to cells, synthesize antibacterial molecules, and interact with receptors that stimulate the expression of inflammation-associated genes. Thus, various strains have undergone testing using different approaches, including in vitro and in vivo studies. For example, the ability of *L. fermentum* strains to ferment dietary fiber, aiming to produce short-chain fatty acids (SCFA), has been assessed. These molecules can bind to receptors on lymphocytes and modulate their responses. Moreover, other strains have been evaluated for their antibacterial capabilities and tolerance to acidic conditions and high bile salt concentrations. Additionally, *L. fermentum* has been utilized in experiments with cellular cultures. For instance, studies have investigated the maturation of dendritic cells and the production of cytokines following inoculation with probiotics. Similarly, this species has been employed to examine the viability of macrophages and the production of inflammatory factors during pathogenic

infections. Furthermore, the protective capacity of this probiotic against *Salmonella* has been evaluated, with particular attention to the expression of inflammatory cytokines. Additionally, *L. fermentum* bacteria have been tested in studies aimed at activating lymphocytes and inducing cytokine expression. Due to its anti-inflammatory properties, some strains have been used in studies involving colitis, hepatic injury, inflammation, and cholesterol levels in aging mice.

Other studies have delved into the effects of probiotics on various health conditions, including hypertension, endothelial and intestinal barrier dysfunction, and bacterial infections. The outcomes of clinical research are pivotal in determining which probiotic strains are suitable for the development of functional foods. Three strains, namely *L. fermentum* ME-3, PCC, and CECT5716, have undergone thorough evaluation. These bacteria have been examined for their ability to modulate the gut microbiota and mitigate pathogenic conditions. In addition, probiotic interventions have been investigated for their effects on respiratory diseases and vaccination against the influenza virus H_1N_1. Similarly, the potential benefits of *L. fermentum* have been explored in the context of mastitis and gastrointestinal infections. Undoubtedly, the utilization of this species has provided valuable insights into the responses associated with inflammatory diseases across cell lines, animal models, and human trials.

L. fermentum bacteria are integral members of the normal animal microbiota, encompassing animals utilized for food production. Poultry, as well as pigs, cattle, and fish, are primarily bred for meat and other products. The advancement of the animal industry has relied on efficient breeding practices, optimal nutrition, and the management of infectious diseases. In particular, the control of infectious diseases poses a significant challenge to animal production as it diminishes animal health and productivity. While antibiotics and vaccines have historically mitigated such issues, their overuse has led to the emergence of antibiotic-resistant bacteria. Therefore, the use of antibiotics as growth enhancers has been restricted or banned in various countries. As a result, the industry is exploring novel alternatives such as

probiotics, prebiotics, algae and plants, and bacteriophages. *L. fermentum* strains have emerged as promising alternatives due to their ability to influence intestinal physiology in production animals. They have been shown to improve gut architecture, enhance epithelial conditions, promote microbiome diversity, and reduce the effects of pathogens. Therefore, in animal husbandry, this species represents an appealing alternative for enhancing intestinal health while mitigating infections.

This book provides a thorough examination of *L. fermentum*, covering its biological and technological features. It explores the interactions between this bacterium and eukaryotic cells, as well as highlights the health advantages of probiotics. By offering molecular insights and practical applications, readers can develop a deeper understanding of this intriguing microorganism and its potential implications for human and animal health.

2. The Intestinal Tract, a Trip Across the Gut

On average, humans consume between one and three kilograms of food per day, totaling over 300 kilograms annually. This ingested food traverses the digestive system, spanning over 9 meters and involving more than 20 specialized cells that convert raw materials into nutrients and energy, with waste expelled as feces. The gastrointestinal tract serves as the conduit for this process, extending from the mouth to the anus and encompassing organs such as the esophagus, stomach, and intestines. Alongside the actual tract, accessory organs including the gallbladder, liver, pancreas, salivary glands, and tongue contribute to digestion. The gastrointestinal tract hosts a diverse array of microorganisms, collectively known as gut microbiota, which play a crucial role in maintaining homeostasis by influencing cell metabolism and immune response. Additionally, specialized cells within the gastrointestinal tract secrete hormones such as gastrin and secretin, which regulate gastric acid secretion and water content to facilitate digestion.

The human gastrointestinal tract is segmented into two primary regions: the upper and lower tracts (Figure 1). The upper tract encompasses the mouth, pharynx, esophagus, stomach, and duodenum, while the lower tract comprises most of the small intestine and the large intestines. The small intestine, a tubular structure, features circular folds, villi, and microvilli that enhance its surface area, facilitating nutrient absorption into the bloodstream. It is divided into three sections: the duodenum, jejunum, and ileum. The duodenum, approximately 25 cm long, receives acidic chyme from the stomach, which is mixed with bile and pancreatic juices for digestion. Brunner's glands secrete bicarbonate to neutralize the acidic environment. The jejunum, measuring

around 2.5 m long, is the midsection where nutrients are absorbed into the bloodstream. Lastly, the ileum is responsible for the absorption of certain vitamins, bile acids, and remaining nutrients.

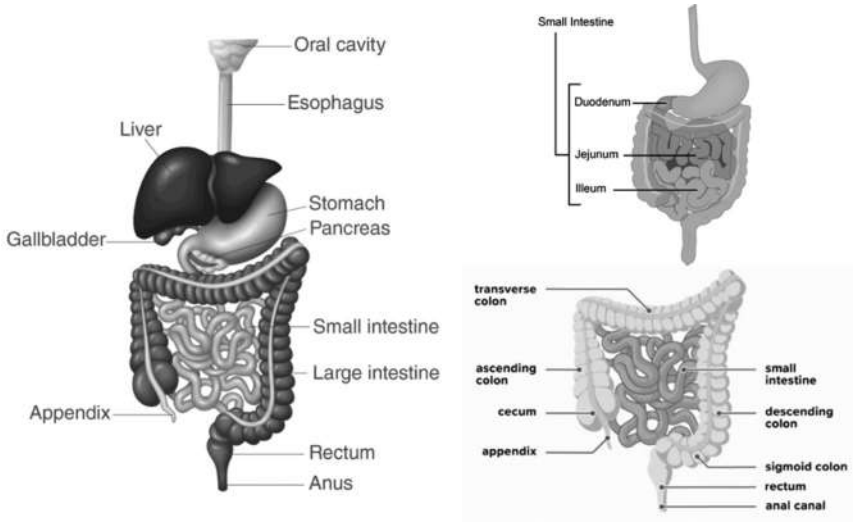


Figure 1. The gastrointestinal tract. Adapted from *"Gastrointestinal Tract"*. (n.d.). BYJU'S. Retrieved November 15, 2023 from https://byjus.com/biology/gastrointestinal-tract/; Agarwal, A. (2018, April 23). *"Small Intestine"*. KNOW YOUR BODY. Retrieved November 16, 2023 from https://www.knowyourbody.net/small-intestine.html; Sethi, S. & Seladi-Schulman, J. (2023, March 13). *"What's the Length of Your Small and Large Intestines?"*. HEALTHLINE. Retrieved November 16, 2023 from https://www.healthline.com/health/digestive-health/how-long-are-your-intestines.

The large intestine, also referred to as the colon, extends from the cecum to the rectum and anal canal, measuring approximately 1.5 meters in length with a mucosal surface area of 2 square meters. Its primary role involves the absorption of water and ions. Divided into nine parts, it includes the cecum connected to the appendix, which has been suggested to potentially serve as a reservoir for beneficial bacteria rather than being vestigial (Figure 1). The ascending colon, located between the transverse colon and the cecum, absorbs remaining water and nutrients from indigestible material, forming stool. The transverse colon crosses the abdomen, connecting to the descending colon via the left colic flexure. The descending colon continues the process of water

and salt absorption before transitioning into the sigmoid colon, where waste is stored until elimination in solid form. Due to its looped structure, gas expulsion occurs without feces excretion.

The rectum, the final segment of the large intestine, spans approximately 15 to 20 centimeters and extends from the end of the sigmoid colon to the anal surface. Its primary function is the temporary storage of feces, which are propelled from the descending colon via regular muscle contractions called peristalsis. As fecal material accumulates, stretch receptors in the rectal walls stimulate the urge to defecate. Subsequently, the anal canal connects the rectum to the anus, the terminal opening of the canal. The anus is surrounded by voluntary muscle that regulates the release of feces, contracting normally but relaxing during defecation. During fetal development, the gut originates from the endoderm and can be divided into three segments: the foregut, midgut, and hindgut. Each segment gives rise to specific gut structures and associated components. For example, the foregut contributes to the esophagus, stomach, part of the duodenum, gallbladder, liver, and pancreas. The midgut gives rise to the lower duodenum, jejunum, ileum, cecum, appendix, ascending colon, and part of the transverse colon. Conversely, the hindgut contributes to the last part of the transverse colon, along with the descending colon, rectum, and part of the anal canal.

The gastrointestinal tract's wall structure comprises four histological layers: adventitia or serosa, muscular layer, submucosa, and mucosa (Figure 2). The outermost layer, adventitia, consists of various connective tissue layers. Connective tissue, one of the four primary tissue types alongside epithelial, muscle, and nervous tissues, typically contains fibers, ground substance, and cells. Cells include leukocytes, macrophages, adipocytes, mast cells, and fibroblasts, while ground substance is a gel-like structure located in the extracellular space, comprising water with organic molecules including polysaccharides and glycoproteins secreted by fibroblasts. Furthermore, elastic and collagen fibers are present. Adventitia covers retroperitoneal portions of the tract, such as part of the duodenum, ascending and descending colon, and mid-rectum. Intraperitoneal sections are covered with serosa, including the

stomach, part of the duodenum, appendix, cecum, transverse and sigmoid colon, and upper rectum. Serosa, a smooth tissue membrane lining cavity walls, secretes a serum-like fluid, serving as a lubricant to reduce friction during muscle movement.

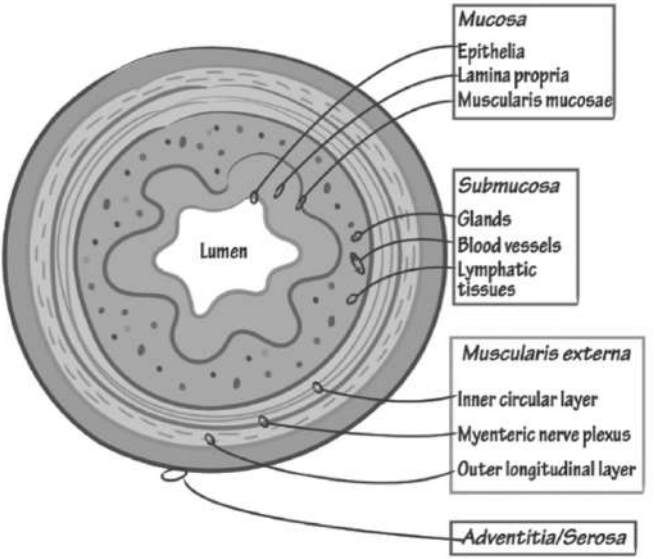


Figure 2. Histological layers of the gastrointestinal tract. Adapted from *"Gastrointestinal Tract Tunics"*. (n.d.). GLOSSARY. Retrieved November 21, 2023 from https://ditki.com/course/gross-anatomy/glossary/term/gastrointestinal-tract-tunics.

The muscular layer consists of an inner circular and an outer longitudinal layer, with the myenteric plexus situated between them to provide motor innervation. Peristalsis, the synchronized contraction of these layers, propels food through the digestive tract. This intrinsic gut activity is initiated by pacemaker cells that generate electrical signals and trigger action potentials. The submucosa comprises a layer of connective tissue containing blood vessels, lymphatics, and nerves branching into the mucosa. It houses the submucosal plexus, regulating peristaltic activity, blood flow, and secretion of water and electrolytes. The mucosa comprises three layers: the epithelium, lamina propria mucosae, and muscularis mucosae. The muscularis mucosae, a smooth muscle layer, facilitates interaction between the epithelium and luminal contents via

contraction and relaxation. Meanwhile, the lamina propria mucosae, abundant in lymphocytes and macrophages, contributes to the immune response. Inflammation, known as a risk factor for cancer development, is associated with macrophages releasing pro-inflammatory factors implicated in colitis-induced cancer.

The epithelium forms the inner lining of the mucosa, which covers the luminal surface of both the small and large intestines in the digestive tract. Comprised of epithelial cells, it serves to absorb substances while also acting as a barrier against harmful agents. Disruptions in intestinal function can lead to various conditions and illnesses. The cells within this epithelial layer are interconnected by various cellular junctions, presenting a continuous appearance. Renewal of these cells occurs approximately every 4 to 5 days, with stem cells located at the base of the crypt dividing to produce new cells that migrate upwards and differentiate. Eventually, these cells undergo apoptosis and are shed into the lumen, ensuring the continual renewal of the epithelial lining. The small intestine features anatomical structures that significantly increase the absorptive surface area, including circular folds, villi, and microvilli. Circular folds are transverse folds that expand the surface area threefold and slow down the movement of luminal contents. Villi further increase the surface area tenfold, while microvilli, tiny finger-like protrusions covering the apical pole of enterocytes, amplify the surface area 20-fold (Figure 3).

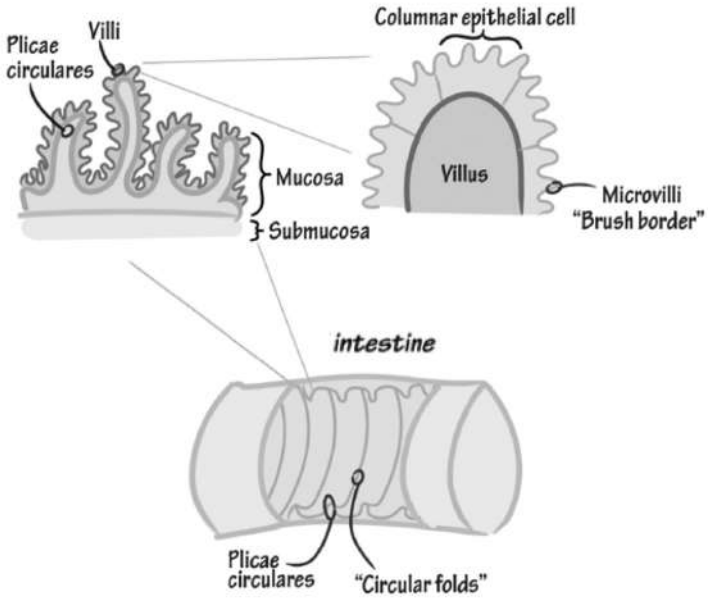


Figure 3. The intestinal epithelium. Adapted from *"Intestinal Folding: Villi & Microvilli"*. GLOSSARY. Retrieved November 21, 2023 from https://ditki.com/coursebiochemistry/glossary/physiological-process/intestinal-folding-villi-microvilli.

The stem cells located in crypts give rise to seven distinct cell types, each following specific differentiation pathways as they migrate out of the crypt (Figure 4). This differentiation process involves the differential expression of at least 50 genes. Enterocytes, the most prevalent cell type, are responsible for absorbing ions, sugars, peptides, lipids, amino acids, and other solutes. To facilitate nutrient uptake, these cells synthesize catabolic enzymes on their luminal surface. Goblet cells, conversely, specialize in secreting mucus, a protective film composed of various substances including inorganic salts and proteins. Mucins, crucial components of mucus, form gel-like structures via a multistep process. Initially, mucins dimerize at the carboxy-terminal regions, followed by association with other proteins at the amino-terminal sites. These heavily glycosylated proteins retain water and are resistant to protease action.

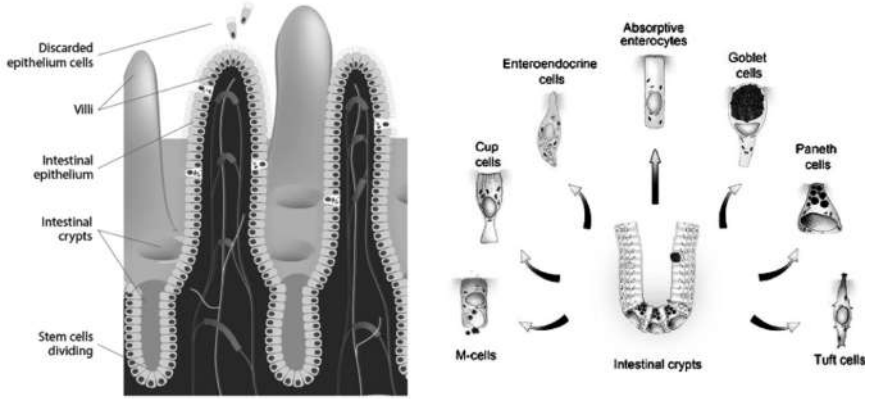


Figure 4. Intestinal epithelial cell types. Adapted from *"New Stem Cell Mechanism in Your Gut"*. Feigl, M. (2022, July 13). EUREKALERT. Retrieved November 27, 2023 from https://www.eurekalert.org/news-releases/958563; *"p62 Knock-Out Mice: Investigation on Gastrointestinal Tract"*. Romito, L. (2019). [Bachelor's thesis, University of Camerino]. https://www.researchgate.net/publication/357574094_p62_knock-out_mice_investigation_on_gastrointestinal_tract.

Enteroendocrine cells represent another specialized group, responsible for producing various gastrointestinal hormones. For instance, secretin regulates water balance, while pancreozymin aids in fat and protein digestion. Paneth cells, on the other hand, focus on synthesizing antimicrobial peptides that target bacterial membranes. Initially, these positively charged molecules interact with negatively charged bacterial membranes, forming pore complexes that induce membrane depolarization and cell lysis. Microfold cells, also known as M cells, facilitate the transport of antigens from the lumen to the mucosal-associated lymphoid tissue, while tuft cells play a role in immune responses. Lastly, cup cells have been identified for their involvement in host-microbe interactions.

The epithelial cells are interconnected by four types of junctions (Figure 5). Gap junctions, situated near the basal membrane, unite adjacent cells through hexameric complexes of connexins. These complexes create channels in the membranes, facilitating the passage of ions, molecules, and electrical impulses. Desmosomes, composed of transmembrane proteins, form strong connections

between cells, with cadherins, linker proteins, and keratin contributing to their structure. Zonula adherens, also known as adherens junctions, include interactions between catenins and cadherins, aiding in cell migration, polarity, and junction assembly. Tight junctions, or zonula occludens, located near the apical membrane, consist of claudins and occludins. These complexes connect adjacent cells extracellularly and regulate polarity, signaling, and vesicle trafficking intracellularly. Despite forming a tight seal, these junctions can be altered to allow the passage of solutes between cells, particularly ions and water-soluble compounds.

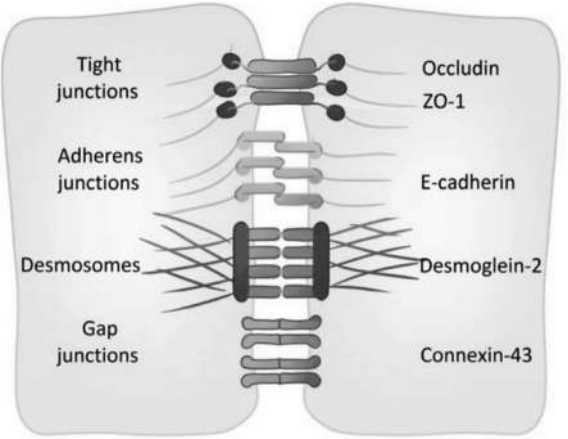


Figure 5. Types of cell junctions. Adapted from *"Comparative Analysis of Cell–Cell Contact Abundance in Ovarian Carcinoma Cells Cultured in Two- and Three-Dimensional In Vitro Models"* by O. M. Kutova, L. M. Sencha, A. D. Pospelov, O. E. Dobrynina, A. A. Brilkina, E. I. Cherkasova, I. V. Balalaeva, 2020, Biology, 9, p. 446.

ZO-1, Zonula Occludens-1.

The apical surface of enterocytes features microvilli, forming a striated or brush border, protected by a pericellular matrix called the glycocalyx. This matrix consists of oligosaccharide side chains associated with glycolipids and glycoproteins. Digestive enzymes, such as hydrolases, facilitate the breakdown of sugars and proteins, with resulting monomers transported across the epithelium to the bloodstream. Moreover, electrolytes and water are absorbed through the membrane, either via bulk flow or selective

permeability routes. Selective permeability occurs through the transcellular route, where specialized transporter proteins mediate solute translocation, or the paracellular route, primarily responsible for water and solute transport, regulated by junction complexes in the lateral membrane of epithelial cells (Figure 6).

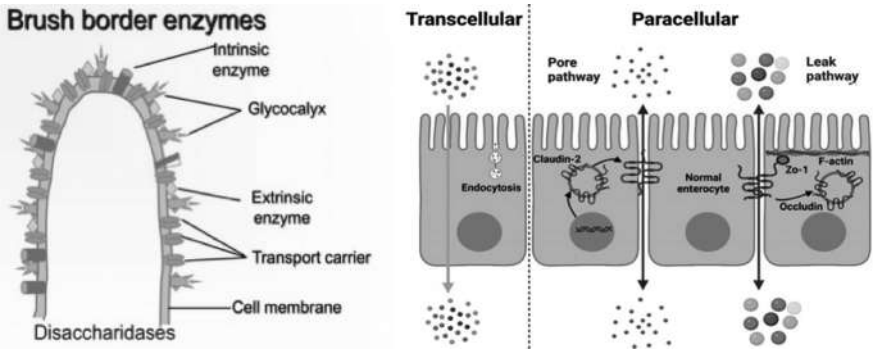


Figure 6. Brush border and membrane selective permeability. Adapted from *"Intestinal Barrier Functions in Hematologic and Oncologic Diseases"* by E. Haroun, P. A. Kumar, L. Saba, J. Kassab, K. Ghimire, D. Dutta, S. H. Lim, 2023, *Journal of Translational Medicine*, 21, p. 233; *"Gastrointestinal Physiology: (3) Digestion and Absorbtion"*. (n.d.). QUIZLET. Retrieved November 30, 2023 from https://quizlet.com/de/776549297/gastrointestinal-physiology-3-digestion-and-absorbtion-flash-cards/.

In addition to acting as a physical barrier between the internal and external environments, the intestinal epithelium is crucial for maintaining balance of the immune system. It is closely associated with the gut-associated lymphoid tissue (GALT), which is a component of the mucosa-associated lymphoid tissue (MALT) responsible for defending against microbial invasion. GALT contains various immune cells, including T and B cells, macrophages, and dendritic cells, distributed throughout the submucosal membrane. Particularly, specialized M cells sample antigens from the intestinal lumen and facilitate immune surveillance. The lamina propria mucosa, a layer of connective tissue beneath the epithelial cells, houses lymphatic circulation. GALT can be classified into organized (e.g., Peyer's patches) and diffuse (e.g., lamina propria lymphocytes and intraepithelial lymphocytes) structures, both contributing to

immune defense. Additionally, factors such as the acidic pH of the stomach and antibodies in mucus further inhibit pathogen invasion (Figure 7).

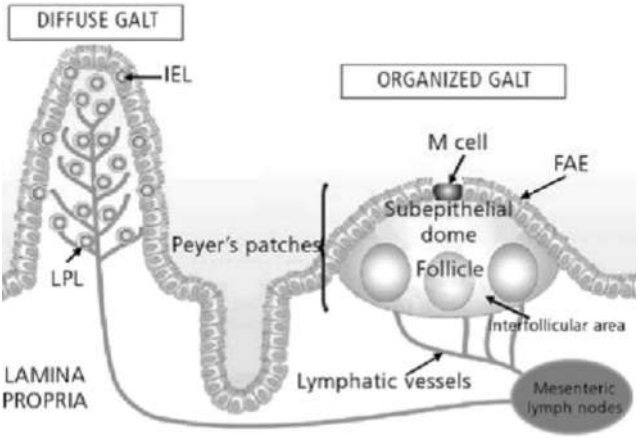


Figure 7. Elements of gut-associated lymphoid tissue, GALT. Adapted from *"The Bowel: A Key Component of the Immune System"* by E. Ramiro-Puig, F. J. Pérez-Cano, C. Castellote, A. Franch, M. Castell, 2008, *Revista Española de Enfermedades Digestivas*, 100, pp. 29-34. GALT, Gut-associated lymphoid tissue; FAE, Follicle-associated epithelium; IEL, Intraepithelial lymphocytes; LPL, Lamina propria lymphocytes.

In humans, the microbiome's composition is established early, often at birth, shaping a symbiotic relationship with the host. Beneficial microbes not only aid in synthesizing vitamins and absorbing nutrients like short-chain fatty acids but also contribute to metabolizing various compounds crucial for health, such as bile acids and sterols. Disruptions in this balanced microbiota can lead to inflammatory disorders. The digestive tract harbors diverse microbial communities, with the colon hosting the highest density, predominantly anaerobic bacteria, while aerobic bacteria are prevalent in the cecum. Additionally, other microorganisms including viruses, archaea, protists, and fungi inhabit different regions, although their functions remain less understood.

In the stomach, harsh acidic conditions create an inhospitable environment for many organisms. However, species like *Lactobacillus*, *Streptococcus*, *Staphylococcus*, and *Helicobacter* have adapted to survive in this ecosystem.

Helicobacter spp., specifically gram-negative spiral bacteria, colonize the gastric mucosa, leading to inflammation and ulcers. Moving into the duodenum, the most proximal part of the small intestine, gram-positive bacteria predominate due to its proximity to the stomach. In contrast, the more distal portions like the jejunum and ileum have alkaline conditions that support the growth of gram-negative bacteria, particularly from the *Enterobacteriaceae* family. The dominant phyla in the gut microbiota include Pseudomonadota, Verrucomicrobiota, Actinomycetota, Bacteroidota, and Bacillota (Firmicutes), with the latter two representing about 90% of the population, mainly found in the colon. Any disruption in this microbiota, caused by factors such as parasites, stress, or antibiotics, can lead to pathological conditions, due to the multitude of beneficial functions these microbes perform for the host.

Some bacteria, termed pathogenic, contribute to inflammatory conditions and the development of certain diseases such as colon cancer and obesity. *H. pylori*, for example, can induce stomach ulcers by penetrating the epithelium, triggering an inflammatory response. Inflammatory bowel diseases (IBD), which encompass conditions including ulcerative colitis and Crohn's disease, are closely linked to the gut flora and its interaction with the host. Individuals with these disorders typically exhibit lower gut microflora diversity compared to healthy individuals. In Crohn's disease, Proteobacteria and *Enterococcus* spp. are dominant, while ulcerative colitis is associated with over-representation of Proteobacteria and Actinobacteria. Additionally, reduced microbiome diversity is observed in irritable bowel syndrome, characterized by abdominal pain and irregular bowel movements. This condition is marked by low levels of lactic acid bacteria and high levels of facultative anaerobic bacteria including *Escherichia coli*.

As the host must interact with a variety of microorganisms, several mechanisms for defense against and tolerance to microbes have evolved (Figure 8). Defense involves limiting microbial entry, facilitated by the presence of a mucus layer and the synthesis of antimicrobial peptides, as well as the production of secretory immunoglobulin A by B cells. Epithelial cells, macrophages, and dendritic cells serve as innate microorganism sensors.

Microbial recognition is mediated by a specific group of receptors known as pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), NOD-like receptors (NLRs), and C-type lectin receptors. Signaling through these PRRs activates pathways that induce microbial killing and trigger the expression of proinflammatory cytokines by T_{H1} and T_{H17} cells. Conversely, in immune tolerance and homeostasis, activation of these receptors in macrophages and dendritic cells does not induce expression or secretion of proinflammatory cytokines. In Peyer's patches, dendritic cells interact with T cells, leading to the differentiation of regulatory T cells (T_{reg}) mediated by interleukin-10 (IL-10), transforming growth factor-β (TGF-β), and retinoic acid.

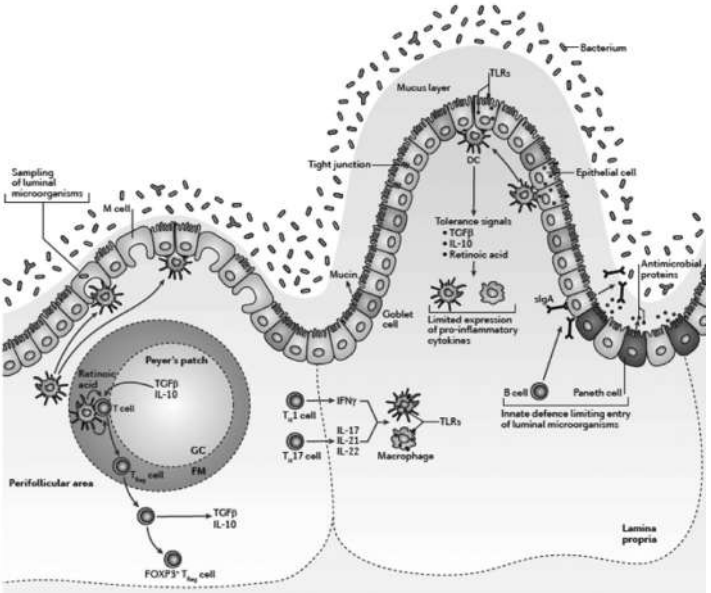


Figure 8. Intestinal interaction with microorganisms. Adapted from *"Emerging Molecular Insights into the Interaction Between Probiotics and the Host Intestinal Mucosa"* by P. A. Bron, P. Van Baarlen, M. Kleerebezem, 2012, *Nature Reviews Microbiology*, 10, pp. 66-78. GC, Germinal center; FM, Follicular mantle; Th, Helper T cell; FOXP3, Forkhead Box P3; TLR, Toll-like receptor; IL, Interleukin; IFN, Interferon; TGF, Transforming growth factor; sIgA, Secretory immunoglobulin A.

As discussed previously, the overgrowth of certain bacteria can provoke an immune response characterized by heightened expression of proinflammatory cytokines. This disruption in the microbiota's balance, known as dysbiosis, alters their functional compositions and metabolic activities. It has been suggested that individuals with alleles linked to inflammatory bowel disease experience an increase in T_{H17} cells and a decrease in T_{reg} cells due to an excess of bacteria that trigger proinflammatory reactions, or a scarcity of beneficial bacteria that induce anti-inflammatory cytokine expression. Dysbiosis may be alleviated by administering specific bacterial taxa, with probiotics being a potential solution. Probiotics, mostly comprising gram-positive bacteria, produce molecules that can signal through PRRs, modulating the immune response. Additionally, probiotics can competitively exclude potentially pathogenic bacteria from the epithelium and enhance the function of the epithelial barrier. Let's now explore how these bacteria can positively impact gut health.

3. Probiotics and Their Characteristics

The World Health Organization (WHO), in collaboration with the Food and Agriculture Organization (FAO), initially defined probiotics as "live microorganisms that, when consumed in adequate amounts, confer a health effect on the host" (FAO/WHO 2001). However, this definition has been revised more recently and now states: "products that deliver live microorganisms with a suitable viable count of well-defined strains with a reasonable expectation of delivering benefits for the well-being of the host" (Hill et al., 2014, pp. 506-514).

The beneficial effects of probiotics stem from their unique structural features and ecological roles. First, probiotics produce various bacteriostatic compounds such as antibacterial peptides and organic acids, which inhibit the growth of harmful bacteria. Second, they enhance the host immune response by activating receptors that trigger cytokine expression, thus regulating immunoglobulin production. Additionally, probiotics adhere to epithelial cells via molecules such as exopolysaccharides and lipoteichoic acids (LTA), improving the intestinal barrier and microbiota, thus preventing epithelial dysfunction and dysbiosis. Moreover, their adhesion to epithelial cells competitively excludes potential pathogens, reducing infection risks. However, the efficacy of probiotics depends on factors like dosage, species, and strains, requiring further research to understand their molecular mechanisms for potential commercial applications.

Before digging into the detailed structures of probiotic bacteria, let's review some fundamental concepts regarding bacterial cells. Bacteria are primarily unicellular, free-living microorganisms classified as prokaryotes due to their lack of a defined nucleus. Among the oldest life forms on Earth, they typically measure a few micrometers in length and inhabit diverse environments. Bacteria play pivotal ecological roles by contributing to nutrient cycling (e.g., nitrogen, carbon, sulfur) and organic matter decomposition. They have evolved various relationships with animals and plants, ranging from commensal to parasitic or mutualistic. In fact, humans and other animals harbor vast numbers of bacteria, ranging from 10^12 to 10^14, predominantly in the gut and on the skin. While many are harmless or even beneficial by stimulating the immune system, some are pathogenic and can cause infections, often treated with antibiotics, although the rise of antibiotic-resistant bacteria poses a significant public health concern. Additionally, bacteria find extensive use in diverse fields, including sewage and oil spill remediation, as well as in food production (e.g., yogurt, cheese), fermentation processes, and biotechnology.

Bacteria exhibit a diverse range of sizes, typically from 0.5 to 5.0 μm in length, although exceptional cases exist. Particularly, some bacteria, like *Thiomargarita magnifica*, can be visible to the naked eye and reach lengths of almost 2 cm, while others, such as *Mycoplasma* species, are among the smallest bacteria, comparable in size to the largest viruses (0.3 μm). Moreover, bacteria display various shapes: cocci for spherical forms, bacilli for rod-shaped bacteria, curved rods (e.g., *Vibrio*), and spirals (e.g., *Spirochaetes*) (Figure 9). Although most bacteria exist as single cells, some form associations with others. For example, *Neisseria* spp., responsible for gonorrhoea and meningococcal diseases, occur as diploids, while *Streptococcus* and *Staphylococcus* form chains and clusters, respectively. Moreover, certain bacteria such as those of Actinomycetota, Myxobacteria, and *Streptomyces* can form large multicellular structures under specific conditions.

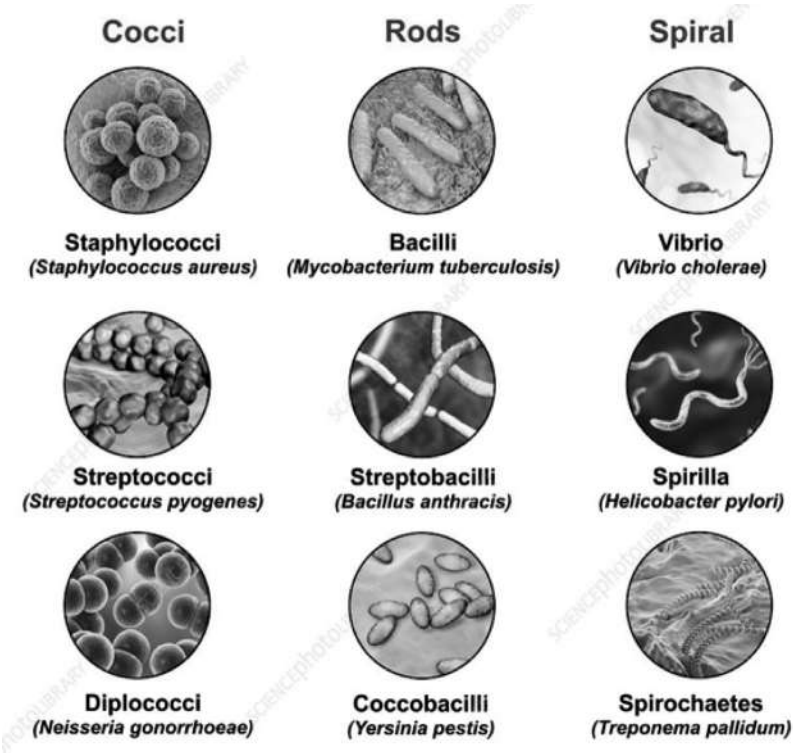


Figure 9. Bacterial arrangements. Adapted from *"Bacteria of Different Shapes"*. Kon, K. (n.d.). SCIENCE PHOTO LIBRARY. Retrieved December 1, 2023 from https://www.sciencephoto.com/media/934700/view/bacteria-of-different-shapes-illustration.

Bacterial cells are enveloped by a phospholipid membrane, serving as a barrier to the external environment. Within the cytoplasm, essential cellular components reside, although membrane-bound organelles are absent in most bacteria, except for some, like cyanobacteria, which possess protein-based organelles such as carboxysomes for carbon fixation. Genetic material is typically organized in a single, often circular, DNA chromosome, known as the nucleoid, which is associated with proteins and RNAs. Additionally, bacteria may harbor small circular DNA molecules called plasmids, which carry extra genetic information. Ribosomes are present in the cytoplasm for protein synthesis. Surrounding the cytoplasmic membrane, bacteria construct a peptidoglycan wall composed of specific polysaccharides linked by peptides.

Bacteria are broadly categorized into two groups based on staining reactions. Gram-positive bacteria produce a thick wall containing additional polymers like teichoic acids, whereas gram-negative bacteria possess a thinner wall encased by a second membrane layer featuring lipopolysaccharides and lipoproteins (Figure 10). Mycobacteria exhibit a distinct structure, featuring a second lipid membrane surrounding a particularly thick wall. They also synthesize significant amounts of mycolic acid, enabling resistance to acid decolorization, earning them the designation "acid-fast bacteria."

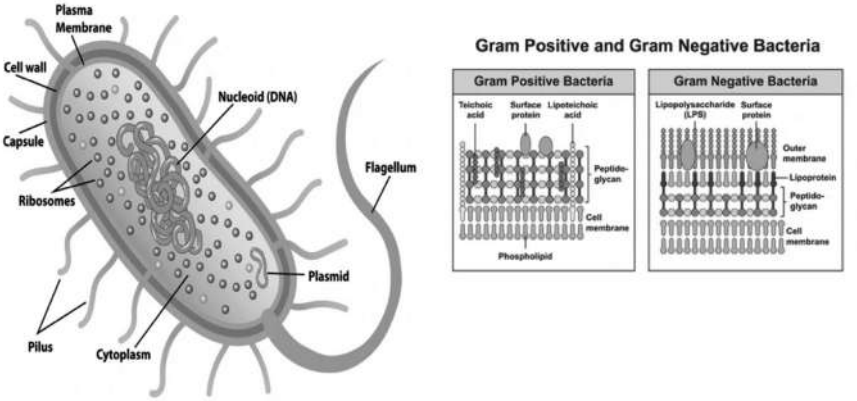


Figure 10. Bacterial cell structure. Adapted from *"Estructuras Celulares Bacterianas"*. (n.d.). SHUTTERSTOCK. Retrieved December 5, 2023 from https://www.shutterstock.com/es/image-vector/bacterial-cell-structures-labeled-on-bacillus-1522904069; *"Diseño Científico de las Diferencias Estructurales entre Bacterias Gram Positivas y Gram Negativas"*. (n.d.). SHUTTERSTOCK. Retrieved December 5, 2023 from https://www.shutterstock.com/es/image-vector/scientific-designing-structural-differences-between-gram-2124045074.

Bacterial mobility is facilitated by flagella, protein structures that may be localized to one side or distributed around the entire cell. Additionally, bacteria can adhere to surfaces using filament proteins called fimbriae. Pili, larger protein structures, are crucial for conjugation, the transfer of genetic material between cells. Many bacteria are enveloped by a pericellular matrix, the glycocalyx, which can range from an amorphous layer of polymers to an organized capsule. This layer provides protection against phagocytic cells,

serves as an antigen, and aids in surface adhesion and biofilm formation. Some gram-positive bacteria have the ability to produce endospores, resilient dormant structures containing genetic material and necessary machinery encased by a cortex and a peptidoglycan layer. Under suitable conditions, endospores can germinate into active bacterial cells.

As previously mentioned, bacterial genes are typically arranged in single chromosomes, along with extrachromosomal DNA molecules called plasmids, which carry genes related to virulence or antibiotic resistance. Bacteria reproduce asexually, resulting in progeny that inherit identical copies of the parent's genome. However, genetic variability can arise from mutations occurring during replication or induced by mutagens. Furthermore, bacteria can transfer genetic information between cells through three mechanisms. Spontaneous acquisition of genetic material, often aided by chemical treatment, is known as transformation. Bacteriophages, viruses that infect bacteria, can integrate foreign DNA into bacterial chromosomes through transduction. Finally, bacteria can transfer genetic information between cells using pili in a process called conjugation (Figure 11).

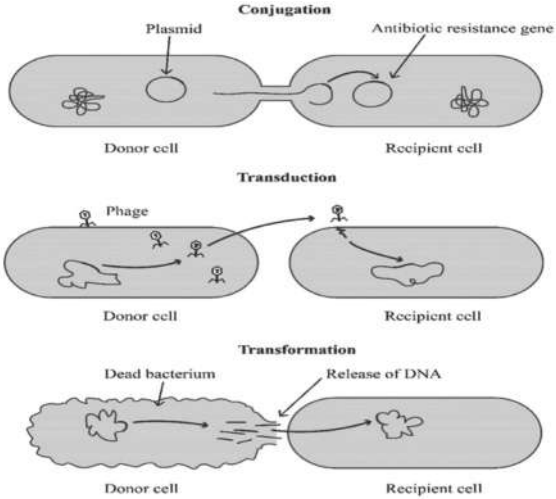


Figure 11. Bacterial horizontal gene transfer. Adapted from *"Occurrence of Antibiotics and Bacterial Resistance Genes in Wastewater: Resistance Mechanisms and Antimicrobial Resistance Control Approaches"* by C. Mutuku, Z. Gazdag, S. Melegh, 2022, *World Journal of Microbiology and Biotechnology*, 38, p. 152.

Bacteria commonly migrate towards each other through a process called quorum sensing, where chemical signals are produced and detected within a population of cells. These signals trigger changes in gene transcription and the expression of various phenotypes. Upon attachment to a surface, bacteria form aggregates embedded in an extracellular matrix consisting of diverse polymers such as lipids, proteins, and polysaccharides. These structures, known as biofilms, may also incorporate microorganisms other than bacteria (Figure 12).

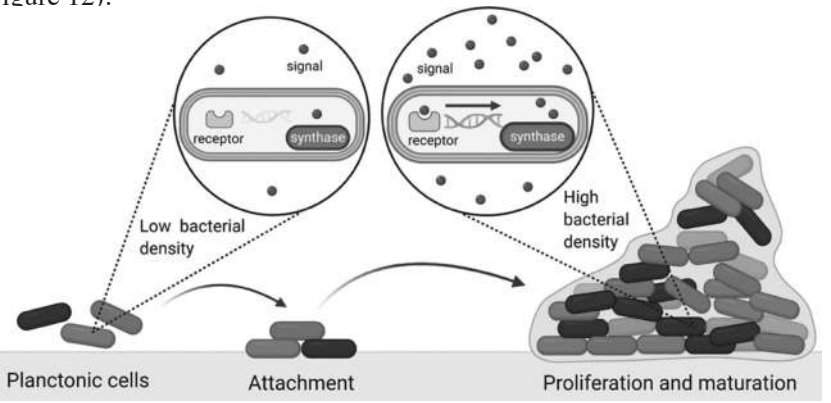


Figure 12. Quorum sensing in bacteria. Adapted from *"Quorum Sensing Systems as a New Target to Prevent Biofilm-Related Oral Diseases"* by A. Muras, N. Mallo, P. Otero-Casal, J. M. Pose-Rodríguez, A. Otero, 2022, *Oral Diseases*, 28, pp. 307-313.

Bacteria form complex associations with their hosts, which can range from negative to neutral or positive. In parasitism, one organism harms another, with pathogenic bacteria causing various diseases in humans, such as tetanus (*Clostridium tetani*), leprosy (*Mycobacterium leprae*), and tuberculosis (*M. tuberculosis*). Treatment for infections caused by these bacteria typically involves antibiotics, but their overuse has led to the emergence of multidrug-resistant strains. In contrast, bacteria can also exist in a commensal relationship, where they are part of the host's normal flora without causing harm. For example, the skin, respiratory tract, and gastrointestinal tract harbor various bacterial species. However, when these bacteria colonize other body sites, they can lead to complicated infections. *E. coli*, typically found in the gut, can cause urinary tract infections, while streptococci, common in the oral cavity,

can contribute to heart diseases. Mutualism involves interactions where two or more species benefit each other. Bacteria in the human intestinal flora are crucial for developing gut immunity, synthesizing vitamins, converting sugars to organic acids, and fermenting complex carbohydrates. Additionally, these bacteria help inhibit the growth of potentially harmful microorganisms.

We have previously discussed that probiotics offer benefits primarily through three mechanisms: the synthesis of bacteriocins, adherence to epithelial cells, and stimulation of the host immune response (Figure 13). Bacteriocins are small, hydrophobic, positively charged proteins typically comprised of around 40 amino acids. These proteins are encoded by genes found in both bacterial plasmids and chromosomes. The expression of these genes is inducible and can be influenced by factors such as bacterial cell density, nutrient availability, and the presence of organic acids in the environment. Once secreted, bacteriocins exert bactericidal or bacteriostatic effects on a variety of related and unrelated bacterial species. Consequently, bacteria producing these proteins must also synthesize proteins that neutralize their effects, either by scavenging them or by competing for their receptors. Gram-positive bacteria are known to produce a diverse array of bacteriocins, which can be classified into different groups based on various characteristics, including genetic background, heat and proteolytic stability, molecular weight, and post-translational modifications (Figure 14).

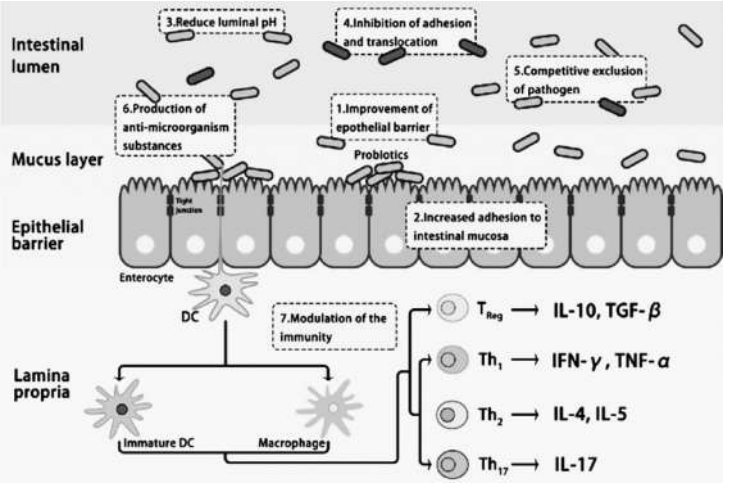


Figure 13. Major defense mechanisms of probiotics. Adapted from *"Interactions Between Intestinal Microbiota and Host Immune Response in Inflammatory Bowel Disease"* by M. Zhang, K. Sun, Y. Wu, Y. Yang, P. Tso, Z. Wu, 2017, *Frontiers in Immunology*, 8, p. 942. DC, Dendritic cell; Th, Helper T cell; IL, Interleukin; IFN, Interferon; TNF, Tumor necrosis factor; TGF, Transforming growth factor.

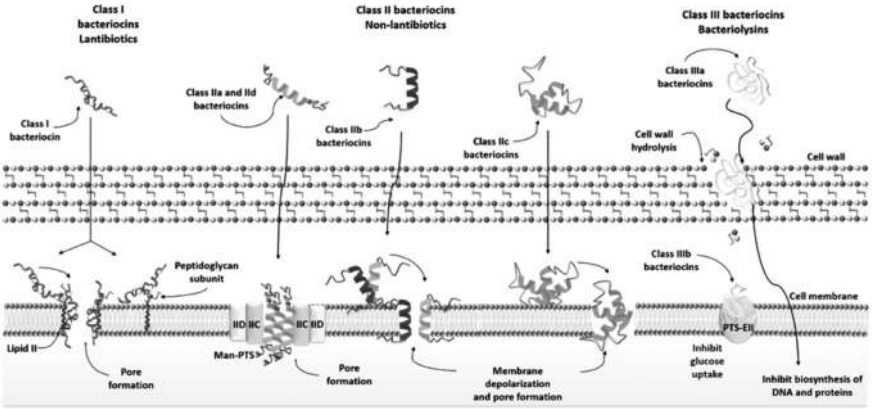


Figure 14. Bacteriocins and their mode of action. Adapted from *"Bacteriocins from Lactic Acid Bacteria. A Powerful Alternative as Antimicrobials, Probiotics, and Immunomodulators in Veterinary Medicine"* by J. C. Hernández-González, A. Martínez-Tapia, G. Lazcano-Hernández, B. Estela García-Pérez, N. S. Castrejón-Jiménez, 2021, *Animals*, 11, p. 979. Man-PTS, Mannose phosphotransferase system; PTS-EII, Phosphotransferase system enzyme II.

Class I bacteriocins, also known as lantibiotics, are small peptides that have undergone post-translational modifications resulting in the formation of the amino acids lanthionine and methyllanthionine. These modifications create cyclic structures within the molecule, increasing its rigidity and resistance to protease hydrolysis. Lantibiotics act primarily in two ways: (I) inhibition of peptidoglycan biosynthesis: Lantibiotics can bind to the carrier molecule lipid II, which transports peptidoglycan monomers from the cytoplasm to the cell wall. By binding to lipid II, lantibiotics inhibit peptidoglycan biosynthesis, leading to cell wall disruption and cell death. (II) Membrane pore formation: Lantibiotics can also tether to lipid II and initiate the formation of pores in the bacterial membrane. These pores disrupt membrane integrity, leading to leakage of cellular contents and ultimately cell death. Class I bacteriocins are further divided into three subclasses: Class Ia: These bacteriocins are characterized by being positively charged, flexible, and hydrophobic. They are associated with pore formation in the bacterial membrane. Class Ib: Class Ib bacteriocins are globular proteins that may carry a net negative charge or no charge. They inhibit key enzymes in susceptible bacteria, leading to cell death. Finally, Class Ic – Sactipeptides – contain an intramolecular linkage between the sulfur group of a cysteine residue and another amino acid. This unique structure contributes to their stability and activity against target bacteria.

Class II bacteriocins, termed non-lantibiotic bacteriocins, lack post-translational modifications and exhibit flexibility and resistance to pH and heat variations. This group comprises four classes: Class IIa: characterized by proteins with a consensus sequence at their N-terminus. Class IIb: consists of two complementary peptides, known as two-peptide unmodified bacteriocins. Class IIc: circular bacteriocins resistant to proteolytic enzymes. Lastly, class IId: single-peptide bacteriocins with disulfide bonds inducing membrane leakage. Class IIa and IId bacteriocins interact with subunits of a mannose permease (Man-PTS), triggering ion passage through the membrane, leading to cell destabilization. Class IIb bacteriocins induce membrane permeabilization, allowing diffusion of monovalent cations like Na^+ or K^+. Class IIc bacteriocins directly interact with the membrane, forming pores that lead to ion efflux and loss of membrane potential.

Class III bacteriocins, characterized by high molecular weights and temperature sensitivity, are divided into class IIIa (lytic) and class IIIb (nonlytic). Class IIIa bacteriocins induce cell wall hydrolysis, leading to cell lysis, whereas Class IIIb bacteriocins interfere with glucose uptake, disrupting membrane potential. Apart from their antibacterial activity, bacteriocins exhibit immunomodulatory effects. For example, nisin, a class Ia bacteriocin, increases T lymphocytes and boosts cytokine expression. However, the full extent of their effects remains unclear. Due to their antibacterial properties, bacteriocins are proposed as novel therapeutics, potentially reducing antibiotic side effects. They can be used alongside antiseptics or antibiotics to eliminate unwanted bacteria. Moreover, they may modulate the immune system and stimulate eukaryotic antimicrobial peptide expression. Bacteriocins produced by pathogenic bacteria are considered virulence factors, as they trigger inflammatory reactions aiding cell infection. Further research is needed to fully understand bacteriocins' effects on the immune response and their potential in infection and disease management.

Probiotics are crucial for reinforcing the gut barrier function by interacting with mucosal and epithelial cells. This interaction stimulates the synthesis of defensive molecules and enhances mucus secretion, further fortifying the barrier against pathogens. Microbial adhesion to mucosal and epithelial cells is a key aspect of colonization, crucial for probiotics to establish themselves in the gut. This adhesion not only prevents the attachment and replication of pathogens but also allows probiotics to compete for nutrients and produce antimicrobial agents. Adhesion mechanisms involve both specific and non-specific interactions. Non-specific interactions, driven by electrostatic and van der Waals forces, as well as hydrophobic interactions and hydrogen bonding, are relatively weak and reversible. In contrast, specific adhesion occurs through the recognition of receptors on epithelial cells by bacterial factors such as proteins (e.g., adhesins), specific polysaccharides, or lipoteichoic acids. This type of interaction is considered stronger and more targeted than non-specific adhesion (Figure 15).

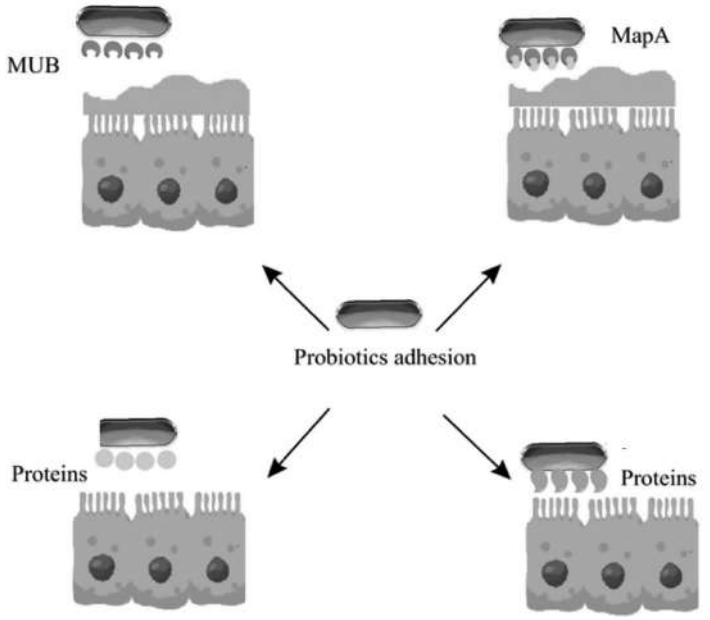


Figure 15. Probiotic adherence to epithelial cells. Adapted from *"Therapeutic and Immunomodulatory Role of Probiotics in Breast Cancer: A Mechanistic Review"* by M. Summer, S. Ali, U. Fiaz, H. M. Tahir, M. Ijaz, S. Mumtaz, R. Mushtaq, R. Khan, H. Shahzad, H. Fiaz, 2023, *Archives of Microbiology*, 205, p. 296. MUB, Mucus-binding protein; MapA, Mucous adhesion-promoting protein.

The gut epithelium is coated with a mucus layer abundant in glycoproteins and glycolipids, providing various sugar molecules for adhesion. Probiotic strains have been found to possess adhesion mechanisms such as mucus-binding proteins (Mub), mannose-specific adhesins (Msa), and lectin adhesins. Lectins, in particular, are carbohydrate-binding proteins that can cause cell agglutination and polysaccharide precipitation. Additionally, some probiotic strains use fimbriae and flagella for adhesion to epithelial cells. When probiotic bacteria interact with epithelial cells, the release of defensins is triggered. Defensins, small cysteine-rich proteins, are fundamental for neutralizing viruses, bacteria, and fungi, while also enhancing gut barrier function. By binding to negatively charged membranes, defensins disrupt microbial metabolism. The adhesion of probiotics to cells is influenced by cell wall characteristics,

host specificity, and gastrointestinal conditions such as pH and bile salt concentration. This adhesion ability allows probiotics to inhibit the invasion of pathogenic microorganisms by displacing them and competing for nutrients. Furthermore, probiotics can modify their environment by secreting organic acids, creating an inhospitable environment for competing microorganisms. Overall, probiotic strains and their combinations offer potential for displacing potential pathogens, particularly those associated with intestinal diseases, by leveraging their adhesion mechanisms and environmental modifications.

The interaction between probiotics and the immune system involves the modulation and regulation of the overall immune response. Gram-positive bacteria, such as many probiotic strains, have a thick cell wall composed of various layers of peptidoglycan and other components, including teichoic acids, lipoproteins, and capsular polysaccharides. These components contain specific molecular patterns recognized by receptors expressed in the intestinal mucosa known as pattern recognition receptors (PRRs), while the bacterial components themselves are termed microbe-associated molecular patterns (MAMPs). Several receptors for these MAMPs have been identified, such as Toll-like receptors (TLRs), which recognize lipoteichoic acids containing diacyl or triacyl glycolipids. TLRs are single-spanning proteins synthesized by various cells including leukocytes, epithelial and endothelial cells, and fibroblasts. Some TLRs are located on the cell membrane, while others are found in intracellular vesicles involved in nucleic acid recognition. Upon activation, these receptors recruit adaptor proteins in the cytosol, initiating signaling cascades that amplify the signal and regulate the transcriptional output of genes associated with cytokine production, cell proliferation, and survival. This modulation of gene expression by probiotics through TLR activation can lead to the upregulation or downregulation of immune responses, ultimately influencing the overall balance of the immune system.

Lipoteichoic acids are recognized by the TLR2–TLR6 and TLR2–TLR1 complexes, while wall teichoic acids primarily induce signaling through TLR2. In addition, TLR2 can interact with peptidoglycan fragments. TLRs are also involved in detecting gram-negative bacteria. Peptidoglycan,

another component of bacterial cell walls, can activate nucleotide-binding oligomerization domain-like receptors (NLRs), which are intracellular proteins expressed in lymphocytes and nonimmune cells. Upon ligand binding, NLRs recruit adaptor proteins that activate downstream signaling pathways, leading to the expression of inflammatory cytokines (Figure 16). Despite the recognition of various bacterial components by PRRs, receptors associated with capsular polysaccharides have not yet been identified. However, the activation of PRRs by probiotics can influence the function of the intestinal epithelial barrier. It has been proposed that probiotics may enhance the synthesis of components of tight junction complexes in response to TLR activation, contributing to the maintenance of intestinal barrier integrity.

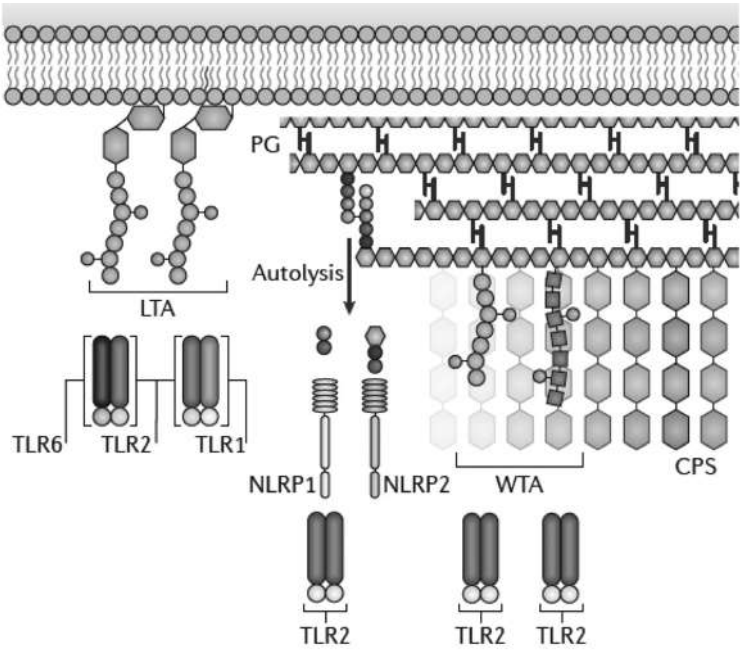


Figure 16. Probiotic-associated molecular patterns. Adapted from *"Emerging Molecular Insights into the Interaction Between Probiotics and the Host Intestinal Mucosa"* by P. A. Bron, P. Van Baarlen, M. Kleerebezem, 2012, *Nature Reviews Microbiology*, 10, pp. 66-78. LTA, Lipotechoic acid; TLR, Toll-like receptor complexes; CPS, Capsular polysaccharide; WTA, Wall techoic acid; PG, peptidoglycan; NLRP, NOD- LRR- and Pyrin domain containing.

In recent years, research has shed light on how probiotic bacteria influence the host, including their ability to enhance intestinal barrier function, modulate the immune response, and competitively exclude potential pathogens. While significant progress has been made in understanding these mechanisms, further research is needed to fully elucidate the molecular pathways involved. Among probiotics, lactic acid bacteria and bifidobacteria are the most commonly studied, but other microorganisms such as *Enterococcus*, *Lactococcus*, and the yeast *Saccharomyces* are also recognized for their potential benefits. Lactic acid bacteria, in particular, play crucial roles in maintaining intestinal homeostasis across various animal species, including humans. Within the lactic acid bacteria genus, *Lactobacillus* is the most extensively studied, with approximately 200 species described. In the upcoming chapter, we will explore the specifics of these bacteria, with a focus on the species of particular interest, *Limosilactobacillus fermentum*.

4. *Limosilactobacillus fermentum*, a Multifunctional Microbe

Lactic acid bacteria (LAB), also known as *Lactobacillales*, exhibit common metabolic and physiological characteristics. They are typically found in both spherical and rod-shaped forms. LAB are classified as gram-positive, non-sporulating, non-respiring bacteria that display a high tolerance to acidic environments. They are commonly present in various decomposing materials such as milk and plant residues. One of the defining features of LAB is their ability to produce lactic acid as the primary end product of carbohydrate fermentation, which is the origin of their name. LAB primarily rely on fermentation rather than aerobic respiration for ATP production. While they can tolerate oxygen, LAB lack catalases and mainly utilize different enzymatic antioxidants to neutralize reactive oxygen derivatives. Glucose fermentation in LAB can occur through two main pathways. Homolactic or homofermentative LAB metabolize glucose into pyruvate under conditions of low oxygen and high glucose levels, leading to the production of ATP and subsequent reduction of pyruvate to lactic acid. In contrast, heterofermentative LAB generate additional metabolites such as acetic acid alongside lactic acid. These bacteria utilize the pentose phosphate pathway, where a five-carbon sugar is cleaved into glyceraldehyde phosphate and acetyl phosphate. While glyceraldehyde phosphate is metabolized into lactic acid, acetyl phosphate is reduced to ethanol. The order *Lactobacillales* encompasses a total of 13 genera, with *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus*, and *Weissella* being among the most prominent and relevant genera within this group (Figure 17).

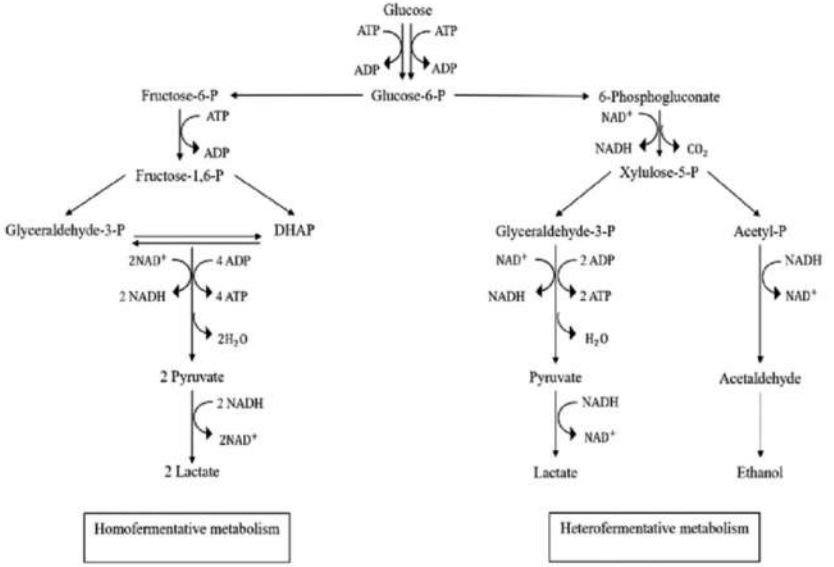


Figure 17. Lactic acid bacterial metabolism. Adapted from *"New Prospect of Algae for Sustainable Production of Lactic Acid: Opportunities and Challenges"* by S. A. Cheah, C. Y. Chai, I. S. Tan, H. C. Y Foo, M. K. Lam, 2022, *Progress in Energy and Environment*, 21, pp. 19-28. ATP, Adenosine triphosphate; ADP, Adenosine diphosphate; NAD, Nicotinamide adenine dinucleotide; DHAP, Dihydroxyacetone phosphate.

Lactic acid bacteria are highly regarded as probiotics due to their biologically active properties, which make them valuable supplements in food formulations. Consequently, they have found widespread use in the food industry. Ongoing research is dedicated to exploring the bioactive compounds synthesized by LAB, such as exopolysaccharides, known for their nutritional benefits, and bacteriocins, which have antimicrobial properties and are utilized as food preservatives. LAB play essential roles in various food processing applications, including cheese and yogurt production, as well as in the fermentation of beverages like beer and wine. However, it is important to note that LAB also contribute to dental decay by producing dextrans from glucose. These carbohydrates facilitate bacterial adhesion to teeth, leading to the formation of dental plaque. Furthermore, the production of lactic acid by LAB within plaque creates an acidic environment that contributes to the

demineralization of enamel, resulting in tooth decay. *Streptococcus mutans* is one of the primary bacterial species associated with this process.

Species of the genus *Lactobacillus* are commonly found in the microbiota of humans and animals, inhabiting various compartments such as the gastrointestinal and female genital tracts. Within these environments, *Lactobacillus* species form biofilms, enabling them to withstand harsh conditions. These bacteria have established mutualistic relationships with their hosts, providing protection against potential pathogens while deriving nutrients from their host organisms. Recent genetic studies have led to the reclassification of several classical members of the genus *Lactobacillus* into other genera (Table 1). Species within the genus *Limosilactobacillus* are known for their heterofermentative and thermophilic characteristics, with the ability to produce exopolysaccharides from glucose. The Latin root "limosus" denotes "slimy," reflecting the biofilm-forming nature of these bacteria. *Limosilactobacillus* species have been isolated from the intestines of humans and various animals, with *L. reuteri* being one of the most well-known species. However, other significant species have also been identified. Among these, *Limosilactobacillus fermentum* stands out as an extensively studied probiotic due to its beneficial properties (Figure 18). *L. fermentum* exhibits antibacterial activity, adhesion to epithelial cells, and the ability to modulate immune response gene expression. In vitro and in vivo experiments have demonstrated the immunomodulatory and anti-inflammatory effects of *L. fermentum* strains. Furthermore, these bacteria are known to contribute to the clearance of pathogens and the protection of the mucosal barrier. Promising results from studies in humans suggest that supplementation with *L. fermentum* may reduce intestinal infections and modulate immune responses. In animal studies involving pigs, birds, and marine animals, its administration has led to improved growth, gut health, nutrition, and overall immune responses. Certain strains have also demonstrated efficacy in antagonizing the effects of harmful microorganisms.

Table 1. *Examples of reclassified genera*

GENUS (SPECIES)	DESCRIPTION
Limosilactobacillus (*L. fermentum*)	⇒ *Limosilactobacillus* species are adapted to vertebrates and form biofilms from exopolysaccharides in the intestine. They are vancomycin-resistant, heterofermentative, and thermophilic.
Lactiplantibacillus (*L. plantarum*)	⇒ *Lactiplantibacillus* species are nomadic, metabolize phenolic acids, are homofermentative, vancomycin-resistant, and can ferment various carbohydrates.
Ligilactobacillus (*L. salivarius*)	⇒ *Ligilactobacillus* species are adapted to their hosts; most are motile and synthesize ureases to tolerate the acidic conditions of the stomach.
Lacticaseibacillus (*L. casei*)	⇒ *Lacticaseibacillus* species are nomadic, resist oxidative stress, and ferment pentoses. They are homofermentative and resistant to vancomycin.
Latilactobacillus (*L. sakei*)	⇒ *Latilactobacillus* species are free-living, homofermentative, and mesophilic.
Lentilactobacillus (*L. buchneri*)	⇒ *Lentilactobacillus* species are nomadic, plant-associated, and adapt to vertebrate hosts. They are heterofermentative, mesophilic, and vancomycin-resistant.

Note. Adapted from "A Taxonomic Note on the Genus *Lactobacillus*: Description of 23 Novel Genera, Emended Description of the Genus *Lactobacillus* Beijerinck 1901, and Union of *Lactobacillaceae* and *Leuconostocaceae*" by J. Zheng, S. Wittouck, E. Salvetti, C. M. Franz, H. M. Harris, P. Mattarelli, ... & S. Lebeer, 2020, *International Journal of Systematic and Evolutionary Microbiology*, 70, pp. 2782-2858.

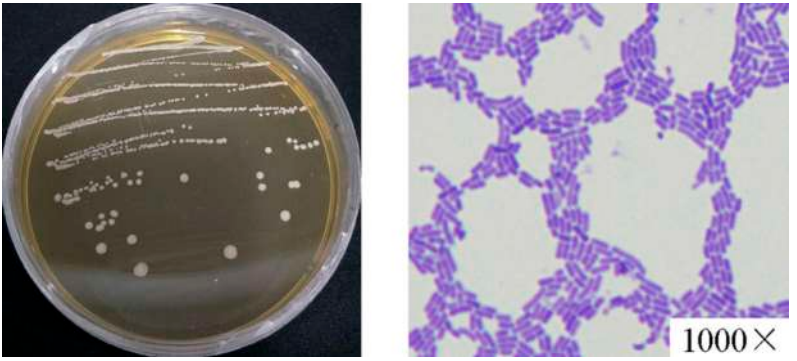


Figure 18. Morphological characteristics of *L. fermentum*. Left hand side: Bacterial culture in the agar plate. Right hand side: Gram staining and cell morphology. Adapted from *"Positive Enhancement of* Lactobacillus fermentum HY01 *on Intestinal Movements of Mice Having Constipation"* by X. Chen, J. L. Song, Q. Hu, H. Wang, X. Zhao, H. Suo, 2018, Applied Biological Chemistry, 61, pp. 39-48.

Lactobacillus fermentum was the original name assigned to this species, although the current name refers to the exopolysaccharides it produces. This species does not sporulate and can utilize various carbohydrate molecules. It is known for its intrinsic resistance to vancomycin, and resistance to other antibiotics such as aminoglycosides, quinolones, and tetracyclines has also been demonstrated. *L. fermentum* strains have been isolated from diverse environments, including the gastrointestinal tract and feces of healthy humans and animals (such as birds and pigs), dairy products, manure, and fermenting plant materials. This species, along with other lactobacilli, can influence the physiology of their hosts as they remain active in the gastrointestinal tract. *L. fermentum* is generally regarded as safe by official food authorities in China, the United States, and Europe, and some strains have been used in the development of commercially available dietary supplements. Table 2 provides a summary of the probiotic properties of selected strains.

Table 2. L. fermentum *strains and their probiotic properties*

STRAIN	PROBIOTIC PROPERTIES
L. fermentum CECT5716	⇒ Isolated from human breast milk; modulation of intestinal immune response; high mucin production; antioxidant capacity; prevention of mastitis; reduction of colitis-associated effects.
L. fermentum DLBSA204	⇒ Isolated from human breast milk; activation of macrophages; upregulation of nitric oxide synthesis; downregulation of proinflammatory cytokines; virus inactivation.
L. fermentum SD11	⇒ Isolated from human oral cavity, fermencin (SD11) synthesis, antibacterial activity.
L. fermentum AGR1487	⇒ Isolated from human oral cavity; activation of the TLR signaling pathway; modulation of immune response.
L. fermentum UCO-979C	⇒ Isolated from the human gut; antibacterial activity against *H. pylori*.
L. fermentum IM12	⇒ Isolated from the human gut; modulation of the NF-κB-STAT3 signaling pathway.
L. fermentum ME-3	⇒ Isolated from human feces, expression of the glutathione system, and downregulation of oxidative stress.
L. fermentum 10	⇒ Isolated from human feces; adhesion to epithelial cells; high bile salt tolerance; autoaggregation; reduced *E. coli* adhesion; antibacterial and antioxidant capacities.
L. fermentum JX306	⇒ Isolated from fermented vegetables; scavenging activity of free radicals; enhanced glutathione peroxidase activity.
L. fermentum GR-3	⇒ Isolated from fermented food; regulation of human hyperuricemia via promotion of uric acid excretion.

L. fermentum LBM97	⇒	Isolated from fermented vegetables; bacteriocin production (LBM97-4 and LBM97-5); activity against *Staphylococcus aureus* and *E. coli*.
L. fermentum BZ532	⇒	Isolated from cereals; bacteriocin production (LF-BZ532); activity against *Listeria* spp. and *Pseudomonas* spp.
L. fermentum J23	⇒	Isolated from cheese; bacteriocin production; activity against *E. coli*, *S. aureus*, *Listeria innocua*, and *S.* Typhimurium.
L. fermentum Y57	⇒	Isolated from artisanal yogurt; modulation of hypercholesterolemia in rats.

Note. Adapted from "An Overview of the Use and Applications of *Limosilactobacillus fermentum* in Broiler Chickens" by M. P. Racines, M. N. Solis, M. A. Šefcová, R. Herich, M. Larrea-Álvarez, V. Revajová, 2023, *Microorganisms*, 11, p. 1944. NF-ϰB-STAT3, Nuclear factor-kappa B and Signal transducer and activator of transcription 3 signaling pathway.

Probiotic bacteria must navigate through diverse stressful environments, such as high levels of bile salts or low pH, in the gastrointestinal tract. *L. fermentum* has demonstrated high viability under these conditions. Additionally, this species can interact with intestinal epithelial cells through auto-aggregation and robust surface hydrophobicity, which are crucial for promoting cell-to-cell interactions. LAB bacteria, including *L. fermentum*, adhere to the intestinal mucosa through various mechanisms involving proteins and bacterial motifs like lipoteichoic acids (LTA), lipopolysaccharides (LPS), and peptidoglycans (PG). Binding proteins such as mucin- and fibronectin-binding proteins, encoded by the *mub* and *fbp* genes respectively, interact with host extracellular factors. Sortases, enzymes common to gram-positive bacteria, modify surface proteins by recognizing and cleaving their carboxy-terminal sorting signals. Many key proteins involved in adhesion and colonization, including pilins and adhesins, are substrates for sortases. The genes encoding these adhesion factors are upregulated when bacteria are exposed to bile, pancreatin, or mucin. Other molecular patterns, such as lipoteichoic acids and LPS, also contribute to bacterial adhesion by interacting with receptors on host cells or stimulating mucin production by goblet cells. Electrostatic and passive forces may contribute significantly to the process of adhesion.

Probiotic bacteria are crucial in competing with potentially harmful microorganisms for a niche in the intestine, thereby maintaining a balanced microbiome and reducing the risk of infections. Additionally, they aid in pathogen elimination through the synthesis and secretion of antimicrobial peptides called bacteriocins. These peptides induce cell deterioration by damaging the membrane or peptidoglycan wall, among other mechanisms. Several strains of *L. fermentum* have been associated with various fermicins that exhibit antibacterial activity against both gram-positive and gram-negative bacteria. Furthermore, this species produces secondary metabolites that modify environmental conditions, such as lactic acid and other organic acids, which lower the pH and inhibit the proliferation of harmful bacteria. These characteristics have been effective in reducing the impact of infections caused by pathogens such as *H. pylori*, *S. aureus*, *Salmonella* spp., *E. coli*, *Campylobacter jejuni*, or *Listeria* spp.

Bacterial invasions can influence the redox state of the host, potentially leading to pathological conditions. Probiotics, including *L. fermentum* strains, synthesize molecular motifs that interact with receptors on eukaryotic cells. These strains possess molecules that activate receptors, triggering the transcription of genes associated with antioxidant responses, thus reducing the effects of oxidative stress. Additionally, *L. fermentum* bacteria produce an active redox peptide that further modulates the oxidative response. This peptide, associated with glutathione, not only directly reduces agents but also acts as a cofactor for enzymes that neutralize electrophilic molecules. These characteristics underscore the importance of this species in developing supplements aimed at mitigating the effects of potential pathogenic infections.

Several strains of probiotics can activate receptors on various cells, including macrophages, dendritic cells, immune cells, and epithelial cells. These interactions initiate signaling pathways that activate transcription factors, ultimately leading to the expression of different cytokines. These proteins can influence T cell polarization. On the bacterial side, these interactions are mediated by lipoteichoic acids, polysaccharides, or peptidoglycans, which are complex cell wall components synthesized by the concerted action of various

enzymes. In contrast, eukaryotic cells use Toll-like receptors 2 and 4, as well as NOD2-binding proteins, to detect bacterial molecular patterns. As discussed in Chapter 3, these proteins can recruit adaptor proteins that transduce the signal and activate the transcription of cytokines and other genes associated with the immune response.

L. fermentum, as a species with probiotic characteristics, holds significance in biotechnology. These bacteria not only modulate the immune response but also contribute to maintaining a balanced microbiome and intestinal architecture. Therefore, they have undergone various testing approaches. In vitro studies have shown that *L. fermentum* strains enhance the expression of key immune genes in macrophages, intestinal cells, dendritic cells, and peripheral blood mononuclear cells (PBMCs). In vivo studies have further supported the potential of this probiotic in ameliorating inflammatory diseases in animal models. Human trials involving selected strains have demonstrated the benefits of consuming *L. fermentum* as a food supplement, including reduced risk of gastrointestinal infections, improved immune response, and modulation of cholesterol levels in infants, pregnant women, and healthy adults. These findings are detailed in Chapter 5. Moreover, this bacterial species has found application in animal production. For example, in terrestrial animals such as birds and pigs, the administration of certain strains not only enhances gut health but also mitigates the effects of pathogenic bacteria while modulating the immune response. Similarly, in marine animals including fish and shrimps, the use of probiotics has led to a reduction in pathogenic effects from both biotic and abiotic factors. Chapter 6 provides a comprehensive summary of the evidence supporting these benefits.

5. Therapeutic Uses and Applications of *Limosilactobacillus fermentum*

L. fermentum are probiotic bacteria with well-defined characteristics that allow them to adhere to cells, produce antibacterial compounds, and most importantly, activate receptors that stimulate the expression of genes associated with inflammation. As such, various in vitro and in vivo studies have provided crucial insights into the benefits of using probiotics in the context of inflammatory diseases. Prior to proceeding further, it would be advantageous to review the Annex to update your knowledge on immune cells and cytokines. This will improve the accessibility of the chapter.

In vitro studies, conducted outside living organisms, typically in a test tube, offer the advantage of precise manipulation of biological materials in controlled environments. The findings from these studies help predict responses in animal models and inform their translation into human applications. Numerous reports have highlighted the in vitro properties of *L. fermentum*. For instance, studies have demonstrated its ability to ferment dietary fiber, resulting in the production of butyrate, lactate, acetate, and other SCFAs. This fermentation process has been linked to improved glucose metabolism and regulation of the immune response. Propionic acid, another SCFA produced during fermentation, has been shown to increase the number of mucosa-associated T cells, including regulatory T cells, by binding to metabotropic receptors in lymphocytes and modulating their response. Moreover, strains of this probiotic exhibit significant antibacterial activity against various pathogens, such as *Salmonella*, *E. coli*, and *Listeria*. Culture supernatants containing organic acids produced by *L. fermentum* create an acidic environment that

inhibits the growth of these bacteria. It is essential for probiotics to withstand the acidic conditions of the stomach and the alkaline environment of the intestines. Strains of this probiotic have demonstrated higher tolerance to low pH levels compared to other species including *L. salivarius*, *L. rhamnosus*, and *L. jensenii*. Additionally, some of them, including HA6, have shown the ability to grow even in the presence of high concentrations of bile salts. Furthermore, *L. fermentum* bacteria have been assessed for their tolerance to antibiotics, with vancomycin resistance being commonly observed. However, most strains remain sensitive to commonly used antibiotics such as gentamicin, ampicillin, and chloramphenicol. These characteristics highlight the potential of these bacteria as probiotics with versatile applications in promoting gut health and combating pathogenic infections.

Cell lines derived from animal cells have the unique ability to be cultured and propagated indefinitely, providing invaluable tools for studying specific cellular processes. These cell lines originate from primary cultures initiated from cells, tissues, or organs of animals, serving as essential models for investigating changes in cellular biology, structure, and genetic background under controlled laboratory conditions. Dendritic cells (DCs) represent a specialized subset of immune cells responsible for antigen presentation and the initiation of adaptive immune responses. DC lines have been instrumental in elucidating the biology of these cells, particularly in the context of antigen presentation. Studies have shown that administration of *L. fermentum* can induce the maturation of dendritic cells derived from monocytes, leading to enhanced synthesis of major histocompatibility complex (MHC) class II molecules and key costimulatory factors such as CD40 and CD80, along with certain cytokines. These findings highlight the immunomodulatory effects of the probiotic on dendritic cells, shedding light on its potential mechanisms of action in regulating immune responses and contributing to host defense against pathogens.

Macrophages, originating from bone marrow cells, are essential components in various physiological processes, including inflammation, innate and adaptive immunity, and the clearance of infections, both intracellular and extracellular,

as well as necrotic, apoptotic, and senescent cells. They, along with neutrophils, constitute the frontline defense against potential pathogens like *Salmonella*, *Helicobacter*, *Mycobacterium*, and *Candida*. When exposed to different species of *Lactobacillus*, macrophages demonstrate enhanced phagocytic and antibacterial capabilities compared to untreated cells. Specifically, supplementation with *L. fermentum* UCO-979C improves cell viability and reduces the release of cytotoxicity indicators during *H. pylori* infection, while also downregulating cytokine expression. These cytokines, particularly involved in signaling pathways and nitric oxide (NO) production, are crucial for bacterial clearance, virus inactivation, and tumor toxicity. Dysregulated NO expression is associated with inflammatory conditions like gastric injury and pulmonary diseases. Additionally, priming the IFN-γ response effectively protects the host against pathogenic *Salmonella* infections. Other strains, such as *L. fermentum* DLBSA204 and *L. fermentum* CECT5716, activate macrophages, inducing the expression of signaling molecules including NO, TNF-α, and IL-10. Furthermore, *L. fermentum* CECT5716 modulates the balance of cytokines in macrophages challenged with bacterial LPS, reducing the expression of factors like IL-1β and IL-6. Clearly, treatment with these probiotic bacteria positively influences macrophage function by impacting key signaling factors, particularly cytokines (Table 3).

Peripheral blood mononuclear cells (PBMCs) encompass lymphocytes and monocytes. Distinguished by their round nuclei, they are distinct from erythrocytes/platelets, which lack nuclei, and granulocytes, which have multi-lobed nuclei. In humans, PBMCs primarily consist of lymphocytes, followed by monocytes and a small proportion of dendritic cells. Despite their morphological similarities, lymphocytes vary in function: B cells specialize in antibody production, T cells in direct cell-mediated recognition of antigens, and natural killer (NK) cells in detecting virus-infected and tumoral cells without prior sensitization.

Administration of *L. fermentum* CECT5716 not only activated NK cells and T cells but also upregulated the expression of various factors, including both type I and type II cytokines. These findings illustrate that this probiotic has the

ability to modulate both innate and acquired immune responses by robustly stimulating a range of cytokines involved in both pro- and anti-inflammatory processes. Specifically, *L. fermentum* CECT5716 influenced the expression of the antiviral factor IFN-γ, which is rapidly produced upon infection. Moreover, this strain induced the expression of a factor involved in myeloid lineage differentiation, thereby enhancing resistance against invading pathogens. Similar activation was observed with another strain, NCIMB701751, albeit to a lesser extent, suggesting that the effects of the probiotic may vary depending on the strain used. In contrast, supplementation with *L. fermentum* B633 led to a reduction in the expression of IL-13 while increasing that of IL-12 and IFN-γ. This modulation of T_{H2} and T_{H1} cytokines could be beneficial for allergic patients, as atopic allergy has been associated with an imbalance in the expression of such factors. However, it is important to note that the effects induced by this strain may be harmful if the immune response becomes disproportionate. For example, treatment with *Bifidobacterium* has been shown to exacerbate conditions associated with autoimmune arthritis in murine models. Therefore, it is crucial to consider dose-dependent effects when evaluating the potential of novel strains.

Enterocytes, which are absorptive epithelial cells lining the inner surface of the intestines, contribute to immune stimulation by synthesizing various chemokines and cytokines upon encountering pathogens or their byproducts. Commercially available enterocyte cell lines, such as Caco2 and HT29 cells, have been instrumental in studying epithelial physiology. When human gastric epithelial cells were challenged with *H. pylori*, *L. fermentum* UCO-979C was found to downregulate inflammatory factors, including IL-8, IL-6, TNF-α, and IL-1β. Additionally, this strain upregulated the expression of TGF-β and inhibited pathogen invasion in the challenged cells. Similar findings have been observed with other species, such as *L. rhamnosus*, *L. acidophilus*, and *L. plantarum*. Furthermore, three different strains of *L. fermentum* (NCIMB-5221, NCIMB-52797, and NCIMB-8829) exhibited anti-proliferative effects against adenocarcinoma cells (Caco-2). Co-culturing of these cells with *L. fermentum* MCC2760 demonstrated that the probiotic modulated the expression of both

pro- and anti-inflammatory cytokines, including IL-6, IL-1β, and IL-10. These findings highlight the ability of *L. fermentum* bacteria to regulate inflammatory factors in various cell lines, with probiotic treatment reducing their expression in pathogen-challenged cells (Table 3). However, the specific effects observed depended on the species and strains used.

Table 3. *Effects of* L. fermentum *on cell lines, animal models, and humans*

CELL LINE TRIALS	
STRAIN	**OUTCOMES**
L. fermentum CECT5716	⇒ In rodent bone marrow-derived macrophages, probiotic administration reduced the expression of proinflammatory factors after LPS stimulation.
L. fermentum UCO-979C	⇒ In human macrophages stimulated with LPS or challenged with *H. pylori*, probiotic exposure not only downregulated the expresion of inflammatory factors, but also upregulated the expression of anti-inflammatory cytokines. ⇒ In human gastric epithelial cells infected with *H. pylori*, proinflammatory cytokine expression was downregulated in the presence of the probiotic. ⇒ In gastric adenocarcinoma human cells, the effects of *H. pylori* infection were reduced in cells treated with the probiotic.
L. fermentum DLBSA204	⇒ In mouse macrophages, the probiotic modulated the expression of T_{HI} cytokines. ⇒ In human lung cells challenged with *S. pneumoniae*, the presence of the probiotic reduced bacterial adhesion to epithelial cells.
L. fermentum MCC2759 and MCC2760	⇒ In Caco-2 colon cancer cells, stimulation with LPS triggered the production of inflammatory factors; these outcomes were reduced after probiotic supplementation.
ANIMAL MODEL TRIALS	
STRAIN	**OUTCOMES**
L. fermentum IM12	⇒ In mice with TNBS-induced colitis, probiotic administration not only downregulated proinflammatory cytokine expression but also inhibited colon shortening and neutrophil infiltration.
L. fermentum HY01	⇒ In mice with DSS-induced colitis, probiotic supplementation reduced the expression of proinflammatory factors in the colon and serum, improved colon length, and reduced mucosal injury.
L. fermentum CECT5716	⇒ In mice with DSS-induced colitis, downregulation of colon inflammatory cytokines and dysbiosis regulation. ⇒ In rats with TNBS-induced colitis, modulation of colon inflammatory factors and reduction of colonic damage were observed. ⇒ In rats with stress-induced intestinal barrier dysfunction, probiotic supplementation reduced the permeability of the small intestine and increased the expression of zonula occludens. ⇒ In mice suffering from tacrolimus-induced endothelial dysfunction and hypertension, probiotic supplementation reduced the expression of plasma inflammatory factors.
L. fermentum ZYL0401	⇒ In mice with LPS-induced hepatic injury, the downregulation of ileal inflammatory factors was observed after treatment with the probiotic.

L. fermentum Suo	⇒ In mice with HCl/ethanol-induced gastric injury, the probiotic not only reduced the extent of injury but also suppressed the expression of inflammatory factors.
L. fermentum MTCC5898	⇒ In aging mice challenged with pathogenic *E. coli*, probiotic treatment increased plasma antibody levels and decreased pathogen colonization. ⇒ In aging mice, supplementation of the probiotic enhanced enzymatic antioxidant activities. ⇒ In rats fed a cholesterol-enriched diet, the downregulation of inflammatory cytokines in the liver was assessed after probiotic exposure, which also reduced cholesterol accumulation.
L. fermentum CGMCC1.1880	⇒ In rats fed a high-fat diet, consumption of the probiotic reduced serum triglyceride and proinflammatory cytokine levels while upregulating sIgA levels.
L. fermentum NA4	⇒ In zebrafish embryos with TNBS-induced inflammation, probiotic administration downregulated the expression of inflammatory factors.
HUMAN TRIALS	
STRAIN	**OUTCOMES**
L. fermentum CECT5716	⇒ In infants, the incidence of gastrointestinal and upper respiratory tract infections was higher in the placebo group than in the probiotic group. ⇒ In lactating women, consumption of the probiotic was associated with a lower incidence rate of mastitis. ⇒ In women with breast pain during lactation, a reduction in the *Staphylococcus* load was assessed after consumption of the probiotic. This difference was not observed in the placebo group. ⇒ In women with infectious mastitis, treatment with the probiotic reduced bacterial counts, which was not observed in the control group. ⇒ In healthy adult volunteers, oral consumption of the probiotic enhanced the response to the anti-influenza vaccine.
L. fermentum ME-3	⇒ In clinically asymptomatic participants, consumption of the probiotic not only reduced total cholesterol levels but also improved the conditions associated with cardiovascular diseases.
L. fermentum PCC	⇒ In healthy adults, enhancement of influenza antigen titers after vaccination was observed. Respiratory symptoms observed for only 2 days (vs. 5 days under normal conditions). ⇒ In competitive cyclists, symptoms of respiratory diseases were reduced in people consuming the probiotic compared to control conditions. ⇒ In healthy elite male distance runners, a reduction in respiratory symptoms was observed in individuals consuming the probiotic; these differences were not detected in the placebo group. No changes in salivary IgA levels were observed.

Note. Adapted from "*Lactobacillus fermentum* and Its Potential Immunomodulatory Properties" by Y. Zhao, K. Hong, J. Zhao, H. Zhang, Q. Zhai, W. Chen, 2019, Journal of Functional Foods, 56, pp. 21-32. PBMC, Peripheral blood mononuclear cells; Caco-2, Colon cancer cells; TNBS, 2,4,6-trinitrobenzene sulfonic acid; DSS, Dextran sulfate sodium; LPS, Lipopolysaccharide.

Animal models are invaluable tools in biomedical research as they enable the study of biological processes and diseases that are common to humans. These non-human species, which encompass a wide range of organisms, can mimic various aspects of human physiology and pathology to some extent. In the context of intestinal inflammation, animal models are fundamental for understanding the multifaceted factors contributing to this condition. Intestinal inflammation can arise from a multitude of factors, including dysregulation of the immune response, alterations in the intestinal lining, changes in the composition of the microbiota, as well as genetic and environmental factors. Animal models provide researchers with the opportunity to investigate these complex interactions in a controlled setting, allowing for the identification of underlying mechanisms and the development of potential therapeutic interventions. Research on *L. fermentum* has shown its ability to mitigate key inflammatory markers in colitis, including TNF-α, IL-17, NO, IFN-γ, IL-10, IL-4, IL-17, STAT3, and NF-ϰB. For example, *L. fermentum* Suo reduced gastric injury in mice and lowered serum levels of TNF-α, IL-12, IFN-γ, and IL-6. Furthermore, higher doses of the probiotic led to more pronounced effects. Another strain, IM12, alleviated TNBS-induced colitis severity by suppressing NF-ϰB and STAT3. Exposure to *L. fermentum* HY01 reduced expression levels of cyclooxygenase and NO synthase in DSS-induced colitis mice, associated with inflammatory and neurodegenerative conditions. TNBS, or trinitrobenzene sulfonic acid, is a water-soluble compound that binds to protein tissue, leading to inflammation. DSS, which stands for dextran sulfate sodium, induces inflammation and is harmful to colonic epithelial cells.

Systemic inflammation in mice is associated with reduced levels of prostaglandin (PG) E2, a lipid crucial for protecting the gut epithelial barrier. Pretreatment with *L. fermentum* CECT5716 in colitis-afflicted mice enhanced PGE2 synthesis, suggesting that probiotics might influence colitis regulatory mechanisms. In mice with LPS-induced hepatic injury, treatment with *L. fermentum* ZYL0401 boosted ileal expression of PGE2 and IL-10. However, *L. fermentum* BGHI14 did not alter colitis effects in TNBS-treated female rats, while *L. fermentum* ACA-DC 179 reduced colonic inflammation

in TNBS-exposed mice. Moreover, zebrafish embryos exposed to TNBS and supplemented with *L. fermentum* NA4 exhibited downregulated inflammatory factors like TNF-α and IL-1β (Table 3).

Inflammatory dysregulation can exacerbate various conditions including hypertension, obesity, stress, and elevated cholesterol levels, particularly with aging. Using *L. fermentum* MTCC 5898 to ferment milk fed to aging mice demonstrated improved antioxidant enzyme activity and modulation of IL-4, IL-10, and IFN-γ levels in splenocytes, thus enhancing the overall immune function. This strain also reduced TNF-α and IL-6 levels and mitigated dyslipidemia in mice fed a cholesterol-rich diet. Similarly, *L. fermentum* CECT5716 alleviated intestinal barrier dysfunction in stressed rats by reducing permeability and upregulating the expression of ZO-1, a key protein in intestinal integrity. In addition, it regulated blood pressure and endothelial dysfunction in male mice with hypertension induced by Tacrolimus, an immunosuppressive agent, and decreased plasma inflammatory markers. Another strain, *L. fermentum* CGMCC1.1880, mitigated the effects of a cholesterol-rich diet in rats, resulting in weight loss, reduced serum triglyceride levels, and increased sIgA levels. These findings suggest that *L. fermentum* strains hold promise for modulating inflammatory processes in individuals with associated conditions.

Inflammatory disorders often arise from fungal or bacterial infections, with pathogenic bacteria and their lipopolysaccharides having long-term effects on the host immune response. In aging mice infected with pathogenic *E. coli*, consumption of milk fermented with *L. fermentum* MTCC 5898 reduced colonization in the intestine, spleen, and liver, while enhancing serum IgA and IgG1 production. However, *L. fermentum* I5007 did not alleviate *E. coli*-induced diarrhea in piglets, although it did upregulate peripheral blood CD4 T cells. Additionally, certain strains of *L. fermentum* have shown promise in combating *Salmonella* infection. For example, the ME-3 strain prevented the development of typhoid nodules in the livers of male mice, with upregulation of IFN-γ and IL-10. Furthermore, the crude exopolysaccharide of *L. fermentum* Lf2 improved survival rates and induced sIgA production in the intestines of mice infected with *Salmonella*. These findings highlight the potential of

probiotic supplementation in mitigating the consequences of pathogenic infections.

Clinical research findings are crucial for identifying strains suitable for inclusion in functional foods. However, studies involving *L. fermentum* are relatively scarce, with only three strains, ME-3, PCC, and CECT5716, being tested in human trials. *L. fermentum* ME-3, known for its antimicrobial and antioxidant properties, as well as its ability to modulate the immune response, has shown promising results. For instance, consumption of this probiotic has been associated with improved abundance and diversity of lactic acid bacteria and the overall gut microbiome in healthy adults. Furthermore, it has demonstrated potential in reducing the risk of gut diseases. In addition, a study indicated that supplementing the diet with *L. fermentum* ME-3 for one month resulted in decreased LDL and total cholesterol levels, as well as reduced expression of IL-6, in healthy individuals.

Ingestion of *L. fermentum* PCC has been shown to increase the expression of serum IFN-γ and sIgA in the gut. Prophylactic use of this strain in distance runners has been associated with reduced frequency and severity of respiratory illnesses. Similarly, competitive cyclists who consumed the probiotic exhibited enhanced inhibition titers of serum hemagglutinin antibody to influenza virus (H_1N_1) both before and after vaccination, a response also observed in healthy individuals. Moreover, *L. fermentum* PCC treatment has been found to alleviate the severity and extent of dermatitis, characterized by skin inflammation, redness, and itching, possibly through modulation of TNF-α and IFN-γ responses in T_{H1} cells.

L. fermentum CECT5716, a patented strain isolated from human milk, has garnered attention from bioresearch companies for nearly two decades due to its antimicrobial, antioxidant, and immunomodulatory properties. Supplementation with this probiotic not only enhances the T_{H1} response but also boosts the production of neutralizing antibodies following influenza vaccination, highlighting its potential in bolstering systemic defenses against infections. Additionally, this strain has demonstrated efficacy in protecting

against gastrointestinal infections in infants aged 1–6 months, as evidenced by lower bacterial detection rates in probiotic consumers compared to non-consumers. Moreover, *L. fermentum* CECT5716 shows promise in modulating various aspects of mastitis, characterized by inflammation of the breast or udder commonly associated with *Staphylococcus* or *Streptococcus*. In controlled experiments involving over 600 women, consumption of this probiotic prevented the development of lactational mastitis.

In another study, this strain was found to decrease the number of *Staphylococcus* strains in milk, both in healthy mothers and those experiencing mastitis and breast pain. This reduction in *Staphylococcus* count is associated with decreased inflammation, as evidenced by an overall downregulation of IL-8, a key indicator of mastitis, in treated individuals. These findings underscore the diverse benefits offered by the limited strains used in human trials, particularly in addressing mastitis, dermatitis, and respiratory diseases (Table 3). Furthermore, *L. fermentum* has demonstrated utility in enhancing the efficacy of anti-influenza vaccines. However, the efficacy and safety of these strains require thorough evaluation, and larger trials need to be designed to fully understand the molecular pathways and identify potential side effects. This chapter has provided insights into the mechanisms through which various strains of *L. fermentum* can modulate the immune response under diverse conditions (Figure 19).

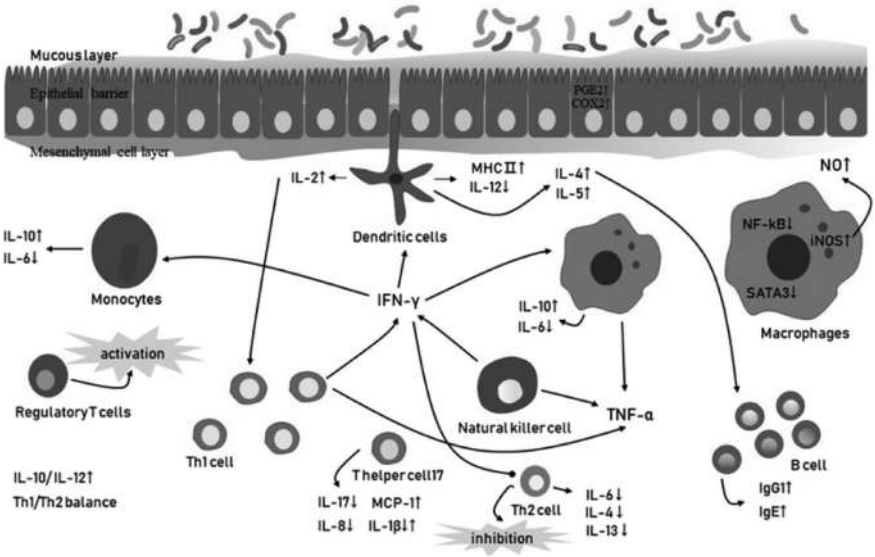


Figure 19. *L. fermentum* bacteria modulate the immune response. Adapted from "Lactobacillus fermentum *and Its Potential Immunomodulatory Properties"* by Y. Zhao, K. Hong, J. Zhao, H. Zhang, Q. Zhai, W. Chen, 2019, *Journal of Functional Foods*, 56, pp. 21-32. PGE2, Prostaglandin E2; COX2, Cyclooxygenase-2; IL, Interleukin; IFN, Interferon; TNF, Tumor necrosis factor; MHC, Major histocompatibility complex; Th, Helper T cell; Ig, Immunoglobulin; MCP, Monocyte chemoattractant protein; NF-ϰB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NO, Nitric oxide; iNOS, Inducible nitric oxide synthase.

Probiotic treatment amplifies the anti-inflammatory response while reducing the expression of key cytokines linked to inflammatory diseases. Both in vitro and in vivo studies have illustrated the significant role of *L. fermentum* strains in pathogen clearance, immune response enhancement, and maintenance of intestinal barrier integrity. However, despite these promising findings, clinical trials involving *L. fermentum* remain limited. Therefore, further research is warranted to unravel the molecular mechanisms underlying these effects and to explore the potential of other bioactive strains.

6. *Limosilactobacillus fermentum* as an Additive for Animal Feed

As outlined in the preceding chapter, *L. fermentum* possesses distinct attributes that render it beneficial for therapeutic use in inflammatory conditions. These bacteria are well-adapted to the intestinal environment, displaying both commensal and transient characteristics. *L. fermentum* is commonly found in the gastrointestinal tract of not only humans but also various other vertebrates. It inhabits the intestines of animals involved in food production, including poultry, pigs, cattle, and fish. Poultry species such as broiler chickens and quails are primarily bred for meat consumption, whereas laying hens are bred for egg production. Moreover, pigs and cattle are raised for meat production, among other purposes. The fish industry encompasses activities related to the cultivation, processing, storage, and distribution of fish as a significant protein source.

The efficiency of the animal industry hinges on effective management strategies encompassing breeding selection, optimal nutrition, and disease control. While antibiotics and vaccines have historically mitigated the impact of infectious diseases, concerns over antibiotic resistance have prompted a shift away from their prophylactic and growth-enhancing use. Thus, there is a growing interest in exploring alternative approaches to combat pathogenic threats and enhance animal performance. Probiotics, prebiotics, bacteriophages, algae, plants, and organic acids have emerged as promising alternatives, with probiotics offering notable benefits within the intestinal ecosystem. As discussed in Chapter 4, *L. fermentum* stands out for its ability to modulate

animal physiology by stimulating the immune system, promoting cytokine expression, and producing compounds that inhibit harmful microorganisms. Additionally, these probiotics can colonize the intestinal epithelium, competitively excluding pathogens. Hence, this probiotic holds significant potential for application in animal biotechnology, with various strains demonstrating efficacy in improving gut health by enhancing architectural integrity, epithelial function, and microbial diversity while antagonizing potential pathogens (Table 4).

Table 4. L. fermentum *strains and their important roles in animal production*

VERTEBRATES		
STRAIN		**IMPORTANT OUTCOMES**
L. fermentum CRL 2085	⇒	Enhanced digestion of plant-derived polysaccharides in cattle.
L. fermentum I5007	⇒	Improved jejunal architecture, enhanced growth performance, downregulation of ileal proinflammatory interleukins, and reduction of *Clostridium* spp. counts in piglet colons.
L. fermentum K9-2	⇒	Reduction in *Clostridium* spp. abundance in piglets.
L. fermentum AD1	⇒	Enhancement of growth performance parameters, reduction of *E. coli* counts in feces, and increment of lactic acid bacteria and *Enterococci* in the cecum and feces of quails.
L. fermentum CCM 7158	⇒	Reduction of yolk cholesterol in laying hens.
L. fermentum Biocenol CCM 7514	⇒	Improved intestinal architecture and goblet cell counts across the small intestine; upregulation of cecal anti-inflammatory interleukins; increase in immunoglobulins and CD8 cells; alleviation of gut damage; and modulation of the cecal inflammatory response caused by *C. jejuni*, *C. coli*, and *S.* Infantis in broiler chickens.
L. fermentum 1.2029	⇒	Increased jejunal goblet cell count; upregulation of *muc2* in the jejunum and ileum; modulation of proinflammatory cytokines in the jejunum; alleviation of necrotic lesions induced by *C. perfringens* in broiler chickens.
L. fermentum NKN51	⇒	Reduction of *E. coli* counts, enhancement of the lactobacillus population, improvement of jejunal architecture and growth performance in broiler chickens.
L. fermentum 1744 (ATCC 14931)	⇒	Improved intestinal architecture, reduced absorption and accumulation of heavy metals in the liver, gills, and muscle, and increased serum immunoglobulin levels in fish.
L. fermentum URLP18	⇒	Enhancement of growth performance parameters, increased numbers of lactic acid bacteria, and improved survival rates associated with *A. hydrophila* in fish.

INVERTEBRATES	
STRAIN	**IMPORTANT OUTCOMES**
L. fermentum SWP-AFFS02	⇒ Increased SCFA concentration in the gut, improved growth performance, and reduced oxidative stress and cell damage in shrimp.
L. fermentum LW2*	⇒ Enhanced growth performance and reduced mortality by *V. alginolyticus* in shrimp.
L. fermentum GR-3	⇒ Alleviation of intestinal mucosal lesions and reduction heavy metal accumulation in crayfish.

Note. Adapted from "An Overview of the Use and Applications of *Limosilactobacillus fermentum* in Broiler Chickens" by M.P. Racines, M.N. Solis, M.A. Šefcová, R. Herich, M. Larrea-Álvarez, V. Revajová, 2023, *Microorganisms*, *11*, p. 1944. *Administered along with *L. pentosus* BD6, *Bacillus subtilis* E20, and *Saccharomyces cerevisiae* P13. SCFA, Short-chain fatty acids; CD, Cluster of differentiation.

In Chapter 3, we explored the multifaceted role of the intestine, highlighting its function as a crucial barrier against pathogens and toxins while also serving as a vital center for immunity and nutrient absorption. Maintaining a balanced intestinal microbiota is paramount for overall health and disease prevention. For example, in broiler chickens, oral administration of *L. fermentum* Biocenol CCM 7514 was found to enhance villus height and the villus height-to-crypt depth (VH:CD) ratio in both the duodenum and ileum, along with an increase in goblet cell numbers, correlating with improved body weight. Similarly, *L. fermentum* 1.2029 promoted goblet cell proliferation in the jejunum and induced mucin overexpression. Furthermore, *L. fermentum* KGL4 positively altered the intestinal microbiota by reducing coliform numbers and increasing lactobacilli counts. Similarly, *L. fermentum* NKN51 supplementation increased lactobacilli in the cecum while reducing *E. coli* levels. These probiotics also improved jejunal villus morphology and ultimately led to enhanced body weight and feed conversion efficiency. Furthermore, administration of *L. fermentum* 1.2133 increased lactic acid bacteria diversity in the ileum and cecum, illustrating the diverse beneficial effects of *L. fermentum* strains on intestinal health in poultry.

Alternatively, *L. fermentum* has been utilized in the development of multi-strain treatments. For example, a probiotic blend containing *Enterococcus* and *Saccharomyces* reduced enterobacteria while increasing lactic acid bacteria

in the ileum, ultimately optimizing growth in poultry. Similarly, a mixture comprising two *L. fermentum* strains, CICC 20176 and GGMCC 0843, enhanced the villus height-to-crypt depth (VH:CD) ratio in the jejunum and ileum when administered to broiler chickens. In pigs, multi-strain mixtures containing *L. fermentum* DSM 20016 and NC1, alongside other beneficial bacteria like *L. plantarum*, *L. acidophilus*, and *Enterococcus faecium*, improved growth parameters such as body weight gain and feed conversion ratio. These treatments also enhanced digestibility, reduced diarrhea incidence, and optimized intestinal microbiota by increasing lactic acid bacteria while decreasing *E. coli* counts. Additionally, in fish, *L. fermentum* 1714 treatment enhanced villus height and decreased heavy metal accumulation in gills, liver, and muscle, while *L. fermentum* R3 Biocenol CCM8675 supplementation increased short-chain fatty acid production in the gut. Inoculation with *L. fermentum* PTCC 1638 and URLP 18 also led to improved growth parameters, including body weight gain and feed conversion ratio.

Nutrition is pivotal in regulating fat metabolism and maintaining a delicate balance between pro- and antioxidants. Excessive production of reactive oxygen and nitrogen species can disrupt the immune response, leading to pathological inflammation. In broiler chickens, supplementation with *L. fermentum* CCM7158 improved overall antioxidant status and reduced serum triglyceride levels. Similar effects were observed with *L. fermentum* KGL4, which also lowered LDL cholesterol levels, raised HDL cholesterol levels, and promoted overall body weight gain. Quails treated with *L. fermentum* AD1 exhibited comparable outcomes. In cattle, *L. fermentum* CRL 2085 supplementation enhanced metabolic pathways involved in plant polysaccharide digestion and positively influenced the composition of the fecal microbiome.

In Chapter 4, we explored the immunomodulatory capabilities of *L. fermentum*. These bacteria activate specific receptors, regulating the expression of various cytokines to modulate the inflammatory response. For instance, in broiler chickens, *L. fermentum* Biocenol CCM7514 regulated cecal expression of IL-13 and IL-14, while reducing inflammatory factors like IL-15, IL-16, IL-17RA, and CXCL-12. This strain also increased the percentage of IgM

plasma cells and intraepithelial CD8 cells. Another mixture, containing *L. fermentum* Js and *Saccharomyces cerevisiae*, boosted intraepithelial CD8, CD4, and CD3 cell populations in the jejunum and upregulated TLR2 and TLR4. Additionally, a probiotic blend with *L. fermentum* CICC20176 and *Bacillus subtilis*, fermented with rapeseed meal, raised serum IgG and IgM levels. In fish, *L. fermentum* 1714 enhanced serum IgM, while *L. fermentum* URLP 18 positively influenced IL-8 and TGF-β expression in the kidneys and intestine.

In broiler chickens, *L. fermentum* demonstrates antimicrobial activity by outcompeting other microorganisms and producing bacteriocins and metabolites. For instance, administration of *L. fermentum* Biocenol CCM7514 enhanced the immune response against *C. jejuni* and *C. coli*, known pathogens associated with inflammation and weight loss. Chickens treated with the probiotic exhibited higher levels of cecal CD8 and IgA plasma cells compared to those infected with *C. coli*. Additionally, the probiotic downregulated the expression of inflammatory cytokines like IL-15 and IL-16. Similarly, *L. fermentum* prevented detrimental effects on intestinal villi height and crypt depth induced by *C. jejuni* challenge in chickens.

Certain *Salmonella* serovars can cause intestinal mucosal damage, adversely affecting animal growth. For example, *S.* Infantis has been shown to decrease the VH:CD ratio in the small intestine. However, oral administration of *L. fermentum* Biocenol CCM7514 not only alleviated these effects but also improved baseline conditions. Additionally, the presence of this probiotic increased the number of intestinal goblet cells and serum IgM levels. Similarly, *L. fermentum* 12133 mitigated the effects induced by *S.* Pullorum, which disrupts intestinal homeostasis by altering the microbiota and causing villi lesions. Another serovar, *S.* Enteritidis, is known to induce hemorrhagic lesions and elevate the expression of inflammatory factors. These effects were attenuated by treating birds with a probiotic mixture containing *L. fermentum*, *L. reuteri*, and *L. salivarius*, which also boosted the overall immune response.

Clostridium species are known to cause intestinal barrier damage, increase the expression of inflammatory cytokines, alter the microbiota, and reduce immune capacity in birds. However, oral inoculation with *L. fermentum* 12029 has been shown to protect animals against these adverse effects. Moreover, avian cholera, caused by *Pasteurella* species, can increase morbidity and mortality by affecting the intestinal microbiota and overall fitness. A probiotic mixture containing *L. fermentum* has been effective in relieving these outcomes, improving the number of lactic acid bacteria, and reducing cholesterol and glucose levels. Similar approaches have been successful in treating quails and laying hens. In quails, exposure to *L. fermentum* CCM7158 reduced the number of cecal and fecal *Salmonella* species, while in laying hens, administration of *L. fermentum* CJL-112 protected against the effects of the H_9N_2 subtype of influenza A virus, particularly reducing viral shedding from the gastrointestinal and respiratory tracts. In pigs, the administration of *L. fermentum* 15007 and K9-2 reduced the counts of *Clostridium* species and downregulated the expression of IL-1β triggered by pathogens. In fish, *L. fermentum* URLP 18 and 1714 increased survival rates in animals challenged with the pathogen *Aeromonas hydrophila*, responsible for Motile Aeromonas septicemia.

The beneficial effects of *L. fermentum* extend beyond vertebrates to include shrimps and crayfish, which play crucial roles in ecosystems for both humans and marine animals. For example, *L. fermentum* GR-3 has been shown to reduce the accumulation of heavy metals and associated intestinal lesions in these crustaceans, while also restoring dysbiosis and reducing oxidative stress. Another strain, *L. fermentum* LW2, provides protection against *Vibrio cholerae*, reducing mortality and enhancing growth. Moreover, administration of *L. fermentum* SWP-AFFSD2 increases fatty acid concentration in the gut and improves body weight and feed conversion ratio in shrimps and crayfish. These findings emphasize the beneficial impact of *L. fermentum* on intestinal health and animal growth. Furthermore, they highlight the potential of certain strains to protect animals from pathogenic threats by adhering to the epithelium, producing antimicrobial compounds, and enhancing the host immune response (Figure 20).

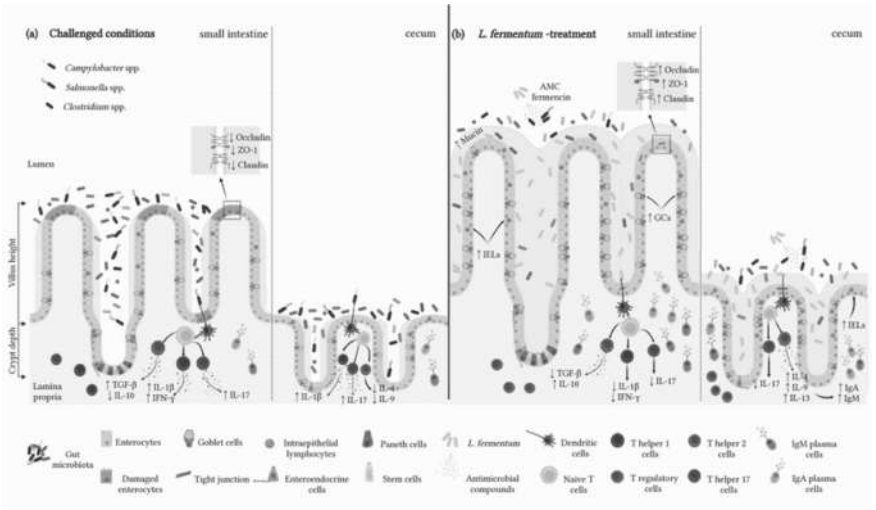


Figure 20. Interaction among *L. fermentum*, epithelial cells, and microbiota. Adapted from *"An Overview of the Use and Applications of* Limosilactobacillus fermentum *in Broiler Chickens"* by M. P. Racines, M. N. Solis, M. A. Šefcová, R. Herich, M. Larrea-Álvarez, V. Revajová, 2023, *Microorganisms*, 11, p. 1944.

The summarized in vivo studies emphasize the advantageous outcomes of incorporating *L. fermentum* into animal nutrition regimens. These benefits extend to bolstering animal health, immune function, and growth. Additionally, the probiotic's ability to counteract pathogenic and opportunistic microorganisms is notable for mitigating their adverse impacts. Strains of *L. fermentum* have exhibited prowess in enhancing the ratio of villus height to crypt depth (VH:CD), shaping intestinal microbiota composition, fortifying intestinal integrity, and mitigating inflammation. Given that animals are raised for various products such as meat and eggs, the use of vaccines and antibiotics has been pivotal for large-scale production. However, concerns over antibiotic resistance have prompted a shift away from antibiotic use in animal husbandry. *L. fermentum* emerges as a favorable alternative to promote animal well-being, as its administration positively influences the intestinal environment and fosters improved growth. Thus, integrating this probiotic into supplements shows potential for optimizing animal performance and safeguarding against infectious diseases.

7. Conclusion

All the food that we consume must traverse the digestive tract, where specialized cells transform it into nutrients, energy, and waste products. The gastrointestinal tract spans from the mouth to the anus and comprises both upper and lower parts. The lower part encompasses the large and small intestines, with the latter further divided into the duodenum, jejunum, and ileum. Upon reaching the small intestine from the stomach, food mixes with digestive enzymes, bile, and pancreatic juices. Sugars, vitamins, fatty acids, and amino acids are absorbed here. The large intestine, subdivided into various segments, is responsible for water and ion absorption, with the resulting waste material expelled as feces

The tract can be divided into four layers: the mucosa, submucosa, muscular layer, and adventitia or serosa. The adventitia is composed of various layers of connective tissue, whereas the serosa constitutes a smooth tissue membrane secreting fluid that works as a lubricant for muscle movement; the synchronized contraction of muscles (peristalsis) allows the movement of food through the tract. The submucosa comprises connective tissue and nerves that extend into the mucosa and regulate peristalsis, blood flow, water, and electrolyte secretion. Finally, the mucosa surrounds the lumen and is made of three components: the muscularis, lamina propria, and epithelium. The smooth muscle comprising the muscularis augments the interaction between the epithelium and the contents of the lumen. The lamina propria, on the other hand, represents a crucial location for the immune response because it is composed of connective tissue rich in lymphocytes and macrophages. The epithelium is composed of various cell types. Enterocytes, for example, are specialized in the absorption

of sugars, ions, vitamins, lipids, peptides, and amino acids, whereas goblet cells are responsible for the secretion of mucus. In contrast, enteroendocrine cells produce hormones that regulate homeostasis and digestion, and Paneth cells synthesize antimicrobial peptides. Moreover, M cells are dedicated to antigen sampling, and cup and tuft cells play important roles in host–microbe interactions and immune responses, respectively.

A group of protein complexes is responsible for joining adjacent cells. Gap junctions, for example, are located near the basal membrane and permit the movement of ions and other small molecules; they also allow the passage of electrical impulses. Likewise, desmosomes and zonula adherens are complexes that join cells, although they interact with components of the cytoskeleton. Finally, tight junctions are located near the apical membrane, and although they form a thin and tight pass, they allow the passage of solutes between cells. Microvilli are found in the apical membranes of enterocytes, which are protected by a matrix composed of glycoproteins and glycolipids. Hydrolases process sugars and proteins so that their constituents can be absorbed by specialized transporters. Similarly, water and electrolytes are absorbed, although this could also be achieved paracellularly. The intestinal epithelium not only blocks the entry of pathogens but also contributes to the development of intestinal immunity. In fact, gut-associated lymphoid tissue offers protection against invaders, which can encounter lymphocytes, phagocytes, and antigen-presenting cells. Additionally, acidic conditions in the stomach are toxic to various microorganisms, and the antibodies present in the mucus can also neutralize dangerous agents.

Microorganisms can be pathogenic, commensal, or mutualistic. Some microbes metabolize various beneficial compounds, such as sterols, vitamins, and short-chain fatty acids, whereas pathogenic bacteria can induce pathological inflammatory conditions. Furthermore, the low diversity of gut microbiota appears to exacerbate these conditions. Undoubtedly, the host must manage the presence of various microorganisms that must be either attacked or tolerated. Dendritic cells, macrophages, and epithelial cells constantly sense the presence of microbes; these cells express a group of receptors capable of

recognizing particular patterns of bacteria. When encountering a pathogen, these receptors are activated and induce the expression of inflammatory factors that ultimately contribute to its elimination. However, when encountering commensal bacteria, they do not trigger the expression of inflammatory factors; instead, they synthesize a variety of anti-inflammatory proteins. Evidently, the growth of pathogenic bacteria induces inflammation, which might disrupt the functional composition of the microbiota, as has been observed when beneficial bacteria are underrepresented. Thus, it has been proposed that such conditions can be reversed by the administration of suitable bacteria. Indeed, in certain cases, probiotic administration has proved effective for restoring homeostasis.

Probiotics act by synthesizing antibacterial compounds, adhering to epithelial cells, and stimulating the immune system. Bacteriocins are proteins that can disrupt the cell walls and membranes of related and unrelated species. Hence, bacteriocin-producing bacteria must also produce proteins that neutralize their effects, either by interacting with them or by competing for receptors. Bacteriocins are classified into three groups: Class I bacteriocins, also called lantibiotics; Class II bacteriocins, known as non-lantibiotics because they have not undergone any post-translational modifications; and Class III bacteriocins. Bacteriocins are also known for their immunomodulatory capacities; some have proved useful for increasing the number of lymphocytes and modulating the expression of cytokines, whereas others induce the expression of antimicrobial peptides in eukaryotic cells. In general, bacteriocins have been suggested as potential novel therapeutics that could be used in combination with conventional drugs to reduce their side effects.

Probiotic bacteria can adhere to mucosal and epithelial cells, enhancing their role as a barrier. This interaction not only triggers the expression of defensive molecules and stimulates mucus production but also excludes potential pathogenic microorganisms. Bacterial adhesion involves both specific and non-specific interactions. The mucus layer, rich in glycoproteins and glycolipids, provides various sugar moieties for adhesion. Indeed, probiotic strains produce mucus-binding proteins and mannose-specific adhesins, with lectin adhesins also identified in some strains. Fimbriae and flagella have also been associated

with adhesion processes. The interaction between probiotics and epithelial cells stimulates the synthesis and release of defensins, which play a significant role in clearing pathogens. In fact, the attachment of probiotic bacteria not only displaces unwanted microorganisms but also reduces the availability of nutrients. Moreover, some probiotics modify the environment by secreting organic acids, making it less conducive to potential invaders.

Finally, the presence of probiotic bacteria modulates the immune response. Gram-positive bacteria possess a thick cell wall composed of various layers of peptidoglycan and other polymers, including teichoic acids, lipoproteins, and capsular polysaccharides. These molecules contain motifs that are recognized by receptors expressed in the intestinal mucosa. MAMPs signal through a group of receptors, conveniently known as pattern recognition receptors. For instance, lipoteichoic acids interact with Toll-like receptor complexes commonly synthesized by fibroblasts, leukocytes, epithelial cells, and endothelial cells. Once activated, these receptors recruit adaptor proteins that, in turn, activate other signaling molecules influencing cytokine expression, cell proliferation, and cell survival. The signaling pathways activated by these receptors also improve the conditions of the intestinal epithelial barrier, such as increased synthesis of tight junction complexes. Lactic acid bacteria (LAB) are among the most common probiotics, although other microorganisms can also be classified as such, including gram-negative bacteria or fungi. LAB are native inhabitants of the intestines, contributing to maintaining homeostasis. Various species of the genera *Lactobacillus* are residents of the gastrointestinal tract of several animals, thus developing mutualistic relationships with their hosts.

Various strains of *L. fermentum* have undergone rigorous testing to assess their capability to survive harsh environments, demonstrating optimal results, particularly in tolerating low pH levels and high concentrations of bile salts. Generally, lactic acid bacteria employ diverse molecules to adhere to host cells. For instance, *L. fermentum* strains utilize binding proteins that interact with various host extracellular factors, including fibronectin- or mucin-binding proteins. Apart from these proteins, sortases play a pivotal role in

bacterial adhesion. Additionally, other bacterial molecular patterns such as lipoteichoic acid (LTA), lipopolysaccharides (LPS), and peptidoglycan (PG) may contribute to cellular interactions, while passive and electrostatic forces also significantly influence this process.

The presence of probiotics reduces the likelihood of colonization by potential pathogens by competing for the intestinal niche. They achieve this by readily attaching to cells and secreting antimicrobial peptides that destabilize membranes and bacterial cell walls. Specific bacteriocins produced by *L. fermentum*, categorized as fermicins, have demonstrated activity against both gram-positive and gram-negative bacteria. Furthermore, as mentioned earlier, these probiotics produce organic acids that acidify the environment, inhibiting the growth of potentially harmful microorganisms such as *H. pylori*, *S. aureus*, *E. coli*, and *Candida* spp. Pathogenic infections often disrupt the host's redox state. *L. fermentum* strains are capable of activating receptors that stimulate the expression of genes associated with the antioxidant response. Additionally, they produce the glutathione complex, which can neutralize harmful oxidizing molecules.

L. fermentum not only attaches to cells and produces compounds with antibacterial activities but also has the capability to stimulate key receptors on a variety of cells, ultimately modulating gene expression. Therefore, numerous studies have investigated the ability of *L. fermentum* strains to mitigate the effects of inflammatory diseases. In vitro studies have demonstrated that strains of this probiotic produce short-chain fatty acids (SCFA) from fiber, which in turn stimulate the production of T cells. Studies utilizing dendritic cells have revealed that exposure to the probiotic promotes monocyte differentiation into dendritic cells (DCs) while increasing the expression of key cytokines. Similarly, in macrophages, *L. fermentum* strains have shown efficacy in increasing their phagocytic activities and antibacterial properties. For instance, *L. fermentum* UCO-979C modulated the expression of factors considered crucial in gastric and pulmonary diseases. Additionally, *L. fermentum* DLBSA204 has been shown to activate macrophages and elicit the expression of various inflammatory and anti-inflammatory genes; similar outcomes were observed with the CECT5716 strain in cells challenged

with bacterial lipopolysaccharide (LPS). Once again, administration of *L. fermentum* prompted the activation of natural killer (NK) cells and T cells with concomitant expression of both type I and type II cytokines.

As expected, in vivo studies have demonstrated that the administration of *L. fermentum* strains can attenuate inflammatory processes in various conditions, including colitis, gastric issues, and hepatic injury. Administration of *L. fermentum* strains has shown positive effects in mice fed a high-cholesterol diet by reducing dyslipidemia and increasing serum antibody levels. Moreover, this probiotic has been observed to not only prevent dysfunction of the intestinal barrier by mitigating intestinal permeability but also regulate blood pressure in animals suffering from hypertension. In mice infected with *E. coli*, *L. fermentum* MTCC 5898 reduced colonization by the pathogen in the liver and spleen, while *L. fermentum* ME-3 mitigated the effects of *Salmonella* infection.

Unfortunately, clinical investigations utilizing *L. fermentum* strains are limited. However, the obtained results demonstrate the potential of probiotics to modulate the immune reaction associated with inflammatory conditions. For example, consumption of *L. fermentum* ME-3 not only reduced the expression of inflammatory genes but also enhanced the diversity and abundance of lactic acid bacteria while lowering cholesterol levels in healthy adults. Another strain, PCC, when ingested by distance runners, alleviated the severity of respiratory illnesses and mitigated the effects of dermatitis. Supplementation with *L. fermentum* PCC and CECT5716 improved the immune response following influenza vaccination. The latter strain is also beneficial for protecting infants against gastrointestinal infections. Its consumption not only prevents the development of mastitis in lactating women but also reduces the number of staphylococci in milk from women suffering from mastitis and downregulates the expression of inflammatory proteins associated with such conditions. However, clinical studies are scarce, and only a handful of strains have been used in this context. Thus, further research must be oriented toward unraveling the mechanisms of known effects and evaluating other strains with potential bioactive properties.

L. fermentum, a bacterial strain with probiotic effects, has found application in animal biotechnology aimed at improving performance. In species such as broiler chickens, pigs, and fish, strains of *L. fermentum* have demonstrated the ability to enhance growth parameters, including feed conversion ratio and body weight. Additionally, the probiotic has been observed to positively influence intestinal architecture and reduce the abundance of pathogenic bacteria. Achieving optimal nutritional levels is essential for animal production, and the application of *L. fermentum* strains has proven beneficial for modulating triglyceride and polysaccharide metabolism in broiler chickens and quails. In broiler chickens specifically, treatment with *L. fermentum* has been associated with a reduction in the expression of inflammatory factors, coupled with an increase in the levels of cytokines associated with antibody production. This modulation of cytokine expression has further resulted in an augmentation of lymphocyte subpopulations. Moreover, elevated serum antibody levels have been observed not only in chickens but also in fish following exposure to various strains of *L. fermentum*. Furthermore, treatment with these probiotic bacteria has been shown to enhance the immune response against infections caused by *Campylobacter* spp. Similarly, administration of *L. fermentum* has been effective in alleviating intestinal stress induced by various *Salmonella* serovars. These protective effects have also been observed in quails, where probiotic exposure led to a decrease in the adverse effects of *Salmonella* infection. The beneficial characteristics of *L. fermentum* extend to viral infections as well. For example, the probiotic has been demonstrated to mitigate the effects of H_9N_2 viral infection. In pigs, intestinal damage caused by *Clostridium* spp. was attenuated in animals supplemented with a probiotic mixture. In aquaculture, promising outcomes have been achieved with the administration of *L. fermentum*. In fish, survival rates against bacterial septicemia caused by *A. hydrophila* improved following treatment, while in shrimp, a reduction in heavy metal accumulation and oxidative stress was observed. Additionally, in these crustaceans, *L. fermentum* reduced the pathogenic impact of *V. cholerae*.

The studies summarized here have highlighted the beneficial effects of using *L. fermentum* strains in terms of nutrition, health, and immune response. Particularly, *L. fermentum* exhibits the ability not only to counteract the effects of pathogenic bacteria but also to enhance intestinal health and microbial diversity. Furthermore, certain strains have been shown to positively modulate the immune response by increasing anti-inflammatory factors while reducing inflammatory ones. Nevertheless, future investigations should not only focus on assessing the effects of probiotic treatment but also on elucidating the underlying molecular mechanisms. It is essential to understand how *L. fermentum* exerts its beneficial effects at the molecular level to optimize its use in various applications. Indeed, *L. fermentum* strains should be recognized as key components in the development of nutritional supplements aimed at improving intestinal health and preventing colonization by harmful agents. As we explore further into the study of these beneficial gut inhabitants, we will further unveil their significance and their contributions to maintaining the overall balance within the body.

References

Chapter 1.

Allaire, J. M., Crowley, S. M., Law, H. T., Chang, S. Y., Ko, H. J., & Vallance, B. A. (2018). The intestinal epithelium: Central coordinator of mucosal immunity. *Trends in Immunology*, *39*(9), 677-696.

Britannica, T. Editors of Encyclopaedia (2024, January 11). *Gastrointestinal Tract*. ENCYCLOPEDIA BRITANICA. https://www.britannica.com/science/gastrointestinal-tract.

Collado, M. C., Gueimonde, M. & Salminen, S. (2010). Chapter 23 - Probiotics in Adhesion of Pathogens: Mechanisms of Action. In R. R. Watson & V. R. Preedy (Eds.), *Bioactive foods in promoting health* (pp. 353-370). London, UK: Academic Press.

Hernández-González, J. C., Martínez-Tapia, A., Lazcano-Hernández, G., García-Pérez, B. E., & Castrejón-Jiménez, N. S. (2021). Bacteriocins from lactic acid bacteria. A powerful alternative as antimicrobials, probiotics, and immunomodulators in veterinary medicine. *Animals*, *11*(4), 979.

Hollister, E. B., Gao, C., & Versalovic, J. (2014). Compositional and functional features of the gastrointestinal microbiome and their effects on human health. *Gastroenterology, 146*(6), 1449-1458.

Racines, M. P., Solis, M. N., Šefcová, M. A., Herich, R., Larrea-Álvarez, M., & Revajová, V. (2023). An overview of the use and applications of *Limosilactobacillus fermentum* in broiler chickens. *Microorganisms*, *11*(8), 1944.

Rodríguez-Sojo, M. J., Ruiz-Malagón, A. J., Rodríguez-Cabezas, M. E., Gálvez, J., & Rodríguez-Nogales, A. (2021). *Limosilactobacillus fermentum* CECT5716: Mechanisms and therapeutic insights. *Nutrients*, *13*(3), 1016.

Zhao, Y., Hong, K., Zhao, J., Zhang, H., Zhai, Q., & Chen, W. (2019). *Lactobacillus fermentum* and its potential immunomodulatory properties. *Journal of Functional Foods*, *56*, 21-32.

Chapter 2.

Agarwal, A. (2018, April 23). *Small Intestine*. KNOW YOUR BODY. Retrieved November 16, 2023 from https://www.knowyourbody.net/small-intestine.html.

Allaire, J. M., Crowley, S. M., Law, H. T., Chang, S. Y., Ko, H. J., & Vallance, B. A. (2018). The intestinal epithelium: Central coordinator of mucosal immunity. *Trends in Immunology*, *39*(9), 677-696.

Arranz, E., Peña, A. S., & Bernardo, D. (2013). Mediators of inflammation and immune responses in the human gastrointestinal tract. *Mediators of Inflammation*, *13*, 865638.

Britannica, T. Editors of Encyclopaedia (2024, January 11). *Gastrointestinal Tract*. ENCYCLOPEDIA BRITANICA. https://www.britannica.com/science/gastrointestinal-tract.

Bron, P. A., Van Baarlen, P., & Kleerebezem, M. (2012). Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. *Nature Reviews Microbiology*, 10(1), 66-78.

BYJU'S. (n.d.). *Gastrointestinal Tract*. Retrieved November 15, 2023 from https://byjus.com/biology/gastrointestinal-tract/.

Cani, P. D. (2018). Human gut microbiome: Hopes, threats and promises. *Gut*, *67*(9), 1716-1725.

Egorov, V. I., Schastlivtsev, I. V., Prut, E. V., Baranov, A. O., & Turusov, R. A. (2002). Mechanical properties of the human gastrointestinal tract. *Journal of Biomechanics*, *35*(10), 1417-1425.

Feigl, M. (2022, July 13). *New Stem Cell Mechanism in Your Gut*. EUREKALERT. Retrieved November 27, 2023 from https://www.eurekalert.org/news-releases/958563.

GLOSSARY. (n.d.). *Gastrointestinal Tract Tunics*. Retrieved November 21, 2023 from https://ditki.com/course/gross-anatomy/glossary/term/gastrointestinal-tract-tunics.

GLOSSARY. (n.d.). *Intestinal Folding: Villi & Microvilli*. Retrieved November 21, 2023 from https://ditki.com/course/biochemistry/glossary/physiological-process/intestinal-folding-villi-microvilli.

Haroun, E., Kumar, P. A., Saba, L., Kassab, J., Ghimire, K., Dutta, D., & Lim, S. H. (2023). Intestinal barrier functions in hematologic and oncologic diseases. *Journal of Translational Medicine*, *21*(1), 1-19.

Hollister, E. B., Gao, C., & Versalovic, J. (2014). Compositional and functional features of the gastrointestinal microbiome and their effects on human health. *Gastroenterology, 146*(6), 1449-1458.

Kamada, N., Chen, G. Y., Inohara, N., & Nunez, G. (2013). Control of pathogens and pathobionts by the gut microbiota. *Nature Immunology*, *14*(7), 685-690.

Kutova, O. M., Sencha, L. M., Pospelov, A. D., Dobrynina, O. E., Brilkina, A. A., Cherkasova, E. I., & Balalaeva, I. V. (2020). Comparative analysis of cell–cell contact abundance in ovarian carcinoma cells cultured in two- and three-dimensional in vitro models. *Biology*, *9*(12), 446.

QUIZLET. (n.d.). *Gastrointestinal Physiology: (3) Digestion and Absorbtion*. Retrieved November 30, 2023 from https://quizlet.com/de/776549297/gastrointestinal-physiology-3-digestion-and-absorbtion-flash-cards/.

Ramiro-Puig, E., Perez-Cano, F. J., Castellote, C., Franch, A., & Castell, M. (2008). The bowel: a key component of the immune system. *Revista Espanola de Enfermedades Digestivas: Organo Oficial de la Sociedad Espanola de Patologia Digestiva*, *100*(1), 29-34.

Romito, L. (2019). *p62 Knock-Out Mice: Investigation on Gastrointestinal Tract*. [Bachelor's thesis, University of Camerino]. https://www.researchgate.net/publication/357574094_p62_knock-out_mice_investigation_on_gastrointestinal_tract.

San Roman, A. K., & Shivdasani, R. A. (2011). Boundaries, junctions and transitions in the gastrointestinal tract. *Experimental Cell Research*, *317*(19), 2711-2718.

Sethi, S. & Seladi-Schulman, J. (2023, March 13). *What's the Length of Your Small and Large Intestines?* HEALTHLINE. Retrieved November 16, 2023 from https://www.healthline.com/health/digestive-health/how-long-are-your-intestines.

Chapter 3.

Bron, P. A., Van Baarlen, P., & Kleerebezem, M. (2012). Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. *Nature Reviews Microbiology*, *10*(1), 66-78.

Collado, M. C., Gueimonde, M. & Salminen, S. (2010). Chapter 23 - Probiotics in Adhesion of Pathogens: Mechanisms of Action. In R. R. Watson & V. R. Preedy (Eds.), *Bioactive foods in promoting health* (pp. 353-370). London, UK: Academic Press.

FAO/WHO. Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. 2001. Available online: http://www.fao.org/3/a-a0512e.pdf (accessed on 10 December 2023).

Fijan, S. (2014). Microorganisms with claimed probiotic properties: an overview of recent literature. *International Journal of Environmental Research and Public Health*, *11*(5), 4745-4767.

Hernández-González, J. C., Martínez-Tapia, A., Lazcano-Hernández, G., García-Pérez, B. E., & Castrejón-Jiménez, N. S. (2021). Bacteriocins from lactic acid bacteria. A powerful alternative as antimicrobials, probiotics, and immunomodulators in veterinary medicine. *Animals*, *11*(4), 979.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., ... & Sanders, M. E. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*, 506-514.

Kon, K. (n.d.). *Bacteria of Different Shapes*. SCIENCE PHOTO LIBRARY. Retrieved December 1, 2023 from https://www.sciencephoto.com/media/934700/view/bacteria-of-different-shapes-illustration.

Muras, A., Mallo, N., Otero-Casal, P., Pose-Rodríguez, J. M., & Otero, A. (2022). Quorum sensing systems as a new target to prevent biofilm-related oral diseases. *Oral Diseases*, *28*(2), 307-313.

Mutuku, C., Gazdag, Z., & Melegh, S. (2022). Occurrence of antibiotics and bacterial resistance genes in wastewater: resistance mechanisms and antimicrobial resistance control approaches. *World Journal of Microbiology and Biotechnology*, *38*(9), 152.

SHUTTERSTOCK. (n.d.). *Diseño Científico de las Diferencias Estructurales entre Bacterias Gram Positivas y Gram Negativas*. Retrieved December 5, 2023 from https://www.shutterstock.com/es/image-vector/scientific-designing-structural-differences-between-gram-2124045074.

SHUTTERSTOCK. (n.d.). *Estructuras Celulares Bacterianas*. Retrieved December 5, 2023 from https://www.shutterstock.com/es/image-vector/bacterial-cell-structures-labeled-on-bacillus-1522904069.

Summer, M., Ali, S., Fiaz, U., Tahir, H. M., Ijaz, M., Mumtaz, S., ... & Fiaz, H. (2023). Therapeutic and immunomodulatory role of probiotics in breast cancer: A mechanistic review. *Archives of Microbiology*, *205*(8), 296.

Tojo, R., Suárez, A., Clemente, M. G., de los Reyes-Gavilán, C. G., Margolles, A., Gueimonde, M., & Ruas-Madiedo, P. (2014). Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis. *World Journal of Gastroenterology*, *20*(41), 15163.

Zhang, M., Sun, K., Wu, Y., Yang, Y., Tso, P., & Wu, Z. (2017). Interactions between intestinal microbiota and host immune response in inflammatory bowel disease. *Frontiers in Immunology*, 8, 942.

Chapter 4.

Archer, A. C., & Halami, P. M. (2015). Probiotic attributes of *Lactobacillus fermentum* isolated from human feces and dairy products. *Applied Microbiology and Biotechnology*, *99*(19), 8113-8123.

Archer, A. C., Kurrey, N. K., & Halami, P. M. (2018). In vitro adhesion and anti-inflammatory properties of native *Lactobacillus fermentum* and *Lactobacillus delbrueckii* spp. *Journal of Applied Microbiology*, *125*(1), 243-256.

Chaplin, A. V., Shkoporov, A. N., Efimov, B. A., Pikina, A. P., Borisova, O. Y., Gladko, I. A., & Kafarskaia, L. I. (2015). Draft genome sequence of *Lactobacillus fermentum* NB-22. *Genome Announcements*, *3*(4), 10-1128.

Cheah, S. A., Chai, C. Y., Tan, I. S., Foo, H. C. Y., & Lam, M. K. (2022). New prospect of algae for sustainable production of lactic acid: Opportunities and challenges. *Progress in Energy and Environment*, *21*, 19-28.

Chen, X., Song, J. L., Hu, Q., Wang, H., Zhao, X., & Suo, H. (2018). Positive enhancement of Lactobacillus fermentum HY01 on intestinal movements of mice having constipation. *Applied Biological Chemistry*, *61*, 39-48.

de Souza, B. M. S., Borgonovi, T. F., Casarotti, S. N., Todorov, S. D., & Penna, A. L. B. (2018). *Lactobacillus casei* and *Lactobacillus fermentum* strains isolated from mozzarella cheese: Probiotic potential, safety, acidifying kinetic parameters and viability under gastrointestinal tract conditions. *Probiotics Antimicrobial Proteins*, *11*, 382-396.

Halimi, S., & Mirsalehian, A. (2016). Assessment and comparison of probiotic potential of four *Lactobacillus species* isolated from feces samples of Iranian infants. *Microbiology and Immunology*, *60*(2), 73–81.

Jang, S. Y., Heo, J., Park, M. R., Song, M. H., Kim, J. N., Jo, S. H., & Oh, S. (2017). Genome characteristics of *Lactobacillus fermentum* strain JDFM216 for application as probiotic bacteria. *Journal of Microbiology and Biotechnology*, *27*(7), 1266-1271.

Khalil, E. S., Abd Manap, M. Y., Mustafa, S., Alhelli, A. M., & Shokryazdan, P. (2018). Probiotic properties of exopolysaccharide-producing lactobacillus strains isolated from Tempoyak. *Molecules*, *23*(2), 398.

Racines, M. P., Solis, M. N., Šefcová, M. A., Herich, R., Larrea-Álvarez, M., & Revajová, V. (2023). An overview of the use and applications of *Limosilactobacillus fermentum* in broiler chickens. *Microorganisms*, *11*(8), 1944.

Rodríguez-Sojo, M. J., Ruiz-Malagón, A. J., Rodríguez-Cabezas, M. E., Gálvez, J., & Rodríguez-Nogales, A. (2021). *Limosilactobacillus fermentum* CECT5716: Mechanisms and therapeutic insights. *Nutrients*, *13*(3), 1016.

Zhao, Y., Hong, K., Zhao, J., Zhang, H., Zhai, Q., & Chen, W. (2019). *Lactobacillus fermentum* and its potential immunomodulatory properties. *Journal of Functional Foods*, *56*, 21-32.

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M., Harris, H. M., Mattarelli, P., ... & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*, *70*(4), 2782-2858.

Chapter 5.

Ale, E. C., Perezlindo, M. J., Burns, P., Tabacman, E., Reinheimer, J. A., & Binetti, A. G. (2016). Exopolysaccharide from Lactobacillus fermentum Lf2 and its functional characterization as a yogurt additive. *Journal of Dairy Research*, *83*(4), 487-492.

Archer, A. C., Kurrey, N. K., & Halami, P. M. (2018). In vitro adhesion and anti-inflammatory properties of native *Lactobacillus fermentum* and *Lactobacillus delbrueckii* spp. *Journal of Applied Microbiology*, *125*(1), 243-256.

Anderson, R. C., Ulluwishewa, D., Young, W., Ryan, L. J., Henderson, G., Meijerink, M., ... & Roy, N. C. (2016). Human oral isolate *Lactobacillus fermentum* AGR1487 induces a pro-inflammatory response in germ-free rat colons. *Scientific Reports*, *6*(1), 20318.

Barone, R., Rappa, F., Macaluso, F., Caruso Bavisotto, C., Sangiorgi, C., Di Paola, G., & Marino Gammazza, A. (2016). Alcoholic liver disease: A mouse model reveals protection by *Lactobacillus fermentum*. *Clinical and Translational Gastroenterology*, *7*, e138.

Bond, D. M., Morris, J. M., & Nassar, N. (2017). Study protocol: Evaluation of the probiotic *Lactobacillus fermentum* CECT5716 for the prevention of mastitis in breastfeeding women: A randomised controlled trial. *BMC Pregnancy Childbirth*, *17*(1), 148.

García, A., Navarro, K., Sanhueza, E., Pineda, S., Pastene, E., Quezada, M., & González, C. (2017). Characterization of *Lactobacillus fermentum* UCO-979C, a probiotic strain with a potent anti-*Helicobacter pylori* activity. *Electronic Journal of Biotechnology*, *25*, 75-83.

Maldonado-Lobon, J. A., Gil-Campos, M., Maldonado, J., Lopez-Huertas, E., Flores-Rojas, K., Valero, A. D., & Olivares, M. (2015). Long-term safety of early consumption of *Lactobacillus fermentum* CECT5716: A 3-year follow-up of a randomized controlled trial. *Pharmacological Research*, *95*, 12-19.

Rodríguez-Sojo, M. J., Ruiz-Malagón, A. J., Rodríguez-Cabezas, M. E., Gálvez, J., & Rodríguez-Nogales, A. (2021). *Limosilactobacillus fermentum* CECT5716: Mechanisms and therapeutic insights. *Nutrients*, *13*(3), 1016.

Toral, M., Romero, M., Rodriguez-Nogales, A., Jimenez, R., Robles-Vera, I., Algieri, F., & Duarte, J. (2018). *Lactobacillus fermentum* improves tacrolimus-induced hypertension by restoring vascular redox state and improving eNOS coupling. *Molecular Nutrition & Food Research*, *62*(14), e1800033.

Vanhaecke, T., Aubert, P., Grohard, P. A., Durand, T., Hulin, P., Paul-Gilloteaux, P., & Neunlist, M. (2017). *L. fermentum* CECT 5716 prevents stress-induced intestinal barrier dysfunction in newborn rats. *Neurogastroenterology & Motility*, *29*(8), e13069.

Zhao, Y., Hong, K., Zhao, J., Zhang, H., Zhai, Q., & Chen, W. (2019). *Lactobacillus fermentum* and its potential immunomodulatory properties. *Journal of Functional Foods*, *56*, 21-32.

Chapter 6.

Giang, H. H., Viet, T. Q., Ogle, B., & Lindberg, J. E. (2010). Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. *Livestock Science*, *129*(1-3), 95-103.

Madreseh, S., Ghaisari, H. R., & Hosseinzadeh, S. (2019). Effect of lyophilized, encapsulated *Lactobacillus fermentum* and lactulose feeding on growth performance, heavy metals, and trace element residues in rainbow trout (*Oncorhynchus mykiss*) tissues. *Probiotics and Antimicrobial Proteins*, *11*, 1257-1263.

Mansilla, F. I., Ficoseco, C. A., Miranda, M. H., Puglisi, E., Nader-Macías, M. E. F., Vignolo, G. M., & Fontana, C. A. (2022). Administration of probiotic lactic acid bacteria to modulate fecal microbiome in feedlot cattle. *Scientific Reports*, *12*(1), 12957.

Mudroňová, D., Nemcová, R., Lauková, A., Koščová, J., Strompfová, V., Györyová, K., ... & Lazar, G. (2006). Effect of *Lactobacillus fermentum* alone, and in combination with zinc (II) propionate on *Salmonella enterica* serovar Düsseldorf in Japanese quails. *Biologia*, *61*(6), 797-801.

Nimalan, N., Sørensen, S. L., Fečkaninová, A., Koščová, J., Mudroňová, D., Gancarčíková, S., ... & Sørensen, M. (2023). Supplementation of lactic acid bacteria has positive effects on the mucosal health of Atlantic salmon (*Salmo salar*) fed soybean meal. *Aquaculture Reports*, *28*, 101461.

Racines, M. P., Solis, M. N., Šefcová, M. A., Herich, R., Larrea-Álvarez, M., & Revajová, V. (2023). An overview of the use and applications of *Limosilactobacillus fermentum* in broiler chickens. *Microorganisms*, *11*(8), 1944.

Strompfova, V., Marcinakova, M., Gancarcikova, S., Jonecova, Z., Scirankova, L., Guba, P., ... & Laukova, A. (2005). New probiotic strain *Lactobacillus fermentum* AD1 and its effect in Japanese quail. *Veterinární Medicína – Czech*, *50*(9), 415-420.

Šefcová, M., Larrea-Álvarez, M., Larrea-Álvarez, C., Karaffová, V., Revajová, V., Gancarčíková, S., ... & Herich, R. (2020). *Lactobacillus fermentum* administration modulates cytokine expression and lymphocyte subpopulation levels in broiler chickens challenged with *Campylobacter coli*. *Foodborne Pathogens and Disease*, *17*(8), 485-493.

Šefcová, M., Larrea-Álvarez, M., Larrea-Álvarez, C., Revajová, V., Karaffová, V., Koščová, J., ... & Herich, R. (2020). Effects of *Lactobacillus fermentum* supplementation on body weight and pro-inflammatory cytokine expression in *Campylobacter jejuni*-challenged chickens. *Veterinary Sciences*, *7*(3), 121.

Šefcová, M. A., Larrea-Álvarez, M., Larrea-Álvarez, C. M., Karaffová, V., Ortega-Paredes, D., Vinueza-Burgos, C., ... & Revajová, V. (2021). The probiotic *Lactobacillus fermentum* Biocenol CCM 7514 moderates *Campylobacter jejuni*-induced body weight impairment by improving gut morphometry and regulating cecal cytokine abundance in broiler chickens. *Animals*, *11*(1), 235.

Šefcová, M. A., Ortega-Paredes, D., Larrea-Álvarez, C. M., Mina, I., Guapás, V., Ayala-Velasteguí, D., ... & Larrea-Álvarez, M. (2023). Effects of *Lactobacillus fermentum* administration on intestinal morphometry and antibody serum levels in *Salmonella*-Infantis-challenged chickens. *Microorganisms*, *11*(2), 256.

Veizaj-Delia, E., Piu, T., Lekaj, P., & Tafaj, M. (2010). Using combined probiotic to improve growth performance of weaned piglets on extensive farm conditions. *Livestock Science*, *134*(1-3), 249-251.

Wang, Y. C., Hu, S. Y., Chiu, C. S., & Liu, C. H. (2019). Multiple-strain probiotics appear to be more effective in improving the growth performance and health status of white shrimp, *Litopenaeus vannamei*, than single probiotic strains. *Fish & Shellfish immunology*, *84*, 1050-1058.

Wang, W., Zijlstra, R. T., & Gänzle, M. G. (2020). Feeding *Limosilactobacillus fermentum* K9-2 and *Lacticaseibacillus casei* K9-1, or *Limosilactobacillus reuteri* TMWL.656 reduces pathogen load in weanling pigs. *Frontiers in Microbiology*, *11*, 608293.

Youn, H. N., Lee, Y. N., Lee, D. H., Park, J. K., Yuk, S. S., Lee, H. J., ... & Song, C. S. (2012). Effect of intranasal administration of *Lactobacillus fermentum* CJL-112 on horizontal transmission of influenza virus in chickens. *Poultry Science*, *91*(10), 2517-2522.

Annex

Batlle, E., & Massagué, J. (2019). Transforming growth factor-β signaling in immunity and cancer. *Immunity, 50*(4), 924-940.

Biedermann, T., Röcken, M., & Carballido, J. M. (2004). TH1 and TH2 lymphocyte development and regulation of TH cell-mediated immune responses of the skin. *Journal of Investigative Dermatology Symposium Proceedings, 9*(1), 5-14.

BIOSCIENCE NOTES. (n.d.). *Cytokines*. Retrieved March 22, 2024 from https://www.biosciencenotes.com/cytokines/.

CUSABIO. (n.d.). *What cells release cytokines?*. Retrieved March 23, 2024 from https://www.cusabio.com/cytokines/What-Cells-Release-Cytokines.html.

Domanska, U. M., Kruizinga, R. C., Nagengast, W. B., Timmer-Bosscha, H., Huls, G., de Vries, E. G., & Walenkamp, A. M. E. (2013). A review on CXCR4/CXCL12 axis in oncology: no place to hide. *European Journal of Cancer, 49*(1), 219-230.

Gaffen, S. L. (2011). Recent advances in the IL-17 cytokine family. *Current Opinion in Immunology, 23*(5), 613-619.

Junttila, I. S. (2018). Tuning the cytokine responses: an update on interleukin (IL)-4 and IL-13 receptor complexes. *Frontiers in Immunology, 9,* 888.

Justiz Vaillant, A. A., & Qurie A. (2022). Interleukin. (August 22, 2022). In StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499840/.

Kak, G., Raza, M., & Tiwari, B. K. (2018). Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. *Biomolecular Concepts*, *9*(1), 64-79.

Kalinski, P. (2012). Regulation of immune responses by prostaglandin E2. *The Journal of Immunology, 188*(1), 21-28.

Lopez-Castejon, G., & Brough, D. (2011). Understanding the mechanism of IL-1β secretion. *Cytokine & Growth Factor Reviews*, *22*(4), 189-195.

Mihara, M., Hashizume, M., Yoshida, H., Suzuki, M., & Shiina, M. (2012). IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clinical Science*, *122*(4), 143-159.

Patidar, M., Yadav, N., & Dalai, S. K. (2016). Interleukin 15: A key cytokine for immunotherapy. *Cytokine & Growth Factor Reviews*, *31*, 49-59.

R&D SYSTEMS. (n.d.). *TH1/TH2 Differentiation*. Retrieved March 21, 2024 from https://www.rndsystems.com/resources/articles/th1-th2.

Richmond, J., Tuzova, M., Cruikshank, W., & Center, D. (2014). Regulation of cellular processes by interleukin-16 in homeostasis and cancer. *Journal of Cellular Physiology, 229*(2), 139-147.

Rolfe, A. J., Bosco, D. B., Wang, J., Nowakowski, R. S., Fan, J., & Ren, Y. (2016). Bioinformatic analysis reveals the expression of unique transcriptomic signatures in Zika virus infected human neural stem cells. *Cell & Bioscience*, *6*(1), 1-13.

Russo, R. C., Garcia, C. C., Teixeira, M. M., & Amaral, F. A. (2014). The CXCL8/IL-8 chemokine family and its receptors in inflammatory diseases. *Expert Review of Clinical Immunology*, 10(5), 593-619.

Sabat, R. (2010). IL-10 family of cytokines. *Cytokine & Growth Factor Reviews, 21*(5), 315-324.

Vignali, D. A., & Kuchroo, V. K. (2012). IL-12 family cytokines: immunological playmakers. *Nature Immunology, 13*(8), 722–728.

Wink, D. A., Hines, H. B., Cheng, R. Y. S., Switzer, C. H., Flores-Santana, W., Vitek, M. P., Ridnour, L. A., & Colton, C. A. (2011). Nitric oxide and redox mechanisms in the immune response. *Journal of Leukocyte Biology, 89*(6), 873-891.

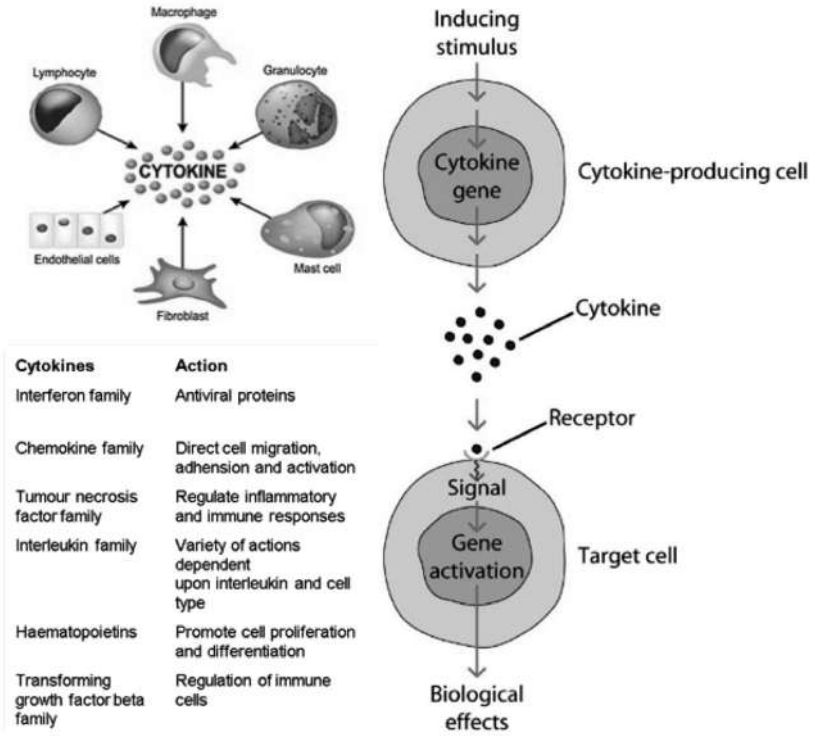
Zelová, H., & Hošek, J. (2013). TNF-α signalling and inflammation: interactions between old acquaintances. *Inflammation Research, 62,* 641-651.

Annex

Cytokines

A successful immune response relies on coordinated communication among various cell types. Lymphocytes, macrophages, granulocytes, mast cells, fibroblasts, and endothelial cells play crucial roles by secreting cytokines, which are glycoproteins influencing immunomodulatory responses, cell differentiation, and migration. Cytokines encompass a variety of types, including interferons, chemokines, tumor necrosis factors, interleukins, haematopoietins, and transforming growth factors (Figure A1). These molecules exert their effects by binding to receptor proteins expressed on target cell membranes, initiating signaling cascades that alter gene expression profiles. Signaling by cytokines and pathogen-associated molecular patterns (PAMPs) activates pathways such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ϰB) and signal transducer and activator of transcription (STAT), ultimately leading to cytokine production (Figure A2). NF-ϰB comprises a group of transcription factors crucial for cell survival and cytokine production. Dysregulation of NF-ϰB is linked to autoimmune and inflammatory diseases, cancer, viral infections, and abnormal immune development. In the cytosol, NF-ϰB is bound to an inhibitory protein, which is degraded upon sensing a signal by membrane receptors. The activated form then translocates to the nucleus, where it binds to DNA and facilitates transcript synthesis. STAT proteins serve as transcription factors involved in cellular differentiation, apoptosis, proliferation, and immunity. Binding of growth factors or cytokines to membrane-bound kinase receptors activates them, leading to STAT phosphorylation. Phosphorylated STATs then translocate to the nucleus, where they bind to DNA and initiate gene transcription. STAT

inactivation occurs through phosphatases, followed by their export to the cytoplasm.



Cytokines	Action
Interferon family	Antiviral proteins
Chemokine family	Direct cell migration, adhension and activation
Tumour necrosis factor family	Regulate inflammatory and immune responses
Interleukin family	Variety of actions dependent upon interleukin and cell type
Haematopoietins	Promote cell proliferation and differentiation
Transforming growth factor beta family	Regulation of immune cells

Figure A1. Characteristics of cytokines. Adapted from "What cells release cytokines?" (n.d.). CUSABIO. Retrieved March 23, 2024 from https://www.cusabio.com/cytokines/What-Cells-Release-Cytokines.html; *"Cytokines."* (n.d.). BIOSCIENCE NOTES. Retrieved March 22, 2024 from https://www.biosciencenotes.com/cytokines/.

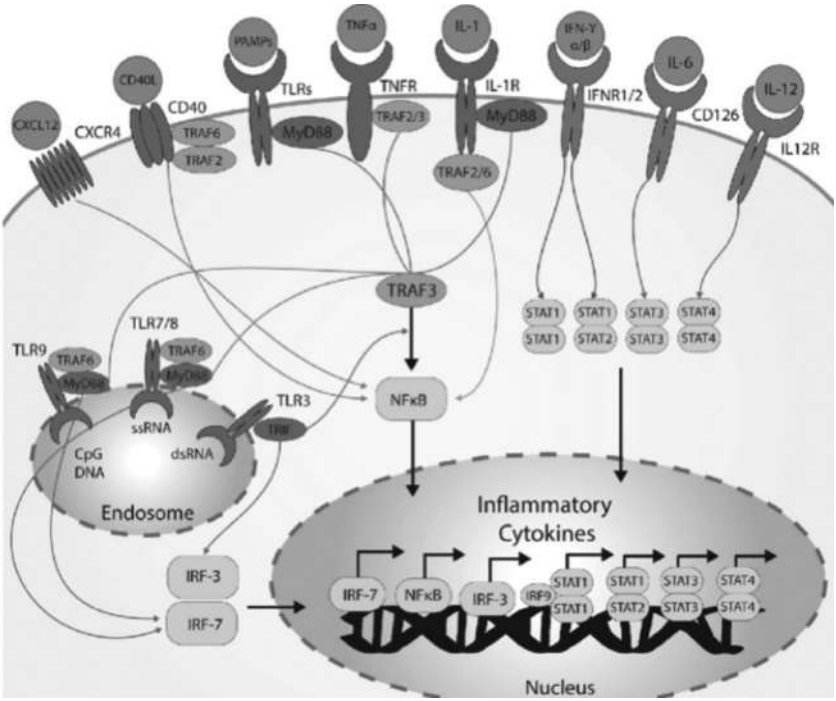


Figure A2. Cytokine and PAMPs signalling. Adapted from *"Bioinformatic analysis reveals the expression of unique transcriptomic signatures in Zika virus infected human neural stem cells"* by A. J. Rolfe, D. B. Bosco, J. Wang, R. S. Nowakowski, J. Fan, Y. Ren, 2016, *Cell & Bioscience*, 6, pp. 1-13. PAMP, Pathogen-associated molecular patterns; CD, Cluster of differentiation; IL, Interleukin; IFN, Interferon; CXCL, Chemokine; CXCR, CXC chemokine receptor; MYD88, Myeloid differentiation primary response 88; TLR, Toll-like receptors; TNF, Tumor necrosis factor; TRAF, Tumor necrosis factor receptor (TNFR)-Associated factor; NF-ϰB, Nuclear factor kappa-light-chain-enhancer of activated B cells; IRF, Interferon regulatory factor; STAT, Signal transducer and activator of transcription.

Activation of the adaptive immune system relies on signals provided by the innate immune response, which are determined by the nature of antigens and the signaling proteins produced. Helper T cells transition from a naïve phenotype to an activated form, with T_{H1} and T_{H2} cells synthesizing specific cytokines associated with distinct immune responses. The T_{H1} response is geared towards eradicating tumors and intracellular pathogens, stimulating

processes including phagocytosis and oxidative stress crucial for pathogen clearance. However, it can also contribute to autoimmune diseases. Conversely, the T_{H2} response targets pathogenic bacteria and extracellular parasites, playing a role in antibody responses, but it is also linked to atopic diseases and allergic reactions. In both responses, antigen-presenting cells activate naïve T_H cells, leading to the development of T_{H0} cells. These cells produce various cytokines that guide the differentiation of naïve cells into either T_{H1} or T_{H2} cells (Figure A3).

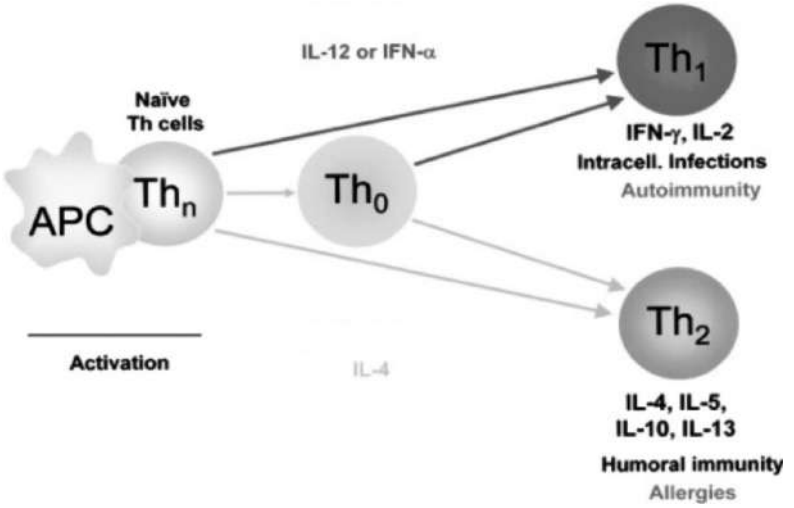


Figure A3. Differentiation of T_{H1} and T_{H2} cells. Adapted from *"TH1 and TH2 Lymphocyte development and regulation of TH Cell–mediated immune responses of the skin"* by T. Biedermann, M. Röcken, J. M Carballido, 2004, *Journal of Investigative Dermatology Symposium Proceedings*, 9, pp. 5-14. APC, Antigen-presenting cell; Th, Helper T cell; IL, Interleukin; IFN, Interferon.

Proinflammatory cytokines are key players in the initiation and regulation of the innate immune response. However, their chronic overproduction is associated with the pathogenesis of various inflammatory diseases, such as cancer and atherosclerosis. Some of the key proinflammatory cytokines mentioned in the main text include:

IFN-γ: This particular soluble cytokine plays a critical role in modulating the immune system. It is primarily released by natural killer cells as a component

of the innate immune response. Additionally, its production by CD4 and CD8 cells contributes to antigen-specific immunity. This cytokine is implicated in various infections caused by viruses, bacteria, and protozoa. Moreover, it is synthesized by antigen-presenting cells like dendritic cells, macrophages, and B cells. Dysregulation of this cytokine's expression is linked to autoimmune and inflammatory conditions.

IL-6: This cytokine is produced by macrophages upon detection of pathogen-associated molecular patterns by specific receptors known as pattern recognition receptors (PRRs). Its signaling triggers the maturation of B cells, differentiation of T cells, and induces systemic inflammatory responses, often working synergistically with other cytokines like TNF-α and IL-1. IL-6 has been implicated in the inflammatory processes observed in a range of diseases, such as diabetes, multiple sclerosis, atherosclerosis, multiple myeloma, prostate cancer, and Alzheimer's disease.

IL-1β: This cytokine is synthesized as a proprotein by macrophages, monocytes, and certain dendritic cells, requiring cleavage by caspases to become active. It serves as a significant mediator of inflammation and contributes to cell proliferation, differentiation, and apoptosis. Elevated levels of this factor are linked to autoinflammatory syndromes and disturbances in intestinal microbiota. Additionally, it is implicated in processes such as carcinogenesis, HIV-1 infection, retinal degeneration, and neuroinflammation.

IL-8: This chemotactic cytokine, or chemokine, is synthesized by macrophages as well as other cell types including epithelial, smooth muscle, and endothelial cells. Initially produced as a large precursor, it undergoes processing by proteases. IL-8 serves multiple functions: it induces chemotaxis, guiding the migration of neutrophils and other granulocytes to the infection site, and it also enhances phagocytosis by these cells upon arrival. Known for its role in inflammation, IL-8 has been implicated in various diseases, including obesity, colorectal cancer, and cystic fibrosis.

IL-12: This interleukin is primarily synthesized by neutrophils, dendritic cells, and macrophages following antigenic stimulation. Its functions are

diverse: it prompts the differentiation of naïve T cells into T_{H1} cells and stimulates the production of IFN-γ and TNF-α by T cells and natural killer cells. Notably, this cytokine is closely tied to the cytotoxic activity of these cells and has been implicated in autoimmune diseases.

IL-17: This cytokine is synthesized by helper T cells (T_{H17}). Upon binding to its receptor, it stimulates the expression of chemokines, cytokines, and prostaglandins in fibroblasts, macrophages, endothelial cells, and epithelial cells. Working in concert with TNF-α and IL-1β, IL-17 promotes inflammation. It has been implicated in various diseases, such as asthma, arthritis, psoriasis, and multiple sclerosis.

IL-17RA: The IL-17A receptor, a glycoprotein, interacts with its ligand with low affinity. This complex is integral to inflammatory and autoimmune diseases.

IL-15: Various cell types, such as nerve cells, macrophages, dendritic cells, monocytes, keratinocytes, and fibroblasts, produce this cytokine. It regulates the proliferation and activation of NK and T cells and is implicated in conditions such as rheumatoid arthritis, celiac disease, and liver disease.

IL-16: This cytokine participates in T cell activation, inhibition of HIV replication, and chemoattraction. Initially synthesized as part of a larger protein, it undergoes proteolytic processing, with its C-terminal region acting as a cytokine and its N-terminal region regulating the cell cycle. Produced by lymphocytes and epithelial cells, it attracts cells expressing the CD4 co-receptor, including eosinophils, monocytes, dendritic cells, and lymphocytes.

TNF-α: This cytokine exists in both transmembrane and soluble forms, with the soluble form resulting from proteolytic processing of the former. It is primarily expressed on the surface of monocytes and macrophages, where it plays a key role in immune cell regulation. Additionally, it acts as a pyrogen, capable of inducing fever, as well as apoptosis, inflammation, and viral replication. Dysregulation of this factor has been linked to conditions such as cancer, psoriasis, and inflammatory bowel disease.

CXCL-12: The C-X-C motif chemokine 12 is generated in two isoforms (a and b) through alternative splicing. It serves as a potent chemoattractant for lymphocytes and is synthesized in multiple organs, encompassing the spleen, thymus, heart, brain, kidney, liver, and lung, as well as in bone marrow tissue and platelets. This chemokine has been implicated in various diseases, including multiple sclerosis and Alzheimer's disease.

Anti-inflammatory cytokines are pivotal in regulating the immune response by controlling the expression of proinflammatory cytokines. Their role is to prevent an exaggerated immune reaction that could result in tissue damage. The following anti-inflammatory cytokines are discussed in the main text:

IL-10: This cytokine is primarily produced by monocytes, with helper and regulatory T cells also contributing, albeit to a lesser degree. Its expression seems to be linked to stimulation by pathogenic or commensal bacteria. IL-10 serves to regulate the expression of T_{H1} cytokines and enhances B-cell survival, proliferation, and antibody production. Moreover, IL-10 mitigates the inflammatory effects of allergic reactions and suppresses the synthesis of pro-inflammatory cytokines.

IL-13: It is a cytokine synthesized by T_{H2} cells, natural killer T cells, eosinophils, and basophils. It serves as a pivotal mediator of the physiological effects induced by allergic inflammation. Additionally, IL-13 has the capacity to stimulate IgE production by B cells and promote goblet cell differentiation in the tracheal epithelium. Remarkably, this cytokine is associated with the expulsion of helminths from the gut, as it induces hypersecretion of glycoproteins and enhances contraction, facilitating the detachment and removal of pathogens from the gut wall. IL-13's involvement in the formation of granulomas is also noteworthy, as it can counteract T_{H1} responses required for dealing with intracellular infections, leading to an accumulation of T_{H2} cells under such conditions.

IL-4: IL-4 is primarily produced by mast cells, basophils, eosinophils, and T_{H2} cells. Its functions extend to stimulating the proliferation of T and B cells, as well as promoting the differentiation of B cells into plasma cells.

Within the realm of immune regulation, IL-4 plays a crucial role in humoral and adaptive immunity by inducing the synthesis of IgE, upregulating the MHC II complex, and reducing the expression of T_{H1} cells and macrophages. Abnormal expression of IL-4 is strongly associated with allergic reactions. Particularly, the presence of IL-4 leads to the polarization of macrophages into M2 cells, which are instrumental in tissue repair and wound healing due to their production of polyamides that stimulate collagen synthesis. Conversely, IL-4 inhibits the polarization of macrophages into M1 cells, which, when activated by IFN-γ or LPS, synthesize proinflammatory cytokines, phagocytize microbes, and initiate immune responses. IL-4 is also implicated in various disorders, including allergies and autoimmune diseases.

IL-14: This factor, synthesized by T cells and certain malignant B cells, serves to stimulate the proliferation of activated B cells while simultaneously inhibiting immunoglobulin secretion.

TGF-β: The transforming growth factor beta (TGF-β), synthesized by various tissues, plays crucial roles in embryo development, cell differentiation, hormone production, and immune response. Especially, TGF-β, in conjunction with IL-6, fosters T_{H17} differentiation, thereby promoting inflammation. TGF-β exists in three isoforms (TGF-β1, TGF-β2, and TGF-β3) and is produced by a diverse array of cell types, including fibroblasts, smooth muscle cells, epithelial cells, macrophages, endothelial cells, lymphocytes, eosinophils, mesothelial cells, and mast cells.

Factors

The following factors are also mentioned in the main text:

NO: Nitric oxide (NO) is crucial for regulating the function, growth, and apoptosis of various immune cells, including T lymphocytes, mast cells, macrophages, natural killer cells, and neutrophils. In high concentrations, NO exhibits toxicity against infectious microorganisms. Additionally, NO serves as a signaling molecule and metabolic regulator, playing a crucial role in inflammation.

PGE2: Prostaglandin E2 (PGE2) has the ability to activate dendritic cells, yet it suppresses their capacity to attract effector, memory, and naïve T cells. Furthermore, PGE2 diminishes T_{H1} and natural killer (NK) cell-mediated immunity while also regulating the activity of macrophages and neutrophils. Additionally, PGE2 stimulates T_{H2}, regulatory T cell (T_{reg}), and T_{H17} responses. This multifaceted factor is produced by various cell types including fibroblasts, endothelial cells, osteoblasts, and macrophages.

Probiotics are microorganisms that, when consumed in adequate quantities, confer health benefits to the host. Their effects extend beyond merely outcompeting harmful microbes; they also bolster the intestinal barrier and regulate immune responses. Lactic acid bacteria, particularly *Limosilactobacillus fermentum*, are commonly associated with probiotic properties and have been utilized in food fermentation and industrial processes. *L. fermentum* stands out for its ability to produce antimicrobial peptides, adhere to cells, and activate immune receptors. Through interactions with immune cells, they modulate crucial pathways in both innate and adaptive immune responses, particularly relevant in conditions such as colitis or inflammatory bowel disease. This manuscript provides an overview of the key characteristics of these probiotic bacteria, including the molecular mechanisms underlying their effects and their applications in both animal models and humans.

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