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Chapter 5

The Synergistic Contribution of *Lactobacillus* and Dietary Phytophenols in Host Health

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Additional information is available at the end of the chapter

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Abstract

Phytophenols are found ubiquitously among all plants. They are important in diets rich in fruits and vegetables because these compounds provide health benefits to the host, ultimately decreasing the incidence of chronic diseases. These compounds act as natural antioxidants and provide anti-inflammatory, antiviral, antibiotic, and antineoplastic properties. Reactive oxygen species (ROS) are produced under normal physiological functions, and low/moderate levels are required for cellular turnover and signaling. However, when ROS levels become too high, oxidative stress can occur. Phytophenols quench ROS and ultimately avoid the damaging effects ROS elicit on the cell. The highest source of bioavailable phytophenols comes from our diet as a component usually esterified in plant fiber. For phytophenols to be absorbed by the body, they must be released by esterases, or other related enzymes. The highest amount of esterase activity comes from the gastrointestinal (GI) microbiota; therefore, the host requires the activity of mutualistic bacteria in the GI tract to release absorbable phytophenols. For this reason, mutualistic bacteria have been investigated for beneficial properties in the host. Our laboratory has begun studying the interaction of *Lactobacillus johnsonii* N6.2 with the host since it was found to be negatively correlated with type 1 diabetes (T1D). Analyses of this strain have revealed two important characteristics: (1) It has the ability to release phytophenols from dietary fiber through the secretion of two strong cinnamoyl esterases and (2) *L. johnsonii* also has the ability to generate significant amounts of H_2O_2, controlling the activity of indoleamine 2,3-dioxygenase (IDO), an immunomodulatory enzyme.

Keywords: *Lactobacillus*, *Lactobacillus johnsonii* N6.2, Indolamine 2,3-dioxygenase, 5-hydroxytryptamine, reactive oxygen species, esterase

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1. Phytophenols

Phytophenols, also called polyphenols or simply phenols, are a unique group of monocyclic and polycyclic phytochemicals found within fruits, vegetables, and other plants as a component of plant fiber. Phytophenols are ubiquitously found as secondary metabolites in plants and are therefore consumed in relatively high quantities. They are a very diverse and multifunctional group of active plant compounds with substantial health potential in many areas, and numerous scientific studies demonstrate that increasing the intake of plant foods rich in fiber can minimize the incidence of modern diseases [1–3].

Consumption of foods and beverages containing phytophenols may impact nutrient levels in the body by preventing their oxidation. Their activity is based on functional groups' capacity to accept a free radical's negative charge [4, 5]. In order to be absorbed by intestinal epithelial cells, phytophenols attached to fiber can only be released by the enzymatic activities of the gastrointestinal (GI) microbiota [6–9] because the phenolic esterase enzymes necessary to release antioxidant phytophenols from plant fiber are not produced by the host GI system. It has been shown *in vitro* that after hydrolysis with purified enzymes, more biologically active compounds can be released, including hydroxytyrosol and elenolic acid from oleuropein [10, 11] and dihydroxyphenyllactic acid from rosmarinic and salvianolic acids [12, 13]. Nevertheless, very little is known about the modifications that these natural compounds undergo after ingestion.

All phytophenols arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid [14]. Often they are present in conjugated forms, with sugar residues linked to hydroxyl groups, although in some cases, direct links of the sugar to an aromatic carbon do exist. In addition, associations with other compounds are also common, including linkages with carboxylic and organic acids, amines, and lipids, as well as associations with other phenols [15].

Plants produce an impressive array of phenolic compounds, and it is thought that these plant-based constituents have a stronger biological antioxidant effect when compared to synthetic antioxidants. This is mainly because phytophenols are part of the normal function of living plants and therefore are thought to have better compatibility with the body [4, 16, 17]. Although there are more than 8000 identified polyphenolic compounds, they can be sorted into four main classes: phenolic acids, flavonoids, stilbenes, and lignans [18]. **Figure 1** illustrates the different groups, which are divided by the number of rings they contain as well as the structural elements that bind these rings together.

Phenolic acids are derivatives of either benzoic acid or cinnamic acid and can thus be divided into two classes. They make up about a third of the polyphenolic compounds found in human diets. These phenolic compounds can be found in all plant-based material, although they are most commonly found in acidic fruits [19]. Flavonoids are the most abundant polyphenolic compounds found in our diet and are also the most well-studied group. More than 4000 varieties have been accounted for, often contributing to the color of flowers, fruits, and leaves [20]. Six subclasses exist, as shown in **Figure 2**, based upon variations in structure: flavonols, flavones, flavanones, flavanols, anthocyanins, and isoflavones.

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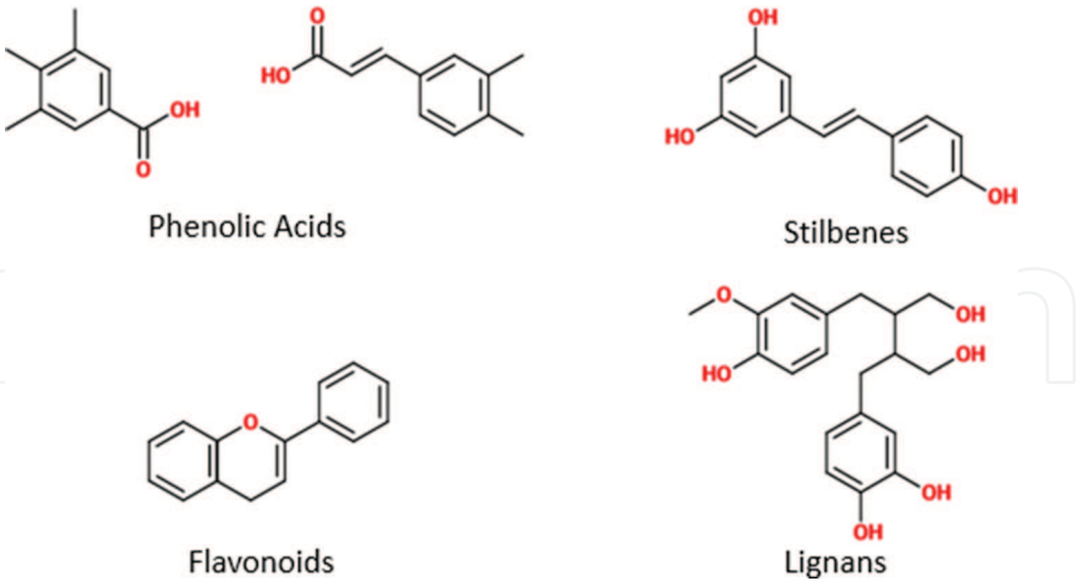


Figure 1. Chemical structures of the different classes of polyphenols, broadly divided into four classes [14].

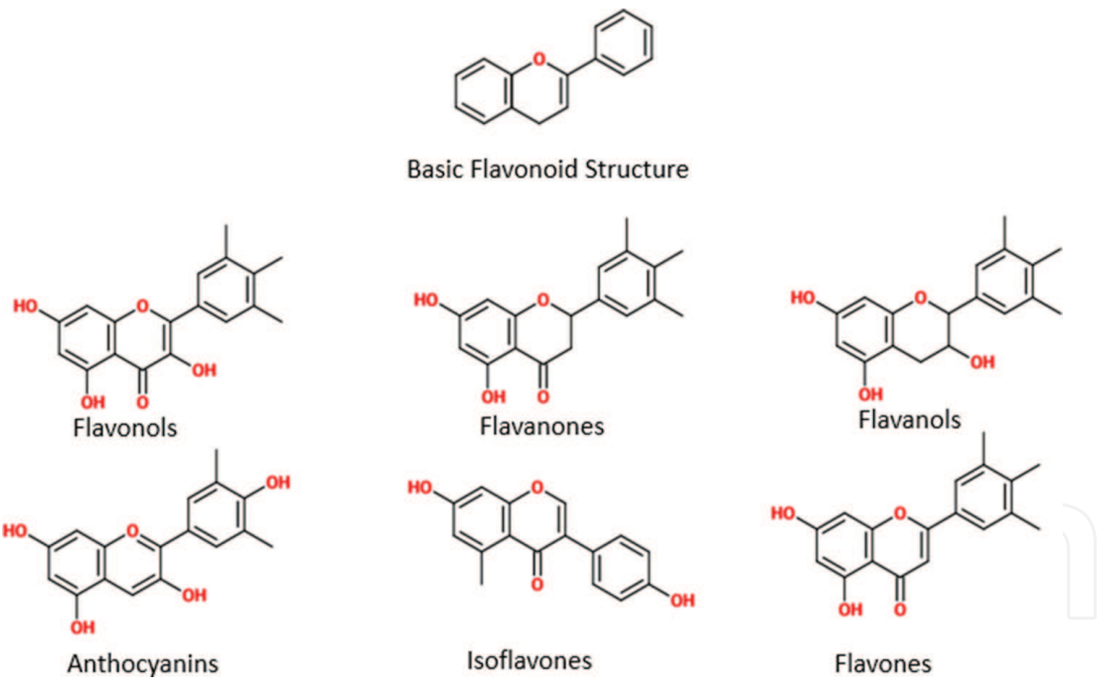


Figure 2. Chemical structures of subclasses of flavonoids [14].

Stilbenes contain two phenyl moieties connected by a two-carbon methylene bridge. Their synthesis is typically initiated as a result of injury or infection in plants, and as a consequence, their occurrence in our diet is much lower than either phenolic acids or flavonoids. The best studied stilbene is resveratrol, found mainly in grapes and as a result also in red wine. Lignans are diphenolic compounds formed by the dimerization of two cinnamic acid residues, as seen in **Figure 1**.

Estimating the total polyphenol content is most accurately done through analysis of every individual phytophenolic compound. Due to the large diversity in phytophenolics, the only way to complete this task is through a compilation of the literature data. Fortunately, the USDA database contains a nearly complete source of food composition data [21–23]. This database combined with other literature sources for the remaining phytophenolic compounds was used to develop the Phenol-Explorer database. This recently developed database is the most complete source on the content of polyphenols in foods, including glycosides, esters, and aglycones of flavonoids, phenolic acids, lignans, stilbenes, and other polyphenols [24].

The occurrence of dietary phenolics in plants is not uniform, even at the cellular level. Insoluble phytophenols are often found in cells walls, while soluble phytophenols are found within the vacuoles of plant cells [25]. In many instances, plant-based foods contain a variable mixture of polyphenols. Some polyphenols, such as flavanones and isoflavones, are found only in specific foods, whereas others such as quercetin are found in nearly all plant products. Conventionally, the outer tissues of a plant contain higher levels of phenolics than the inner tissues [26].

Various other factors can affect the concentration of dietary phytophenols, including ripeness of the plant when harvested, environmental factors, storage, and processing of plant materials [14]. Before harvesting, abiotic factors such as soil type, exposure to sunlight, and amount of rainfall can alter phenolic compounds in plants. In addition, the degree of ripeness when harvested can be positively or negatively correlated with the concentration of polyphenols, depending upon which compound is under observation [27]. Storage of plant-based foods also affects polyphenol levels, and the oxidation of polyphenols over time can be beneficial (as in the case of black tea) or harmful (as in the case of browning of fruit) to polyphenolic compound concentrations [27]. Cooking also has a major effect on phytophenolic compounds, and depending on how the material is processed, cooking may account for a 30–80% loss of phenolic content [28].

Bioavailability is described as the proportion of the nutrient that follows natural pathways to be digested, absorbed, and metabolized in the body. For phytophenols, there is no relationship between the quantity of phenolic compounds found in food and their bioavailability, and every one of the numerous known polyphenols differs in its bioavailability. Furthermore, the most ubiquitous phytophenols found in plant-based foods are not necessarily the same as those that show the highest concentration of metabolites in tissues. Often, polyphenols are present in a form that cannot directly be absorbed by the body, including esters, glycosides, or polymers [29]. Due to the microbial modification of phytophenols during absorption in the intestinal cells and later in the liver, the compounds reaching the bloodstream and bodily tissues are drastically different from those originally ingested. As a consequence, identifying all the metabolites and subsequently evaluating their activity is a difficult task. It is the chemical structure of the phytophenolic compound that determines absorption rate and extent rather than the concentration of the compound found in the diet [30]. Evidence does indirectly suggest that phenols are absorbed to some extent through the gut barrier due to an increase in antioxidant capacity of plasma after ingestion of phytophenol-rich foods [31, 32].

The potential pharmacological properties of these natural plant compounds have been demonstrated *in vitro* and include anti-inflammatory [9], antioxidant [33–35], antineoplastic

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[10, 36], antiviral [37], and antibiotic [38] properties. Although several mechanisms of action combine to provide the widespread health benefits offered by phytophenols, their role as antioxidants is the mostly frequently studied mechanism. The intestinal inflammatory process is primarily a consequence of the overproduction of inflammatory mediators, triggered by an excess of reactive oxygen species (ROS) [39–41].

ROS are the by-products of cellular redox processes in the body. These free radical compounds contain one or more unpaired electrons in their outer orbit, creating instability that leads to significant reactivity. ROS species include superoxide ($O^{2\bullet-}$), hydroxyl ($^{\bullet}OH$), peroxyl ($ROO^{\bullet}$), lipid peroxyl ($LOO^{\bullet}$), and alkoxyl ($RO^{\bullet}$) radicals. Oxygen free radicals can also be converted to other non-radical reactive species, which are dangerous for health due to their tendency to lead to free radical reactions in living organisms. These species include hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($1/2O_2$), and hypochlorous acid (HOCl). ROS are capable of modifying structural proteins or inactivating enzymes, and as a consequence disrupting normal physiologic functions in the body [42–44]. Production of free radicals is a normal part of our physiology and occurs continually to keep the body functioning properly. Processes that generate ROS include activities of the immune system, metabolism, and inflammation responses, along with stress, pollution, radiation, diet, toxins, exhaust fumes, and smoking. [4, 16, 42, 45].

Excessive production of ROS can easily overwhelm both the enzymatic and non-enzymatic antioxidant defense systems, leading to oxidative stress and inflammation. It has been widely discussed in scientific literature that increasing the intake of natural antioxidants minimizes the deleterious effects of ROS [34, 46–48]. Evidence collected from feeding assays using diets rich in antioxidant plant phenolics supports this claim [2, 7, 49]. The intake of phytophenols has been shown to minimize the production of ROS and mitigate their harmful impact on the GI system [3, 33, 50].

Oxidative stress leads to disease through four destructive pathways: membrane lipid peroxidation, protein oxidation, DNA damage, and disturbance of reducing equivalents in the cell [4]. These steps often lead to altered signaling pathways and cell destruction. Oxidative stress has been connected to various diseases such as cancer, cardiovascular diseases, neurological disorders, diabetes, and aging. Each molecule in the body is at risk of damage by ROS, and damaged molecules can impair cellular functioning and lead to cell death, which ultimately results in diseased states [43, 44, 51]. Due to the antioxidant properties of phytophenolic compounds, they are associated with the prevention of a large array of diseases, including cardiovascular disease, cancer, diabetes, rheumatoid arthritis, neurodegenerative diseases, GI diseases, renal disorders, pulmonary disorders, eye disorders, infertility, and pregnancy complications, as well as slowing the progression of aging [4].

Although reduction of ROS has been shown to decrease risk of a huge array of diseases, the classical model of ROS generation and resulting oxidative stress contrasts with some emerging scientific evidence. Benefits of ROS can in fact occur when these species are present in low/moderate concentrations, as part of normal physiological functions [43]. The majority of cells produce superoxide and hydrogen peroxide constitutively, while other cells possess inducible ROS release systems. Beneficial effects can include defense against infectious agents by

phagocytosis, killing of cancer cells by macrophages and cytotoxic lymphocytes, detoxification of xenobiotics by Cytochrome P450, generation of ATP in mitochondria (energy production), cell growth, and the induction of mitogenic responses at low concentrations. ROS also plays a role in cellular signaling, including activation of several cytokines and growth factors, non-receptor tyrosine kinase activation, protein tyrosine phosphatase activation, release of calcium from intracellular stores, and activation of nuclear transcription factors. ROS can also initiate vital actions such as gene transcription and regulation of soluble guanylate cyclase activity in cells [44, 50].

Reactive oxygen species (ROS) are known to play a dual role in biological systems; they are well documented for playing a role as both deleterious and beneficial species [43, 44, 52]. We hypothesize that redox homeostasis in the GI tract is dependent on the dynamic interplay between the generation of ROS and the ROS quencher ability of antioxidant phytophenols released by intestinal microbes. Although there are possible benefits to maintain low levels of ROS in the proper functioning of the body, the diet and lifestyle of the majority results in increased levels of ROS in the body are known to be harmful and can lead to the progression of disease. In this way, it is critical to maintain the proper balance of ROS in the body, and phenolic compounds have been shown to reestablish a healthy level of ROS. Next, we turn to the vital interaction of phytophenols and microflora of the gut system that can lead to creation of redox balance critical to health.

2. Lactic acid bacteria

The group known as lactobacilli is composed of several genera of bacteria (*Leuconostoc, Pediococcus, Lactococcus*, and *Streptococcus*), *Lactobacillus* being the largest order in the phylum Firmicutes and the class Bacilli [53]. These free living lactic acid bacteria flourish in different biological niches such as soil, plants (fruits, beverage, and silage) and fermented foods (cheese, fermented milk, yogurt, meat products, alcoholic beverages, and pickled products). They are also associated with mammals as members of the microbial community characteristic of the oral cavity, GI system, urinary tract, skin, etc. [54–57]. The genus *Lactobacillus* is composed of nutritionally fastidious gram-positive, non-spore-forming rods or coccobacilli, catalase-negative, aerotolerant or anaerobic bacteria. The main characteristic of their homo- or hetero-fermentative metabolism is the production of lactic acid as the primary end fermentation product. The genus *Lactobacillus* is represented by over 212 species described to date, including several industrially relevant microorganisms such as *L. acidophilus, L. bulgaricus, L. casei, L. lactis, L. paracasei, L. plantarum, L. reuteri, L. fermentum, L. salivarius, L. rhamnosus, L. delbrueckii*, and *L. johnsonii*.

The *Lactobacillus* genus is widely studied because of the bacterias' capacity to produce lactic acid. Thus, most studies regarding their physiology were centered on acidifying bacteria such as *L. delbrueckii subsp. bulgaricus*, which in combination with *Streptococcus thermophilus* acidify milk in a few hours. This process is critical to optimize the production of fermented dairy products such as cheese and yogurt, or other non-dairy products such as pickles, sauerkraut, and sourdough bread.

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The extensive use of these bacteria in food and beverage industries drove the scientific attention toward the evaluation of their impact on health, mainly on the GI system's integrity and responsiveness. Regardless, lactic acid bacteria were safely used for centuries to modify food flavor and texture, modern genomics bring back to light the scientific discussion toward their impact on human health [54, 58].

Studies of the human microbiome revealed that lactobacilli could occupy different microhabitats in the human body, such as the buccal cavity and nasal fossa, but they mainly thrive in the gut and the urogenital tract [59]. In women, it was observed that variations of estrogen and glycogen stimulates the growth of lactic acid bacteria. Depletion of vaginal lactobacilli could give rise to adverse microbial flora colonization inducing urogenital infection [60]. Gustafsson *et al*. evaluated the population of lactobacilli in healthy fertile and postmenopausal women in correlation with hormone levels [57]. They demonstrated that *L. crispatus* is the most abundant bacteria and, together with *L. vaginalis, L. jensenii* and *L. gasseri* are responsible in protecting the urogenital tract against vaginal infection. This effect was associated with the capacity of these *Lactobacillus* species to produce H_2O_2, which negatively affects the viability of pathogenic bacteria [61, 62]. The GI system is home to many different kinds of microorganisms, which globally is referred as the gut microbiota. Among these microbes, one of the most abundant groups, in this complex microbial population, is *Lactobacillus*. Although it is not the most abundant genus in the microflora, it is considered one of the most important genus due to potential beneficial effects associated with them. Scientific studies revealed that the *Lactobacillus* abundance in the gut microbiota changes according to the portion of the GI tract. The highest presence of *Lactobacillus* sequences was found in the jejunum and ileum lumen, 16% respect to the total microbiota. Their abundance in the colon/rectal lumen decreased to 9.9%. Surprisingly, *Lactobacillus* sequences were lower than 0.5% in the fecal samples studied [63–66]. The main *Lactobacillus* strains found in feces are *L. acidophilus, L. crispatus, L. gasseri, L. reuteri, L. brevis, L. sakei, L. curvatus, L. casei, L. paracasei, L. rhamnosus, L. delbrueckii, L. brevis, L. johnsonii, L. plantarum*, and *L. fermentum* [67]. In the GI context, these bacteria interact with other intestinal microbes, with food components and with the GI mucosa. The consequences of these interactions are endless; in addition, it is extremely difficult to isolate the effects and study them separately. Probably one of the most complex and interesting systems effected is the host immune system. Commensals can help in educating and maturing the host immune response and prompt the immunological defensive arsenal. All members of the *Lactobacillus* group are classified as GRAS (generally recognized as safe) organisms; consequently, they are considered innocuous or beneficial for health. The specific mechanisms by which these bacteria are considered beneficial are still the subject of important discrepancies and the center of scientific debates.

Lactobacilli are excellent organic acid producers, converting sugars into lactic acid and other by-products such as acetate, ethanol, CO_2, butyrate, and succinate. They produce small molecules, as well, such as H_2O_2, or compounds such as diacetyl, or acetaldehyde [67]. Several of these metabolites are bioactive, with beneficial effects for the human GI. At the same time, they are essential for the dairy industry because they provide flavoring and display natural preservative properties [68]. They help to maintain the integrity of GI layers, favoring the

renewal of the epithelium. A continuous renewal of the GI layers is critical to maintain an adequate barrier function to minimize several significant human diseases, including autoimmunity and cancer. According to recently published studies, the production of low amounts of H_2O_2 at the GI level is beneficial to the host. Besides its well-characterized antimicrobial activity, this molecule could directly down-regulate the early stages of the host inflammatory response and improve epithelial cell restitution and healing via the oxidation of cysteine residues in the host tyrosine phosphatases [62, 69].

Other important metabolites synthesized by *Lactobacillus* species are larger molecules such as polysaccharides (viscosifying agents) [70] and enzymes (proteases, bacteriocins, esterases, and lipases) [6, 71, 72], which improve dairy product quality (flavor development, texture modification) and provide beneficial effects to boost human health [73]. *L. helveticus* is considered one of the most efficient species associated with proteolysis in cheese ripening. *L. helveticus* also produces bioactive peptides with antihypertensive and antimicrobial activity [74]. Indeed, *Lactobacillus* antimicrobial activity is directly related to its ability to secrete bacteriocins. A subset of *Lactobacillus* strains produce these kind of antimicrobial peptides such as *L. sakei* (bavaricin, sakacin) [75], *L. curvatus* (curvaticin), *L. plantarum* (pediocin), *L. salivairus* (bacteriocins) [76], and *L. acidophilus* (acidocin) [77]. These antimicrobials may play an essential role in regulating the composition of the microbial communities within the GI system, influencing the host's health; however, not all of them showed promising effects on human [71].

Maintenance of the GI redox homeostasis is essential in minimizing human diseases. The production of enzymes, which could increase the amount of free and active antioxidant agents in the GI lumen, is another important characteristic associated with several *Lactobacillus* strains. These enzymes, such as esterases and/or lipases, are synthesized by the intestinal microbiota and can release redox quenchers like the above-described phytophenols that are ingested with the host diet. Thus, the ingestion of probiotic bacteria able to produce these enzymes is a healthy and natural alternative to modulate the redox status in the GI tract. Lactobacilli are excellent producers of lipases and esterases, and several of the best producing strains were selected by the dairy industry due to their contribution in cheese ripening. The esterases are active toward a wide range of ester substrates from free fatty acids to tri-, di-, and monoacylglyceride substrates. Cinnamoyl esterases (CE) are one of the most important enzymes involved in releasing antioxidant molecules from dietary fibers. These enzymes break down the ester linkages between hydroxycinnamates and sugars, commonly found in the fiber of dietary plants, releasing phenolics such as hydroxycinnamic, ferulic, coumaric, and caffeic acids with high ROS scavenging activity. Genes encoding various esterases have been described in *L. fermentum* and *L. reuteri, L. leichmanni*, and *L. farciminis*, and the first two species are frequently found in animal and human feces. These enzymes have also demonstrated to be active toward soluble polyphenols such as chlorogenic acid to release caffeic and quinic acids [78]. The accumulation of the enzymatic products released (monophenols) in *Lactobacillus* cultures suggests that these microorganisms do not (or do so extremely slowly) metabolize the phenolic acids released. The enzymatic action correlates directly with increased amounts of phenolics (i.e., caffeic acid) detected in the bloodstream of model animals fed with fibers in combination with probiotics formulated with those strains [78]. Guglielmetti *et al.* studied the

activity of CE produced by *L. helveticus* MIMLh5 on soluble phenolics, such as chlorogenic acid, to enrich food with free caffeic acid [79]. *L. helveticus* enzymes are mainly intracellular, but some of them could be surface-associated as observed in *L. fermentum* [80]. *L. plantarum*, frequently found in plant-derived food products where hydroxycinnamoyl esters are abundant, produces the enzyme Lp_0796 (esterase), which hydrolyzes the four model substrates for feruloyl esterases (methyl ferulate, methyl caffeate, methyl *p*-coumarate, and methyl sinapinate). This esterase is generally present among several *L. plantarum* strains and provides new insights into the metabolism of hydroxycinnamic compounds in this bacterial species [81]. Further studies on *L. plantarum* showed another esterase encoded by the *est_1092* gene is able to hydrolyze hydroxycinnamic esters, such as methyl ferulate or methyl caffeate, and is active on a broad range of phenolic esters [82]. *L. acidophilus* produces a novel CE with high similarity (70%) with the main CE characterized in *L. johnsonii* LJ1228 [72]. Other *L. acidophilus* and *L. johnsonii* strains displayed, as well, high CE activity [79]. One strain of *L. johnsonii* showed high ferulic acid esterase activity, stimulates insulin production, and alleviates symptoms caused by diabetes [83]. However, there is no direct evidence to associate the ability to release phenolics with the capacity to stimulate insulin production. The strain *L. johnsonii* N6.2 presented two different proteins with ferulic acid esterase activity. These enzymes showed high affinities and catalytic efficiencies toward aromatic compounds such as ethyl ferulate and chlorogenic acid [6]. *L. johnsonii* NCC533 also hydrolyzes rosmarinic acid, the main components of rosemary extracts, and it is ascribed to many health benefits.

The released monophenols (caffeic acid or other cinnamic acids) may exert its biological activities on the host, either at the level of the colonic mucosa itself, or in other tissues and organs, possibly after further modification by mammalian enzymes in the liver [80]. The release and solubilization of these phenolics, from fiber, also favor its absorption and further modification by other GI commensals. *In vitro* fermentation assays demonstrate that the fecal microbiota can efficiently metabolize caffeic, chlorogenic, and caftaric acids. With the use of highly sensitive analytical techniques, it was possible to identify two major metabolites: 3-hydroxyphenylpropionic (3-HPP) and benzoic acids (BA) once the original compounds were fully metabolized. Similar metabolic patterns were observed for other polyphenolic acids, suggesting a large and important metabolic flexibility of the gut microbiota [84]. Evidence for a metabolic pathway leading to the formation of BA from 3-HPP is supported by the established quality of intestinal microorganisms to carry out biological dehydroxylation of 3-HPP to 3-phenylpropionic acid, which can itself be further β-oxidized into BA by the colonic microbiota [85]. Alternatively, the absorbed cinnamic and phenylpropionic acids undergo β-oxidation in the liver to produce BA, which is subsequently conjugated to glycine to form hippuric acid in the liver [86].

The capacity of lactic acid bacteria to transform phenolic compounds into smaller novel molecules able to be absorbed in the GI system reoriented modern research to use combinations of probiotics and prebiotic products together. A large variety of dietary fibers were used for this purpose. Yet, the microbial metabolism of the released compounds by different bioconversion pathways, such as glycosylation, deglycosylation, ring cleavage, methylation, glucuronidation, and sulfate conjugation, depends on the microbial strains and substrates used. The

results of such combinations are a large array of new metabolites, many of them recognized as bioactive molecules. This strategy demonstrates to have the potential to produce extracts with a high-added value from plant-based matrices (soybean, apple, cereals, among others).

Studies of apple juice fermentation to manage hyperglycemia, hypertension, and modulation of microbiota composition were also carried out. Apple juice, fermented by *L. acidophilus*, showed outstanding effects enhancing the free radical-scavenging activity in blood samples. *Lactobacillus* fermented samples inhibited *H. pylori in vitro*. However, the fermented extracts did not exert inhibitory effects on the beneficial intestinal species such as *Bifidobacterium longum*. Thus, these data provided biochemical rationale for the development of new fermented food to reduce hyperglycemia (diabetes) and other chronic diseases [87]. The development of probiotics with therapeutic and preventative effects for various diseases and metabolic disorders is the trend of new healthy nutrition. The main limitation for oral probiotics is the harsh conditions of the GI system. For that, the beneficial bacteria have to reach the intestines alive, colonize, and locally release enzymes or bioactive metabolites.

The benefits of *Lactobacillus* intake is not only linked to the capacity to hydrolyze phytophenols inside the lower GI system but also to prehydrolyze those present in plant extract (juice, fruits, etc.) and increased the phenolic content in food and beverages. Predigestion will enhance their absorption once they reach the small intestine to exert their healing properties. For example, the use of three *Lactobacillus* strains (*L. johnsonii* LA1, *L. reuteri* SD2112, and *L. acidophilus* LA-5) improved the bioavailability of the dietary phenolics present in barley and oat flour by 20-fold [88]. The free ferulic acid in the pretreated cereals increased from 1 μg/g dried weight up to 39–56 μg/g dried weight. Comparing the three strains used, *L. johnsonii* demonstrate to be more active in releasing phenolic acids than the other strains. These data showed that cereal fermentation with specific probiotic strains can significantly increase the quantity of free phenolic acids, improving their bioavailability [89]. *L. johnsonii* NCC 533 synthesizes esterases and a hydroxycinnamate decarboxylase responsible for the biotransformation of chlorogenic and caffeic acids. The complete hydrolysis of 5-caffeoylquinic acid *in vitro* occurred during the first 16 h of incubation. After 48 h, caffeic acid was completely transformed to 4-vinylcatechol (4-VC). In this case, the bacteria increased the presence of caffeic acid and simultaneously generated flavor compounds from plant phytophenols [90]. These data provide solid evidence that the same microorganism is able to hydrolyze caffeoyl quinic acids into 4-VC, combining chlorogenate esterase and a hydroxycinnamate decarboxylase activity [6, 91]. Similar results have been reported in the case of some *L. brevis* strains [92].

The ability of lactic acid bacteria to metabolize dietary phytophenols prompts the use of new component combinations in fermented products. Several of these new blends were formulated with plant extracts rich in aromatic compounds. Example of this is the addition of green tea in bioyogurts fermented with selected lactic acid bacteria. Species such as *St. thermophilus, L. acidophilus* LA-5, *B. animalis* subsp. *lactis* BB-12, or acidophilus enhanced the antioxidant capacity of these preparations in dose-dependent manner. Similar studies were carried out with different tea extracts, green, white, and black tea (*Camellia sinensis*) in yogurt combined with *L. acidophilus* LA-5, *Bifidobacterium* Bb-12, *L. casei* LC-01, *S. thermophilus* Th-4, and *L. delbrueckii* ssp. *bulgaricus*. In general, the three types of tea extracts did not significantly affect

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the viability of the bacteria used during storage [93]. The tea extracts could be successfully used as a functional additives in fermented food, adding extra value to the known health benefits of probiotics. Others extracts prepared from olive, garlic, onion, and citrus were also evaluated using similar formulations [94].

3. A model case study, *Lactobacillus johnsonii* N6.2

The intestinal epithelium is one of the most immunologically active surfaces of the body due to the high abundance of microbes and food antigens that are constantly exposed to the GI system. The mucosal surface of the intestinal epithelium is the first line of defense from invading pathogens in the GI tract. Breaching this barrier and subsequently activating aberrant immune signaling have been involved in many diseases, both locally and systematically related. In this context, it has been proposed that there is a complex interplay between gut resident microbiota [95, 96], gut permeability [97], and altered immune function in the development of type 1 diabetes [98].

Currently, our scientific efforts are directed on characterizing a strain of *Lactobacillus* (*L. johnsonii* N6.2). This lactobacilli is abundant in GI microbiome in a line of animals used as a T1D model, in contrast to the scarcity observed in the counterpart diabetes prone animals. Type 1 diabetes (T1D), also referred to as diabetes mellitus type 1, is an autoimmune disease in which pancreatic β-cells produce little to no insulin due to their destruction. Its more commonly known and more prevalent counterpart, type 2 diabetes, occurs when the body becomes resistant to insulin. Both of these conditions result in increased blood glucose levels, called hyperglycemia. Insulin is the hormone responsible for absorbing sugar, in the form of glucose, from circulating blood to be stored in skeletal muscles and fat cells. Although type 1 diabetes has a genetic component and primarily occurs in adolescents and children, it is possible for adults to develop the disease too. Five to 10 percent of diabetes cases in adults are the result of T1D, and an estimated 80 people per day are newly diagnosed with T1D [99, 100]. Unfortunately, recently epidemiological studies have suggested that the incidence of T1D is increasing up to 3–4% globally every year, most notably among youths [101, 102].

L. johnsonii N6.2 was discovered when it was negatively correlated with diabetes development when analyzing the stool samples from BioBreeding diabetes-prone (BB-DP) and BioBreeding diabetes-resistant (BB-DR) rats. Stool embodies a representative microbiome of an individual and is a useful sample for understanding the microbial diversity of the GI tract. Currently, it is estimated that more than 1000 microbial species encompassing more than 100 trillion microorganisms colonize the GI system, collectively outnumbering human genes by 150-fold [103]. These microorganisms grow more in number and diversity progressing through the GI tract. The BioBreeding rat is popular model when studying type 1 diabetes, as it spontaneously develops this disease through its genetic predisposition. After using culture-independent methods, it was found that two genera, *Bifidobacterium* and *Lactobacillus*, showed a higher abundance in BB-DR rats [104]. Quantitative PCR of 16S rRNA revealed a higher abundance of *Bifidobacterium* and *Lactobacillus* in BB-DR samples [104]. However, it was unknown whether the higher abundance of these bacteria was just the common microflora of a "healthy" gut or

if they played a part in preventing the onset of T1D. Further analyses of the *Lactobacillus* strains in the BB-DR rat model revealed that those with CE activity, such as *L. johnsonii* N6.2 and *L. reuteri* TD1, were negatively correlated with T1D development [6].

As it was described before, the release of antioxidant compounds by probiotic bacteria is relevant since an enhanced oxidative stress response triggered by the excessive production of reactive oxygen species is observed in T1D and other diseases [105–107]. This characteristic was relevant in the study because a low dosage of ferulic acid stimulates the release of insulin and alleviates symptoms common to T1D in rodents [83, 108, 109]. Therefore, it would seem plausible that orally administering lactic acid bacteria containing CE qualities would help reduce blood glucose levels and ultimately prevent the onset of diabetes. To confirm this, a feeding experiment of *L. johnsonii* N6.2 and *L. reuteri* TD1 on BioBreeding rats was conducted to determine whether these strains were responsible for the lack of T1D development. While *L. johnsonii* N6.2-fed rats were associated with reduced diabetes onset, *L. reuteri* T1D showed similar diabetes development characteristics as vehicle-fed control groups [110]. A feruloyl esterase screening assay of bacterial stool sample isolates from BB-DR rats on MRS media supplemented with feruloyl esters demonstrates that *L. johnsonii* N6.2 contained the highest feruloyl esterase activity [6]. Enzymatic screening of two purified *L. johnsonii* proteins, Lj0536 and Lj1228, showed high preference and good enzymatic activity using aromatic esters as substrates (**Figure 3**). Lj1228 displayed the best hydrolytic activity with ethyl ferulate, chlorogenic acid, and rosmarinic acid, while Lj0536 showed a preference to ethyl ferulate. Sequence analyses of these proteins revealed a 42% similarity and the classical serine nucleophilic motif characteristic for some feruloyl esterases [111, 112]. Biochemical analyses of these enzymes suggested that they maintain excellent activity in the presence of emulsifiers. Their activity was tested in the presence of conjugated and deconjugated bile salts of which none of the compounds assayed decreased their activity. Interestingly, with increasing concentrations of

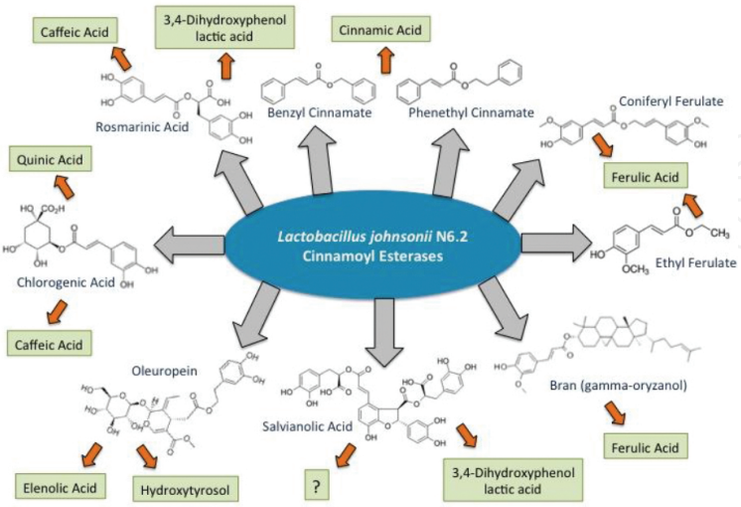


Figure 3. *L. johnsonii* LJ0536 hydrolyze a wide range of substrates. The product(s) of hydrolysis for each substrate are boxed.

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sodium glycocholate, Lj0536 showed increased activity. In this condition, both enzymes were active against a wide variety of substrates, showing the highest affinity toward aromatic esters.

L. johnsonii post-weaning feedings has demonstrated a decreased incidence of diabetes in BB-DP rats compared to vehicle-fed controls and *L. reuteri* TD1-fed rats [110]. With this in mind, it was then determined what type of altered environment *L. johnsonii* created compared to healthy controls and diabetic animals (including those animals from *L. johnsonii* feedings and controls that developed T1D). The first thing that was noticed was the modification of the intestinal microbiota as determined by real-time quantification. While all animals showed an abundance of *Lactobacillus* in stool samples, differences in species seem to differ among feeding groups (*L. johnsonii, L. reuteri*, and vehicle control). Vehicle control animals displayed a predominance of *L. murinus* (65%), while 88% and 92% of *L. johnsonii* and *L. reuteri*, respectively, corresponded to the fed bacteria in each group. Analyses of ileal mucosa unveiled a significant increase of the *Lactobacillus* population in all rats that did not develop diabetes, and a significant increase of enterobacteria was found in all diabetic animals. Since no differences in the microbiota were obtained in stool samples, but were statistically significant in ileal mucosa, the positive effect of *L. johnsonii* N6.2 could be exhibited primarily in the intestinal mucosa [110].

As it was observed that an altered intestinal microbiota was associated with diabetes onset, as previously suggested [95, 96], gut permeability and barrier function were investigated next between *L. johnsonii*-fed, healthy controls, and diabetic animals. It was previously reported that changes in intestinal morphology and permeability, partly due to decreased levels of claudin-1, were observed before the onset of T1D [97]. Claudin-1 is an intercellular tight junction protein responsible for cell-to-cell adhesion in epithelial cell layers. This protein is important in strengthening the physical barrier that keeps the contents of gut lumen from passing into the lamina propria. It has been suggested that unregulated passage of environmental antigens through the intercellular space of the intestinal epithelial could trigger the autoimmune response that contributes to T1D. Expression analysis of the claudin-1 gene in *L. johnsonii*-fed animals exposed its higher abundance when compared to healthy controls or diabetic animals [110]. Furthermore, a significant increase in goblet cells was unveiled in healthy controls and *L. johnsonii*-fed animals compared to those that developed diabetes. Goblet cells produce mucin, the main constituent of the mucosal lining of the GI tract. This feature is important when considering the harsh environment of the GI tract and the constant exposure to potential invading pathogens and inflammatory antigens. The mucosal layer serves as one's first line of defense against these threats by acting as a physical, viscous, and continuously moving layer that rests above epithelial cells. Most harmful substances get trapped in the mucous and before even making it to the epithelial layer, get swept down the intestines. The increase in claudin-1 and goblet cell levels in *L. johnsonii*-fed animals strengthens and physically protects the epithelial cell layer and undoubtedly intensifies intestinal barrier function contributing to the decrease in diabetes onset.

Among the destructive properties of reactive oxygen species (ROS) generated during early disease development is its ability to disrupt the function of epithelial tight junction proteins [113]. To determine the extent of the oxidative stress environment, ileal mucosal hexanoyl-

lysine levels were quantified by ELISA and a significant increase of levels was observed in diabetic animals when compared to healthy controls and *L. johnsonii*-fed animals [110]. Due to the difference in the oxidative environment between diabetic and non-diabetic animals, the expression of genes involved in ROS detoxification pathways were also quantified. It was evident that *L. johnsonii* helps the host to cope with intestinal oxidative stress response as levels of superoxide dismutase 2, catalase, glutathione reductase, and glutathione peroxide were induced in diabetic animals. Meanwhile, superoxide dismutase and glutathione peroxidase were induced in healthy controls compared to *L. johnsonii*-fed groups. Taken collectively, catalase and glutathione reductase were negatively correlated with a healthy status, while superoxide dismutase 2 and glutathione peroxidase were negatively correlated with *L. johnsonii* feeding. Also among the stress response genes assayed was inducible nitric oxide synthase (iNOS), which produces nitric oxide in the presence of ROS. The mRNA levels of iNOS were significantly reduced in *L. johnsonii*-fed rats compared to healthy controls and those that developed diabetes. When further examining the iNOS protein levels via Western blot, *L. johnsonii*-fed rats and healthy controls showed similar levels of detection, suggesting that expression of iNOS is associated with healthy status. Amid the inducers of iNOS expression is INFγ, a pro-inflammatory cytokine [114, 115]. It was hypothesized that a negative correlation existed between pro-inflammatory cytokines, specifically INFγ, and the reduced stress response due to *L. johnsonii* feeding. This hypothesis was proven as diabetic animals showed a significant increase in INFγ gene expression compared to healthy animals; meanwhile, healthy controls and *L. johnsonii*-fed animals did not show any statistical differences.

Since it has been determined that *L. johnsonii* N6.2 feedings can promote a healthy gut microbiota and strengthen epithelial barrier function, it was next examined whether *L. johnsonii* could influence immune function. At the intestinal mucosal layer, resident microbiota and host cells reside in constant homeostasis, epithelial cells tightly controlled by the recognition and tolerance of local bacteria. Host cells recognize the resident microbiota or their associated components through pattern recognition receptors (Toll-like receptor, TLR) and/or by cytoplasmic nucleotide-binding oligomerization domain (NOD)-like receptors, which can subsequently initiate an immune response. Of the first things noticed with *L. johnsonii* administration was the overexpression of pro-inflammatory chemokine mRNA levels, particularly CCL20 (MIP3A), CXCL8 (IL-8), and CXCL10 (IP-10), suggesting that *L. johnsonii* may prime the innate immune system to become more resistant to a subsequent strong inflammatory response [116]. Investigation of the ability of *L. johnsonii* to activate TLR and NOD-like receptor revealed that exposure to *L. johnsonii* created a 4.2- and 10-fold increased expression of TLR7 and TLR9, respectively. Because both of these receptors are involved in nucleic acid recognition, cell free extracts and purified *L. johnsonii* nucleic acid extracts were tested on their ability to induce expression of these TLRs. In both cases, cell-free extracts and purified *L. johnsonii* nucleic acid were able to increase the mRNA levels of TLR7 and TL9, suggesting that the ability for epithelial cells to sense foreign nucleic acids may be involved in the observed increased of some chemokine levels. This also suggests that *L. johnsonii* predominantly exerts its signaling capability through RNA/DNA recognition, as opposed to other cell components, such as peptidoglycan that is sensed by TLR2 and NOD2. Lastly, consequences of TLR9 induction by *L. johnonii* were determined by exploring the expression of Frizzled 5

receptor (fzd5), which is responsible for Paneth cell maturation, and INF-α, which is secreted by TLR9 activity and induces the chemokine CXCL10 [117–119]. Paneth cells are located at the base of intestinal glands throughout the small intestines and secrete antimicrobial peptides. *L. johnsonii* administration showed higher levels of Paneth cells in agreement with the higher levels of fzd5, and a higher level of INF-α, in agreement with the observed increased levels of CXCL10 [116]. As discovered in this study, *L. johnsonii* may be able to prime the immune system by activating an innate immune response early on and therefore protecting the host from more prominent stimuli later on.

As more is studied about *L. johnsonii* N6.2, it has been found that its protective functions are very diverse, supporting its probiotic qualities. In addition to the activation of innate immune response, adaptive immune response stimulation was discovered when diabetes-resistant *L. johnsonii*-fed rats were correlated with a T helper 17 (Th17) cell bias [120]. Th17 cells protect the host from extracellular pathogens by recruiting neutrophils and macrophages to the site of infection. This activity could aid in defending the host from aberrant microflora that could ultimately trigger the autoimmune response leading to T1D. While experimenting with other host-effected pathways, our recent focus has been on the ability of *L. johnsonii* to modulate the tryptophan catabolism pathway. This pathway involves the rate-limiting enzyme indoleamine 2,3-dioxygenase (IDO), which is the first enzyme along the pathway that breaks down tryptophan into kynurenine. Kynurenine is a potent aryl hydrocarbon receptor (AhR) ligand; however, the largest source of AhR ligands is found in the diet among which are vegetable and fruit phytophenols [121–123]. Interestingly, IDO induction has also been linked to AhR [124–126]. AhR is a ligand-activated, basic helix-loop-helix transcriptional activator that is associated with many diseases, including autoimmunity [127–129]. Since it was previously found that *L. johnsonii* associates with the ileal mucosa and its colonization correlated with decreased expression of pro-inflammatory cytokine INFγ, the ileal tissue seemed to suggest the best site for observing *L. johnsonii* effects on host cells [110, 130]. More importantly, INFγ has been noted as a primary inducer of IDO in many cell types. Indeed, while surveying different tissues via quantification of the IDO gene expression, the ileum appeared to have a decreased appearance of IDO transcripts in *L. johnsonii*-fed animals compared to control animals [130]. Healthy control rats expressed a 4.7-fold higher level of IDO transcripts, while diabetic animals expressed 11.8-fold increase in mRNA levels compared to *L. johnsonii*-fed animals [130]. This correlated with an observed decrease in blood serum kynurenine levels through HPLC in *L. johnsonii*-fed animals compared to healthy controls and diabetic animals [130]. However, since this study was performed at 120 days, after diabetes onset, it could not reveal early developmental or physiological effects of the bacterial feeding in the host. To address this, a study was completed to evaluate the effects of *L. johnsonii* feedings in a prediabetic host. As a reliable indicator of IDO activity, systemic kynurenine: tryptophan ratios were quantified via HPLC in 30- and 60-day-old prediabetic BBDP rats. At both ages, serum kynurenine levels decreased significantly from the controls while only at 30 days of age did serum tryptophan levels show a significant increase [130]. To verify that IDO activity was responsible for the reduced systemic kynurenine: tryptophan ratios between bacterial-fed groups and controls, the activity of a related enzyme more commonly found in the liver, tryptophan 2,3-dioxygenase (TDO), was examined. TDO, unlike IDO, was previously reported

to be unresponsive to inflammatory stimuli and is important in homeostatic control of tryptophan levels under normal conditions. However, recently TDO expression has been correlated to neurological diseases, such as Alzheimer's, and various cancers, such as ovarian carcinoma, breast, and gliomas [121, 131]. After examining activity levels from tissue lysates, there was not a significant difference between the TDO activities in *L. johnsonii*-fed rats and vehicle-fed controls. IDO is widely distributed throughout the human host, and therefore, significant levels can be found throughout the GI tract. Since this enzyme, and its downstream catabolite kynurenine, is activated during inflammatory conditions, it could be involved in the inflammatory response associated with diabetes. Interestingly, we have reported that *L. johnsonii* feedings can reduce the expression of INFγ, a pro-inflammatory cytokine, in the ileum of rats after diabetes onset [110]. Upon performing Western blots of lysates of the colon, cecum, duodenum, jejunum, ileum, liver, and pancreas, it was found that the colon and ileum had variable, but overall decreased, levels of IDO in *L. johnsonii*-fed animals compared to control animals [130]. This correlates well with reduced ileal INFγ expression and overall decreased inflammation in *L. johnsonii*-fed hosts. At this point, it appeared that *L. johnsonii* effected IDO activity and subsequent systemic kynurenine concentrations.

L. johnsonii produces an inhibitor of IDO affecting the enzymatic activity and the products synthesized downstream the pathway. Diluted cell-free supernatant (CFS) of *L. johnsonii* was incubated with purified recombinant IDO, and the resulting kynurenine concentrations were quantified. Increasing concentrations of *L. johnsonii* CFS caused an increased inhibition of IDO activity. Furthermore, CFS from *L. johnsonii* N6.2 most potently inhibited IDO when compared to other enteric *Lactobacillus* species CFS effect on IDO activity [130]. This observation stimulated the characterization of *L. johnsonii* N6.2 supernatant in order to locate the IDO inhibitor. It was found that increased concentrations of hydrogen peroxide (H_2O_2) correlated with increased *L. johnsonii* culture incubation time before centrifugation and collection of the CFS [130]. Upon increasing concentrations of catalase, which decreases the pool of H_2O_2, IDO activity increased, supporting the role of H_2O_2 in CFS as an inhibitor of IDO. Likewise, upon increasing concentrations of H_2O_2, IDO activity decreased in a dose-dependent manner. This strongly supported H_2O_2 as an inhibitor of IDO enzyme activity. This enzyme contains heme in the catalytic center to carry out its dioxygenase activity. When the active ferrous centers are oxidized to its inactive ferric form, the dioxygenase activity of IDO is restricted [132]. This causes an accumulation of tryptophan and a decrease in kynurenine levels that can be detected throughout the host. Hydrogen peroxide has the ability to oxidize the reactive heme ferrous centers of IDO, rendering the enzyme inactive. In this current study, the biological relevance of H_2O_2 was tested by measuring the levels of H_2O_2 in the GI tract of *L. johnsonii*-fed animals. Since ileal IDO levels were reduced, the hypothesis was that an increase of hydrogen peroxide would be found in these tissues compared to other sites of the body. This could potentially explain the difference in IDO expression of the ileum compared to other sites of the GI tract. Indeed, when measuring H_2O_2 levels from GI contents, the ileum contained higher levels compared to other sections of the digestive tract [130]. Since *L. johnsonii* most strongly associates with the host mucosa at this site, it further supports the hypothesis of the ability of *L. johnsonii* to produce an inhibitor of IDO [110]. Upon RNA-seq analysis of *L. johnsonii* grown under different aeration conditions, a gene (T285_08005) regulating H_2O_2 production was

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identified. The encoding protein contained a Per-Arnst-Sim (PAS) domain and regulated the H_2O_2 production from heterodimeric FMN reductases, FRedA and FRedB (WP_004898036.1 and WP_011162530.1, respectively) [62].

After experiencing reduced kynurenine production and IDO inhibition in response to *L. johnsonii*, it was hypothesized that other tryptophan metabolite concentrations could be effected. Tryptophan is a precursor to the neurotransmitter 5-hydroxytryptamine (5-HT, serotonin), which is predominantly produced in enterochromaffin cells along the GI epithelium. IDO also has the enzymatic activity to catalyze 5-HT to 5-hydroxykynuramine, aiding in increased 5-HT turnover [133, 134]. Using ELISA, 5-HT levels in ileum tissue lysates and blood serum collected from 60-day-old BBDP rats were quantified. Serotonin levels were significantly elevated, both locally and peripherally, in *L. johnsonii*-fed animals [130].

Figure 4 most accurately summarizes the work our group has done in characterizing *Lactobacillus johnsonii* N6.2, starting with its discovery in 2008 and most recently revealing its regulating effects on IDO. This bacterial genera was correlated with a reduced diabetes onset when comparing BioBreeding diabetes-prone and diabetes-resistant rats [104]. It was identified that *Lactobacillus* strains that contain CE activity were more correlated with diabetes resistance, and through subsequent feeding assays of these potential bacterial targets, *L. johnsonii* N6.2 was identified as being negatively associated with T1D [6, 110]. Since this discovery, *L. johnsonii* N6.2 has been characterized in regard to its diabetes resistance. *L. johnsonii* strengthens gut permeability through a higher abundance of the tight junction claudin-1 levels and goblet cells, and it reduces GI stress by reducing the expression of oxidative stress genes and inflammatory INF-γ levels [110]. Among the most recent and most interesting findings of the qualities of *L. johnsonii* is its ability to modulate IDO activity through its production of H_2O_2 [130]. However, this bacterium's esterase activity has the ability to quench its own H_2O_2 production through the release of phytophenols. These antioxidants have the potential to eliminate part of the pool of produced H_2O_2, along with other even more dangerous ROS that precede chronic diseases. In the case of *L. johnsonii* N6.2, sufficient H_2O_2 production is observed in the ileum, where *L. johnsonii* is localized [110, 130]. Conversely, reduced levels of oxidative stress genes are observed in the ileum of *L. johnsonii*-fed rats [6, 110]. Thus, one of the main probiotic properties of *L. johnsonii* could be its ability to maintain redox homeostasis in the GI tract. This balance is dependent on the dynamic interplay between the generation of H_2O_2 and the ROS quenching ability of antioxidant phytophenols released by this bacterium. The H_2O_2 released by this bacterium in the intestinal lumen would stimulate oxidative stress defense mechanisms in host cells, while controlling the activity of IDO [130, 132]. Meanwhile, enzymes unique to *L. johnsonii* will increase the pool of free, bioavailable antioxidant phytophenols in the intestinal lumen. The phenolic released will differentially quench the most reactive ROS.

Although *L. johnsonii* N6.2 was found and characterized in regards to its correlation with reduced T1D onset, it has the potential to expand its beneficial functions into the realm of other chronic diseases. IDO is an immunoregulatory enzyme whose altered activity has been

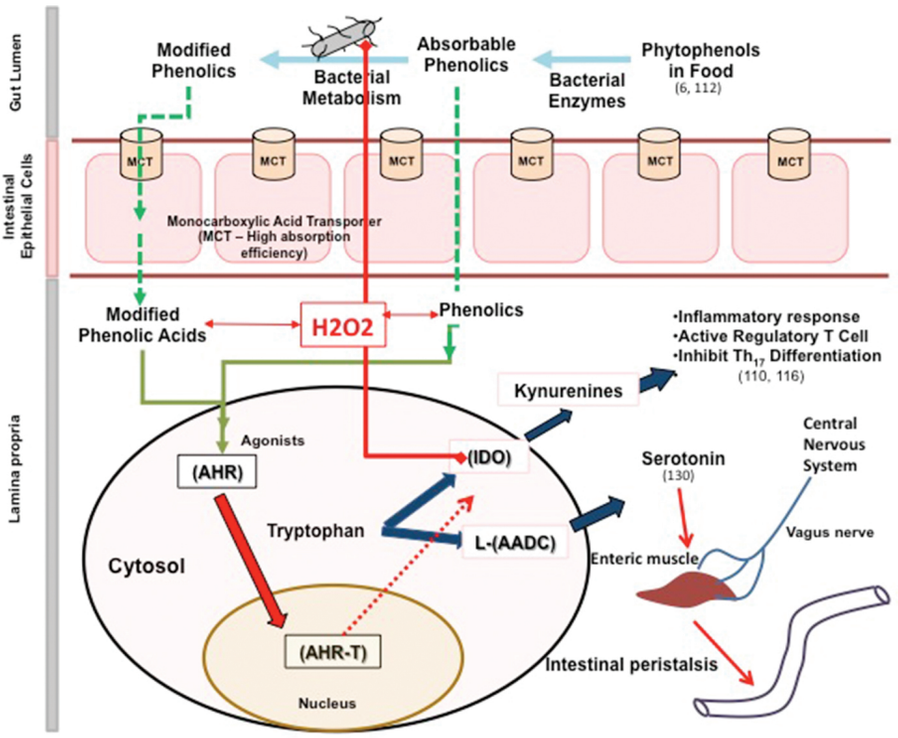


Figure 4. Gastrointestinal (GI) epithelium with proposed mechanisms of phytophenol and H_2O_2 action. This figure summarizes the results published by the group between 2009 and 2014.

observed in a multitude of diseases, including autoimmunity and cancer [135–137]. *L. johnsonii* N6.2 has the ability to regulate IDO activity by inactivating its redox-sensitive heme centers through H_2O_2 production [130]. The effect of this inactivation has the potential to expand over into other chronic disease and reduce their occurrence. This makes regulators of IDO an important immunotherapy target in preventing some of today's most serious diseases. Resident microbiota provide many protective functions for the host, such as outcompeting pathogenic threats, releasing necessary resources from digested foods, and maintaining GI homeostasis. It is to no surprise that disturbances to microfloral composition could dictate disease onset. There are numerous probiotics in the market, encompassing many different genera. These probiotics exert beneficial properties through their unique enzymes, their released metabolites, or a combination of both as in the case of *L. johnsonii* N6.2. Therefore, probiotic administration serves as an important defense in preventing some of the most common and chronic diseases.

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