

REVIEW ARTICLE

GUT MICROBIOTA, PROBIOTICS AND PHYTOCHEMICALS

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ABSTRACT

The human gut is populated by an array of bacterial species with a marked effect on the nutritional and health status of the host. Phytochemicals are bioactive non-nutrient plant compounds, which have raised interest because of their potential effects as antioxidants, antiestrogens, anti-inflammatory, immunomodulatory, and anticarcinogenics. Many phytochemicals are present in plant foods as glycosides or other conjugates and need to be hydrolyzed in order to be absorbed. Gut bacteria can hydrolyze glycosides, glucuronides, sulfates, amides and esters. They also carry out reduction, ring-cleavage, demethylation and dehydroxylation reactions. The hydrolysis of glycosides and glucuronides typically results in metabolites that are more biologically active than the parent compounds. Phytochemicals and their metabolic products may also inhibit pathogenic bacteria while stimulate the growth of beneficial bacteria, exerting prebiotic-like effects. Therefore, the intestinal microbiota is both a target for nutritional intervention and a factor influencing the biological activity of other food compounds acquired orally. Probiotic *lactobacilli* and *bifidobacteria*, bear several glycosyl-hydrolases and can contribute to release the aglycones from glycoconjugated phytochemicals. The significance of this review is to focuses on the complex relationship between Phytochemicals phyto-metabolites and gut microbiota and the consequences of this on human metabolic homeostasis.

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INTRODUCTION

Gut Microbiota

The human colon harbors one of the most diversified and densely populated bacterial ecosystems on Earth, dominated by anaerobic bacteria belonging to the phyla of *Firmicutes* (which include *Clostridiales* and *Lactobacillales*), *Bacteroidetes*, and *Actinobacteria* (which include *Bifidobacteriales*), in numbers exceeding  $10^{11}$  cells/g of intestinal content [1].

The host and the commensal bacteria establish a mutualistic relationship, which has a major impact on the nutrition and overall health status of the host. The colonic microbiota is maintained in a constant-temperature environment and is provided with a broad spectrum of substrates undigested and unabsorbed [2]. On the other hand, the microbiota offers to the host protection against infections, plays a role in the modulation of the immune system, and supplies carbon, energy, vitamins, and bacterial- activated dietary metabolites [3].

In order to be tolerated by our bodies our intestinal flora are adapted for their environment and interact with our immune system [4], binding to intestinal cells and mucus [5] and eliciting specific immune responses [4]

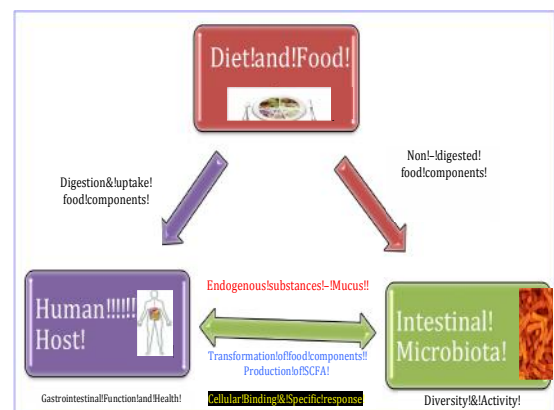


Figure 1 Impact from intestinal interaction

Diet Alters The Gut Microbiota

Diet is known to modulate the composition of the gut microbiota in humans and mice. Long-term dietary habits have a considerable effect on the human gut microbiota. However, this concept is currently being challenged as enterotypes maybe more of a gradient than discrete entities. Each enterotype is dominated by a different genus - *Bacteroides*, *Prevotella* or *Ruminococcus* [7] but not affected by gender, age or nationality [7]. Enterotypes dominated by *Bacteroides* or *Prevotella* are associated with the consumption of a diet rich in protein and animal fat, or carbohydrates, respectively [8].

Changes in daily carbohydrate intake may affect specific groups of colonic bacteria over a short period of time. Consumption of the prebiotic inulin increases the levels of *F. prausnitzii* and *Bifidobacterium sp.* in humans [9]. Human diets that are supplemented with resistant starch have increased faecal levels of *Ruminococcus bromii* and *Eubacterium rectale*, which correlates with fibre fermentation [10]. Consumption of resistant starch also improves insulin sensitivity [11], but the variation in the microbial response to changes in resistant starch between individuals suggests successful dietary interventions need to be personalized [10].

The gut microbiota also reacts to dietary fat. Mice fed on high-fat diets have reduced numbers of *Bacteroidetes*, and increased numbers of *Firmicutes* and *Proteobacteria* [12]. This change is rapid, occurring within 24 hours [13]

A change in diet clearly alters the gut microbiota, and these alterations may contribute to the host's metabolic phenotype. Further metatranscriptomic and proteomic studies should provide insight into the response of microbial function as a result of a dietary shift.

### Probiotics

Probiotics were defined as "live micro-organisms," which, when administered in adequate amounts confers a health benefit on the host [14]. Various bacterial genera most commonly used in probiotic preparations are *Lactobacillus*, *Bifidobacterium*, *Escherichia*, *Enterococcus*, *Bacillus* and *Streptococcus*. Some fungal strains belonging to *Saccharomyces* have also been used [15],[16].

### Properties of a Probiotic

Harmsen *et al.*, [17] describe The criteria of an ideal microorganism as probiotic which including:

1. High cell viability, thus they must be resistant to low pH and acids
2. Ability to persist in the intestine even if the probiotic strain cannot colonize the gut.
3. Adhesion to the gut epithelium to cancel the flushing effects of peristalsis
4. They should be able to interact or to send signals to the immune cells associated with the gut.
5. They should be of human origin
6. Should be nonpathogenic
7. Resistance to processing
8. Must have capacity to influence local metabolic activity

There are a large number of probiotics currently used and available in dairy fermented foods, especially in yogurts. Lactic acid bacteria constitute a diverse group of organisms providing considerable benefits to humankind, some as natural inhabitants of the intestinal tract and others as fermentative lactic acid bacteria used in food industry, imparting flavor, texture and possessing preservative properties. Beyond these, some species are administered to humans as live microbial supplements, which positively influence our health mainly by improving the composition of intestinal microbiota. For this reason, they are called

probiotics. Some selected strains of *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus* and *Saccharomyces* have been promoted in food products because of their reputed health benefits [18],[19].

### The physiological effects related to probiotic bacteria

The physiological effects related to probiotic bacteria include the reduction of gut pH, production of some digestive enzymes and vitamins, production of antibacterial substances, e.g., organic acids, bacteriocins, hydrogen peroxide, diacetyl, acetaldehyde, lactoperoxidase system, lactones and other unidentified substances, reconstruction of normal intestinal microflora after disorders caused by diarrhoeas, anti-biotic therapy and radiotherapy, reduction of cholesterol level in the blood, stimulation of immune functions, suppression of bacterial infections, removal of carcinogens, improvement of calcium absorption as well as the reduction of faecal enzyme activity [20] , [21].

From the safety point of view, the probiotic microorganisms should not be pathogenic, have no connection with diarrhoeagenic bacteria and no ability to transfer antibiotic resistance genes, as well as be able to maintain genetic stability. To be recognized as functional food components, they should demonstrate the following properties: acid- and bile-stability, resistance to digestive enzymes, adhesion to intestine surface, antagonistic activity against human pathogens, anti-carcinogenic and anti-mutagenic activity, cholesterol-lowering effects, stimulation of the immune system without inflammatory effects, enhancement of bowel motility, maintenance of mucosal integrity, improvement of bioavailability of food compounds and production of vitamins and enzymes [22].

Gastrointestinal mucosa is the primary interface between the external environment and the immune system. Whenever intestinal microflora reduces, antigen transport is increased indicating that the normal gut microflora maintains gut defences [23].

### Phytochemicals

Phytochemicals are bioactive nonnutrient chemical compounds found in plant foods, such as fruits, vegetables, grains, and other plant foods. They can be categorized into various groups, i.e., polyphenols, organosulfur compounds, carotenoids, alkaloids, and nitrogen-containing compounds. The polyphenols are some of the most studied compounds and can be further divided into flavonoids (including flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavones), phenolic acids, stilbenes, coumarins, and tannins [24].

### Mechanism of Action of Phytochemicals

Researchers have found that phytochemicals have the potential to stimulate the immune system, prevent toxic substances in the diet from becoming carcinogenic, reduce inflammation, prevent DNA damage and aid DNA repair, reduce oxidative damage to cells, slow the growth rate of cancer cells, trigger damaged cells to self-destruct (apoptosis)

before they can reproduce, help regulate intracellular signaling of hormones and gene expression, and activate insulin receptors [25], [26]. In addition, there likely are health effects of phytochemicals that researchers haven't yet recognized [25].

Much laboratory research has focused on the antioxidant function of phytochemicals. However, their antioxidant activity is reduced in the body during metabolism, and the levels present in blood and tissue are fleeting and quite low [27], [28]. For many of the phytochemicals in food, their antioxidant effects on cell signaling and gene expression may be more important for health benefits than direct antioxidant activity, effects that can be seen even with low concentrations of phytochemicals in plasma and tissues [27]

In addition to being rich sources of phytochemicals, plant foods also are sources of fiber, vitamins, and minerals whose mechanisms have been more clearly elucidated. But identifying which individual compounds are responsible for the benefits associated with phytochemical-rich foods is difficult, if not impossible, because of the interactions that occur with vitamins, minerals, and fiber as well as among the phytochemicals themselves. The unique combination of these compounds may be the key to reduced disease risk, but that formula hasn't yet been identified and tested [29].

**Table 1** Phytochemicals in Foods and Possible Health Benefits [29]

Foods	Phytochemicals	Possible Health Benefits
Soybeans, soy milk, tofu	Isoflavones (genistin, daidzein), types of flavonoids	Reduction in blood pressure and increased blood vessel dilation
Strawberries, red wine, blueberries	Anthocyanins, a type of flavonoid	Blood vessel dilation, induction of cancer cell death, improved insulin sensitivity, neuroprotective effects
Red wine, grape juice, grape extracts, cocoa, peanuts	Proanthocyanidins, a type of flavonoid; resveratrol	Inhibition of LDL oxidation and inflammation
Garlic, onions, leeks, olives, scallions	Sulfides, thiols	Decreased LDL cholesterol, anticancer effects
Carrots, tomatoes and tomato products, other orange, yellow, and red fruits and vegetables	Carotenoids, such as lycopene, and beta-carotene	Neutralization of free radicals that cause cell damage
Cruciferous vegetables such as broccoli and kale, horseradish	Isothiocyanates (sulforaphane)	Neutralization of free radicals that cause cell damage and protection against some types of cancer
Green and black tea, cocoa	Catechins, epicatechins, types of flavonoids	Vasodilation, improved blood flow to the brain, improved insulin sensitivity

**Role of Gut Microbiota in Nutrient Metabolism and Energy Storage**

1. The intestinal microbiota develops an important biochemical activity within the human body by providing additional metabolic functions [30] and regulating the diverse aspects of cellular differentiation and gene expression via host-microbe interactions [31]. The intestinal microbiota provides enzymes involved in the utilization of non-digestible carbohydrates and host-derived glycoconjugates, deconjugation and dehydroxylation of bile acids, cholesterol reduction and biosynthesis of vitamins (K and B group), isoprenoids and amino acids (e.g. lysine and threonine) [30], [31]. In particular, the ability of the commensal microbiota to utilize complex dietary polysaccharides which would otherwise be inaccessible to human subjects and to generate SCFA seems to contribute to the ability of the host to harvest energy from the diet [32]. Specific components of the commensal microbiota also regulate serum lipids and cholesterol by taking part in bile-acid recycling and metabolism. Bacterial enzymes mainly catalyse the

deconjugation and dehydroxylation of bile acids, which alter the solubilization and absorption of dietary lipids throughout the intestine [33]. Faecal commensal bacteria also reduce cholesterol to coprostanol and, thus, increase its excretion in faeces [34].

- In addition, the commensal microbiota and its metabolites regulate the expression of genes involved in the processing and absorption of dietary carbohydrates and complex lipids in the host, favouring fat storage [32], [35].
- Furthermore, microbial colonization reduces the levels of circulating fasting-induced adipose factor in the gut, skeletal muscle and liver levels of phosphorylated AMP-activated protein kinase, which jointly contribute to reducing fat oxidation and enhancing fat storage [36].

**Gut Microbiota and Probiotics**

The use of probiotics to modulate the activity and composition of the gut microbiota to improve the health status is consolidated [37]. *Bifidobacterium* is a genus of high G+C (guanine + cytosine content) Gram-positive bacteria within the phylum of Actinobacteria.

Among nearly 50 species recognized so far, the most represented in the gastrointestinal tract of human adults or infants are *Bifidobacterium pseudocatenulatum*, *B. catenulatum*, *B. adolescentis*, *B. longum*, *B. infantis*, *B. breve*, *B. angulatum* and *B. dentium* [38]. *Bifidobacteria* are abundant gut colonizers and one of the most important health-promoting groups within the colonic microbiota and are largely used as probiotics [39]. They compete with other species of intestinal microbiota and transient organisms for nutrients and attachment sites in the gut. *Bifidobacteria* are anaerobic saccharolytic fermenters producing lactic and acetic acids, which acidify the large intestine against putrefactive and potentially pathogenic bacteria. Furthermore, they participate in the regulation of intestinal microbial homeostasis, interfere with the ability of pathogens to colonize and infect the mucosa, modulate local and systemic immune responses, stabilize and preserve the gastrointestinal barrier function, produce vitamins, repress procarcinogenic enzymatic activities, and promote the bioconversion of a number of dietary compounds into bioactive healthy molecules [39], [40].

The genus *Lactobacillus* includes almost 200 recognized

species of low G+C Gram-positive bacteria within the phylum of *Firmicutes*[41]. Despite their wide phylogenetic and functional diversity, *Lactobacilli* are invariably anaerobic/microaerophilic, aciduric/ acidophilic nonsporulating rods. They are included within the functional group of lactic acid bacteria (LAB), being saccharolytic and strictly gaining energy through the lactic fermentation of carbohydrates. On the basis of the fermentation end-products, they can be classified as obligate homofermentative (giving mainly lactic acid), obligate heterofermentative (giving mainly lactic acid, acetic acid, and CO<sub>2</sub>), or facultative heterofermentative [41].

*Lactobacilli* occur in a variety of habitats where carbohydrate-based substrates are available. At present, the strains of *Lactobacillus* with the greatest relevance for the manufacture of probiotics and functional foods belong to the species *L. acidophilus*, *L. casei*, *L. paracasei*, *L. plantarum*, *L. rhamnosus*, *L. reuteri*, and *L. salivarius* [37], [42].

Several studies provided insights into metabolic, trophic, protective, and immune effects of *bifidobacteria* and *lactobacilli*, and probiotic strains have been specifically selected to alleviate chronic intestinal inflammatory diseases, to prevent and treat pathogen-induced diarrhea, to manage autoimmune and atopic diseases, to lower cholesterol levels, and to exert antioxidant activity. In this context, probiotic strains, selected for the production of specific enzymatic activities, may be exploited to enhance the release of the aglycones, improving the rate of biotransformation toward bioactive metabolites carried out by other intestinal microorganisms [43], [44].

#### Phytochemical Metabolism by Gut Microbiota

Gut bacteria can hydrolyze glycosides, glucuronides, sulfates, amides and esters [45]. They also carry out reduction, ring-cleavage, demethylation and dehydroxylation reactions. The hydrolysis of glycosides and glucuronides typically results in metabolites that are more biologically active than the parent compounds. In contrast, further bacterial degradation and transformation of aglycones can lead to production of more or less active compounds, depending on the substrate being metabolized and the products formed [45][46].

The interaction between phytochemical and gut microbiota is illustrated in figure 4.

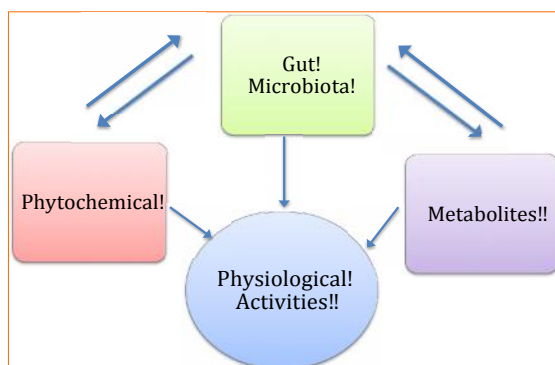


Figure 2 The interaction between phytochemical and gut microbiota

The gut microbiota has proved to be essential for the

production of active isoflavone metabolites with oestrogen-like activity; additionally, the metabolites produced exhibit 5 different anti-inflammatory properties[47]. Similarly, the flavonoid quercetin generated by gut microbial enzymes exerts a higher effect in the down-regulation of the inflammatory responses than the glycosylated form present in vegetables (quercitrin or 3-rhamnosylquercetin) [48]. This effect is exerted by inhibiting cytokine and inducible nitric oxide synthase expression through inhibition of the NF-kappaB pathway both *invitro* and *in vivo*[48]. In contrast, the ellagitannin punicalagin that is the most potent antioxidant found in pomegranate juice is extensively metabolized to hydroxy-6H- dibenzopyran-6-one derivatives, which did not show significant antioxidant activity compared to punicalagin [49]. Phytochemicals and their derived products can also affect the intestinal ecology as a significant part of them are not fully absorbed and are metabolized in the liver, excreted through the bile as glucuronides and accumulated in the ileal and colorectal lumen [50]. For example, the intake of flavonol-rich foods has been shown to modify the composition of the gut microbiota, exerting prebiotic-like effects [51]. Unabsorbed dietary phenolics and their metabolites have been shown to exert antimicrobial or bacteriostatic activities [52]. These metabolites selectively inhibit pathogen growth and stimulate the growth of commensal bacteria, including also some recognized probiotics [52], [53], thus influencing the microbiota composition. Plant phenolic compounds from olives [54], tea [52], wine [53] and berries [55] have demonstrated antimicrobial properties. Tea phenolics have shown to inhibit the growth of *Bacteroides* spp., *Clostridium* spp. (*C. perfringens* and *C. difficile*), *Escherichia coli* and *Salmonellatyphimurium* [52]. The level of inhibition was related to the chemical structure of the compound and bacterial species. In this sense, caffeic acid generally exerted a more significant inhibitory effect on pathogen growth than epicatechin, catechin, 3-O- methylgallic acid, and gallic acid [51].

Polyphenols, a ubiquitous group of secondary plant metabolites originating from the shikimate pathway and sharing at least one aromatic ring structure with one or more hydroxyl groups, represent a large group of natural antioxidants abundant in fruits, vegetables, and beverages [56], [57]. The intact forms of complex dietary polyphenols have limited bioavailability, and this bioavailability is highly variable among individuals and generally far too low to explain the direct antioxidant effects *in vivo*[56]. A large portion of the polyphenols undergoes extensive phase II biotransformation (covalent attachment of a small polar endogenous molecule, such as glucuronic acid, sulfate, or glycine, to form water-soluble compounds) in the small intestine and liver, at which point the polyphenol aglycones are conjugated to glucuronide, sulfate and/or methyl-moieties to facilitate their elimination from the body[57]. Dietary polyphenols are transformed into deglycosylated forms by mammalian  $\beta$ -glucosidases in the gastrointestinal tract before reaching the systemic circulation system [58]. The polyphenol-derived metabolites, such as derivatives of phenyl- propionic, phenylacetic and benzoic acids, with different hydroxylation patterns generated by the action of intestinal microbiota are easily absorbed through the colonic

barrier and can be further transformed in tissues by conjugation with glycine, glucuronic acid or sulfate groups [59].

Another group of compounds that undergo gut bacterial metabolism are the glucosinolates ( -thioglycoside *N*-hydroxysulfates) present in cruciferous vegetables. As with other plant compounds, the glucosinolates themselves are not biologically active, but some of their hydrolysis products, e.g. ITC and indole, are. The plant enzyme myrosinase, a -thioglucosidase, co-occurs in plants producing glucosinolates and in intact plant tissues is located in a compartment separate from the glucosinolates. When the cells in plants are damaged (e.g., cut, ground, or chewed), enzyme and substrate come in contact, releasing the biologically active ITC. If myrosinase has been inactivated (e.g., with cooking), intestinal microbial metabolism of glucosinolates contributes to ITC exposure, albeit at a lower level [60].

Resveratrol, a potent antioxidant found in wine, favoured the increase of *Bifidobacterium* and *Lactobacillus* counts [53] and abolished the expression of virulence factors of *Proteus mirabilis* to invade human urothelial cells [61]. Anthocyanins from berries also have proved to inhibit the growth of pathogenic *Staphylococcus* spp, *Salmonella* spp, *Helicobacter pylori* and *Bacillus cereus* [55]. Phenolics, and flavonoids may also reduce the adhesion ability of *L. rhamnosus* to intestinal epithelial cells [62]. Tea catechins have also been shown to modify mucin content of the ileum which could modulate bacterial adhesion and colonization[63]. Therefore, polyphenols appear to have potential to confer health benefits via modulation the gut microecology.

#### **Effect of Probiotic on Dietary Phytochemicals**

Many reactions that transform naturally occurring phytochemicals into bioactive molecules require the activity of different components of the colonic microbiota. Therefore, probiotic *lactobacilli* or *bifidobacteria*, if properly selected, can affect the kinetics of transformation of these precursors, thus improving the bioavailability and/or biological activity of natural phytochemicals [64].

Many phytochemicals are poorly digested and absorbed in the human small intestine. In this case, they end up in the large intestine, where they are metabolized by the resident microbiota. However, to date, assessment of the effects of probiotics on metabolism of phytochemicals in human gut has been limited to a few studies with isoflavones, which are phytoestrogens abundant in soy foods. Consumption of isoflavones has been associated with changes in sex steroid metabolism and may decrease the risk of breast cancer [65].

Soy isoflavones have received considerable attention. Individuals with isoflavones-rich diets have significantly lower occurrences of cardiovascular disease, osteoporosis, and some cancers. The clinical effectiveness of soy isoflavones may be a function of the ability to biotransform soy isoflavones to the more potent estrogenic metabolite, equol, which may enhance the actions of soy isoflavones, owing to its greater affinity for estrogen receptors, unique antiandrogenic properties, and superior antioxidant activity. However, not all individuals consuming daidzein produce

equol. Only approximately one-third to one-half of the population is able to metabolize daidzein to equol. This high variability in equol production is presumably attributable to interindividual differences in the composition of the intestinal microflora, which may play an important role in the mechanisms of action of isoflavones [66].

Intestinal bacteria play an important role in the metabolism of isoflavones as they release the bioavailable and bioactive aglycone configurations from the food-borne inert glycoside conjugates. In addition, they are able to chemically change the aglycone forms into more potent derivatives such as equol, which binds to estrogen receptors with higher affinity than the parent molecule daidzein. It has been hypothesized that probiotics could metabolize isoflavones or alter intestinal bacteria and enzymes involved in isoflavone metabolism, thereby increasing isoflavone bioactivity[67]. In vitro, Tsangalis *et al.* [68] isolated 4 different  $\beta$ -glucosidase-producing bifido- bacteria able to hydrolyze isoflavone glycoside conjugates, 3 of them also converting daidzein to equol.

Kurzer's group also investigated extensively the ability of probiotics to increase isoflavone bioavailability and bioactivity, using the commercial product DDS Plus (*L. acidophilus* DDS+1 and *B. longum*,  $10^9$  CFU/d of each strain) in a series of randomized, PC trials. In postmenopausal women, urinary 2- hydroxyestrogens and the ratio of 2:16- hydroxyestrone, which is inversely associated with breast cancer risk, increased after 6 wk of soy consumption in those participants who produced equol. But this, as well as plasma isoflavone concentration and equol urinary excretion, was not influenced by probiotics consumption [69- 71].

Raimondi *et al.* [72] demonstrated that Twenty-two strains of *Bifidobacterium*, representative of eight major species of human origin, were screened for their ability to transform the isoflavones daidzin and daidzein. Most of the strains released the aglycone from daidzin and 12 gave yields higher than 90%. The kinetics of growth, daidzin consumption, and daidzein production indicated that the hydrolytic activity occurred during the growth. The supernatant of the majority of the strains did not release the aglycone from daidzin, suggesting that cell-associated -glucosidases (-Glu) are mainly responsible for the metabolism of soybean glyco-conjugates. Cell-associated -Glu was mainly intracellular and significantly varied among the species and the strains. The lack of -Glu was correlated with the inability to hydrolyze daidzin. Although S-equol production by anaerobic intestinal bacteria has been established, information on S-equol-producing *bifidobacteria* is contradictory. It has been suggested that, selected probiotic strains of *Bifidobacterium* can be used to speed up the release of daidzein, improving its bioavailability for absorption by colonic mucosa and/or biotransformation to S-equol by other intestinal microorganisms

Possemiers *et al.* [73] used a *Eubacterium limosum* strain shown to produce in vitro the potent phytoestrogen 8-prenylnaringenin from isoxanthohumol, its inactive precursor contained in hops. In humans, activation of isoxanthohumol to 8-prenylnaringenin depends on intestinal microbial

metabolism, but this occurs only in one-third of individuals. Oral administration of *E. limosum* to rats triggered 8-prenylnaringenin production in those that were germ-free and increased 8-prenylnaringenin production in those that were colonized with a low-level activity of human fecal microbiota. This study supports the idea that probiotic consumption can increase intestinal production of active forms of phytoestrogens, paving the way for balancing exposure to these compounds in an initially heterogeneous human population.

Probiotic strains, and particularly *bifidobacteria*, bear a number of glycosyl-hydrolases, because they have evolved within the colonic ecosystem, where indigestible oligo- and polysaccharides are the major carbon sources for saccharolytic fermentative bacteria. Thus, they may be involved in the release of aglycones from glycoconjugated forms of polyphenols. Among the diverse glycosyl-hydrolases,  $\alpha$ -glucosidases (EC 3.2.1.21) are the most pertinent for the release of the aglycones, because, many phytochemicals are glucoconjugates. In particular, the initial hydrolysis of soy isoflavone glucosides to their respective aglycones is the ratelimiting step in isoflavone absorption, and  $\alpha$ -glucosidase activity has been claimed as relevant in relation to isoflavone bioavailability [74], [75].

*Lactobacilli* are also known to produce  $\alpha$ -glucosidase activity [76].

$\alpha$ -Glucosidase-producing probiotic *lactobacilli* may contribute to the release of the aglycone from several glucoconjugates phytochemicals, thus improving their bioavailability. Nonetheless, the genus *Lactobacillus* has never been investigated for the hydrolysis of glucoconjugates other than the ones of soy isoflavones [64].

Rhamnosidases from *L. acidophilus* and *L. plantarum* efficiently hydrolyzed rutinoides and neohesperidosides of flavonols and flavanones such as rutin, hesperidin, and naringin. Thus, probiotic strains of *L. acidophilus* and *L. plantarum* may contribute to the release of quercetin, hesperetin, and naringenin (from rutin, hesperidin, and naringin, respectively), which are among the most abundant flavonoids occurring in plant foods [77], [78].

Members of the genus *Bifidobacterium* were demonstrated to produce enzyme activities that could potentially participate to the metabolism of ginsenosides through the removal of diverse sugar moieties. In particular,  $\alpha$ -arabinopyranosidase,  $\alpha$ -xylosidase, and  $\alpha$ -glucosidase activities, capable of removing diverse arabinose, xylose, and glucose moieties from ginsenosides, were found and characterized in some strains of *Bifidobacterium*, such as *B. breve* K-110 and a strain designated *Bifidobacterium cholerium* K-103 [79], [80].

Bacterial species belonging to the genera *Lactobacillus* and *Bifidobacterium* were found to be capable of producing esterase activity, hydrolyzing chlorogenic acid, and releasing caffeic acid, a hydroxycinnamic acid with antioxidant properties, which is much more easily digested in the gut [81], [82].

### Phytochemical profiling "phytoprofilng" and metabolic phenotyping "metabotyping"

Phytoprofilng is now one of the most promising approaches to investigate the bioactive components beneficial to human health and has also been proven to be a valuable analytical tool for the identification of secondary metabolites from medicinal plants, particularly for evidence-based development of new phytotherapeutic agents and nutraceuticals [83]. The phytochemical composition and bioefficacy of a given plant are affected by the geographical origin, climatic conditions and cultural practices [84]. Certain cultivars and locations may be more amicable to the accumulation of phytochemicals than others, and these differences must be accounted for in both nutrition research and food processing. Xie *et al.* have performed an UPLC-QTOFMS-based phytoprofilng study of five medicinal Panax herbs including Panax ginseng (Chinese ginseng), Panax notoginseng (Sanchi), Panax japonicus (Rhizoma Panacis Majoris), Panax quinquefolium L. (American ginseng), and Panax ginseng (Korean ginseng) [85]. PCA of the analytical data showed that the five Panax herbs could be separated into five different groups of phytochemicals. They demonstrated the potential of phytochemical profiling for the rapid differentiation and identification of complex plant extracts that contain similar chemical ingredients as well as the potential for the discrimination of subtle variations within the same plant species or strains due to different geographical locations, cultivation, and collection times.

Baba-Moussa *et al.* reported that, essential oils of different medicinal plants including *Clausena anisata*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus camaldulensis*, *Eucalyptus citriodora*, *Lantana camara*, *Lippia multiflora*, *Melaleuca quinquenervia*, *Mentha piperita*, *Ocimum basilicum*, *Ocimum gratissimum*, *Citrus aurantium* and *Xylopi aethiopia* were extracted and tested for their antimicrobial properties against various human infectious pathogenic agents. The essential oils were tested against 5 pathogenic strains (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*) using the agar diffusion and microdilution methods. *Ocimum gratissimum* essential oil was found to have the strongest antimicrobial effect with the inhibition diameter on *Staphylococcus aureus*, while *Lippia multiflora* essential oil was found to have the least antimicrobial effect on *Enterococcus faecalis*. The chromatographic derived phytochemical profiling analysis showed thymol, 1,8-cineole, sabinene, citral, limonene and estragole to be the major components of the essential oils [86].

Kim *et al.* have reported NMR spectroscopy-based phytoprofilng applied to the classification of 11 South American *Ilex* species. PCA, partial least squares-discriminant analysis (PLS-DA) and hierarchical cluster analysis (HCA) of the NMR spectral data were able to differentiate and group the species based on metabolic similarities [87].

In the GC-MS analysis of *Sauropus androgynus* extract the result shows the presence of bioactive compounds which revealed a broad spectrum of many medicinal property and antioxidant activity were identified. The functional group present in these compounds was identified by IR spectral

analysis. This study also helped to identify the formula and structure of biomolecules, which can be used as drugs [88].

In a recent study, an UPLC-QTOFMS-based phytoprofilng was performed on Pu-erh tea, a famous fermented tea produced mainly in the Yunnan province of China. The study revealed that unfermented green tea (Chinese Longjing) was rich in polyphenols and theanine, and Lipton black tea contained more thearubigin (TR) and theaflavic acid, while Pu-erh tea possessed characteristic phytochemicals such as theabrownin (TB, a group of brown color polyphenols) and gallic acid (GA) [89].

The human metabolic system is highly extensive and sophisticated, and the functional integrity of human physiology (homeostasis) ultimately reflected in the phenotype depends not only on nucleotide polymorphism, [90] but also on external factors such as environmental and behavioral influence, and even other genomes from symbiotic organisms such as gut microbiota [91].

Metabonomics, [92] or metabolomics, [93] provides a comprehensive profiling of metabolites in biofluids and tissues and their systematic and temporal changes mainly by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) coupled with multivariate or univariate data analysis [93], [94]

Metabonomic strategies together with advanced chemometric and bioinformatic tools [95], [96] can help track the interaction between phytochemicals and human metabolism, as well as the involvement of the genome and the gut microbiome, in overall human health, and can be considered critical measures of function or phenotype [97].

Metabonomics/metabolomics has been identified as a promising approach to assess nutrients and functional phytochemicals via simultaneous detection of multiple metabolic endpoints in the complex metabolic regulatory system.

Llorach *et al.* have applied an LC-MS-based metabonomics approach for exploring urinary metabolic modifications after cocoa consumption. After overnight fasting, 10 subjects consumed randomly either a single dose of cocoa powder with milk or water, or milk without cocoa. Urine samples collected at baseline and at 0-6, 6-12, and 12-24-h post-cocoa-consumption were analyzed, which revealed an important effect of cocoa intake on urinary metabolome. The metabolomic changes are characterized by 27 metabolites including alkaloid derivatives, both host and microbial metabolites polyphenols and processing-derived products such as diketopiperazines [98].

Xie *et al.* determined the metabolic fate of polyphenolic components in Pu-erh tea in human subjects. Urine samples were collected at 0, 1, 3, 6, 9, 12, and 24 h within the first 24 h of tea intake and once a day during a 2-week daily Pu-erh tea ingestion phase and a two-week "wash-out" phase. The dynamic concentration profile of bioavailable plant molecules (due to in vivo absorption and the hepatic and gut bacterial metabolism) and the human metabolic response profile were

identified and correlated with each other, highlighting the great potential of metabonomic strategy to unravel the complex interactions between multicomponent nutraceuticals and human metabolic system in nutritional studies [99].

The two profiling strategies, metabolic phenotyping (metabotyping) and phytochemical profiling (phytoprofilng), greatly facilitate the measurement of important health determinants and the discovery of new biomarkers associated with nutritional requirements and specific phytochemical interventions.

## CONCLUSIONS AND FUTURE PERSPECTIVES

The gut microbiota exerts an enormous impact on the nutritional and health status of the host via modulation of the immune and metabolic functions. The microbiome provides additional enzymatic activities involved in the transformation of dietary compounds. Food bioactive compounds also exert significant effects on the intestinal environment, modulating the gut microbiota composition and probably its functional effects on mammalian tissues.

Enzymes that assure greater levels of digestion and absorption of food, and probiotic bacteria that keep problems in check, can make a huge difference in one's own health.

This review provides a picture of the potentiality of probiotics to take part in improving the bioavailability and/or biological activity of natural phytochemicals.

The utilization of probiotic strains, selected for the hydrolysis and activation of specific phytochemicals, needs substantial efforts to be validated.

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