JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Review

pubs.acs.org/JAFC

Potential Impact of Probiotic Consumption on the Bioactivity of Dietary Phytochemicals

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ABSTRACT: Many healthy phytochemicals occur in food in the form of esters, glycoconjugates, or polymers, which are not directly bioavailable. Probiotic lactobacilli and bifidobacteria, which have evolved within the colonic ecosystem where indigestible oligo- and polysaccharides are their sole carbon sources, bear several glycosyl-hydrolases and can contribute to release the aglycones from glycoconjugated phytochemicals. Among the glycosyl-hydrolases, β-glucosidases are the most pertinent, because many phytochemicals are glucoconjugates. β-Glucosidase-positive probiotic bacteria were proved to release the aglycones of isoflavones and lignans in vitro, but studies in vivo are scarce. A positive correlation between probiotic consumption and urinary and/or plasma levels of isoflavone or lignan metabolites was not established. However, the strains used in the trials were not validated for the enzymatic properties or for the ability to hydrolyze lignans or isoflavones. Thus, activation of specific phytochemicals by probiotic bacteria still needs substantial efforts to be proved.

KEYWORDS: *phytochemicals, probiotics, Lactobacillus, Bifidobacterium, microbiota, isoflavones, lignans*

INTRODUCTION

Edible plants are dietary sources of hundreds of non-nutritional phytochemicals. Many phytochemicals exert a number of beneficial activities, including antioxidant, antitumoral, anti-inflammatory, and estrogenic-like properties, as demonstrated by numerous epidemiologic, clinical, and experimental studies.[1−3]

The level of bioactive phytochemicals within the body is largely determined by diverse phenomena, such as the digestive transformation of native compounds, absorption in the intestine, hepatic activity, and biliary or urinary excretion.[4,5] The phytochemicals that are not absorbed in the small intestine reach the colon, where they may undergo extensive biotransformation by the resident microbiota.[5−7] This bacterial transformation may lead to the inactivation and/or degradation of phytochemicals or may cause the production of compounds with enhanced biological activity or bioavailability. Examples of the specific conversion of diverse molecules into bioactive metabolites accomplished by the microbiota are the conversion of lignans into enterodiol and enterolactone (ED and EL, respectively) and the conversion of soy isoflavones into *S*-equol.[8−11] During the course of absorption, phytochemicals are conjugated in the intestine and later in the liver, being subjected to methylation, sulfation, and β-glucuronidation. Mammalian conjugates can be secreted in the duodenum with the bile as hydrophilic conjugates or can be effluxed from the enterocytes directly to the gut lumen. Furthermore, the colonic microbiota is involved in the enterohepatic recycling of phytochemicals. In fact, the microbial β-glucuronidase and sulfatases can deconjugate the excreted phytochemicals in the colon, where they can be reabsorbed, leading to a longer presence in the body.[5,12]

Bacterial transformations of phytochemicals that reach the colon modify their absorption and bioavailability. For instance, in vivo studies demonstrated that a diverse availability of bioactive compounds generally occurs among different subjects, this interindividual variation being mainly attributed to differences in the composition of the gut microbiota.[13−17] This review provides an update on current advances on the impact of probiotic consumption on metabolism and bioavailability of phytochemicals. It addresses the genetic and enzymatic features of probiotic bacteria potentially involved in the transformation of phytochemicals and the outcome of in vivo trials carried out with associations of probiotics and phytochemicals.

GUT MICROBIOTA AND PROBIOTICS

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.[11] 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.[18,19] The colonic microbiota is maintained in a constant-temperature environment and is provided with a broad spectrum of substrates undigested and unabsorbed.[18,19] 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.[20,21]

For its energy needs, the bacterial community exploits unabsorbed oligo- and polysaccharides, proteins, and peptides. They are broken down by bacterial enzymes into their

Received: June 25, 2013
Revised: August 27, 2013
Accepted: September 5, 2013
Published: September 5, 2013

oligomeric and/or monomeric components, which are fermented, yielding organic acids (such as lactic, acetic, propionic, and butyric acids), branched-chain fatty acids (such as isobutyric, isovaleric, and 2-methylbutyric acids), H_2, CO_2, ammonia, amines, and several other end-products.[18,19] Short-chain fatty acids (SCFA) are, from a nutritional point of view, the major fermentation products. They affect the metabolism, growth, and differentiation of colonocytes, influence the hepatic control of lipids and carbohydrates, and provide the muscles, kidneys, heart, and brain with energy.[18]

The use of probiotics to modulate the activity and composition of the gut microbiota to improve the health status is consolidated. They are defined as "live microbes which when administered in adequate amounts confer a health benefit to the host".[22,23] Even though probiotic microorganisms were acknowledged within different phyla of bacteria and yeasts, the majority of probiotics in use today are bacteria belonging to species of *Lactobacillus* and *Bifidobacterium* naturally colonizing the human colon.[24]

Bifidobacterium is a genus of high G+C (gunosine + 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*.[25] Bifidobacteria are abundant gut colonizers and one of the most important health-promoting groups within the colonic microbiota and are largely used as probiotics.[26] 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.[26−30]

The genus *Lactobacillus* includes almost 200 recognized species of low G+C Gram-positive bacteria within the phylum of Firmicutes.[31] 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_2), or facultative heterofermentative.[31]

Lactobacilli occur in a variety of habitats where carbohydrate-based substrates are available. They inhabit plants, plant-derived matrices, and fermented foods (such as dairy products and fermented dough, milk, vegetables, and meats) and are found in the commensal microbiota naturally colonizing diverse niches within the body of humans and animals. In particular, several species are endogenous members of the resident microbiota of the hindgut. Many commensal lactobacilli have been proven to exert a number of beneficial health effects and have attracted considerable attention as candidates for the development of probiotics, although the molecular mechanisms behind these beneficial properties are still under investigation.[32−35] 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*.[24,34]

Although at first probiotics were added to foods or consumed as pharmaceutical products to improve the gut microbiota balance, nowadays specific health effects of probiotics have been demonstrated, even though the molecular mechanisms remain largely unknown.[26,34,35] In fact, 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.[36−39] 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.

BIOAVAILABILITY OF NATURALLY OCCURRING CONJUGATED PHYTOCHEMICALS

Many phytochemicals with relevant interest for human health are present in food in the form of esters, glycoconjugates, or polymers, which are not directly bioavailable. The chemical form in which they occur is important because it influences the bioavailability, the biological activity, and, therefore, the physiological effects.[10,40,41] Polyphenols, in particular, are frequently bound to hydrophilic moieties and are generally too polar to be absorbed through passive diffusion by enterocytes in the small intestine.[42] With only a few exceptions, polyphenols bound to sugar moieties cannot be absorbed in their native form and require hydrolysis to their corresponding aglycones.[43]

Isoflavones, which occur abundantly in cotyledons and hypocotyls of soybeans and soy-derived foods, are found as aglycones (daidzein, genistein, and glycitein), glucosides (daidzin, genistin, and glycitin), acetylglucosides (acetyldaidzin, acetylgenistin, and acetylglycitin), and malonylglucosides (malonyldaidzin, malonylgenistin, and malonylglycitin).[5] Due to the lack of active transporters in the intestinal epithelium, both isoflavone glycosides and aglycones are absorbed only via passive diffusion and have poor oral bioavailability.[44] However, isoflavone glycosides are too polar and present lower permeability through the intestinal epithelial membrane, if any, than the corresponding aglycones.[40,45,46]

Likewise, the sugar moiety of glycoconjugated flavonols is a major determinant affecting absorption in the small intestine. Only glucose-conjugated quercetin is rapidly absorbed in the small intestine, probably through a sodium-dependent glucose transporter, whereas the other glycoconjugates (such as rutinose, rhamnose, galactose, arabinose, xylose, and glucuronic acid conjugates) are not.[5,47]

Lignans are involved in plant cell wall formation and ubiquitously occur in many plants. They are found especially in flaxseeds, berries, rye, and a wide range of seeds, fruits, and vegetables.[5,48,49] They can be transformed by colonic microbiota into metabolites that present antioxidative, antiproliferative, antiestrogenic, and antiangiogenic properties. Secoisolariciresinol (SECO), the major dietary lignan, is mostly found as

secoisolariciresinol diglucoside (SDG), which is ester-linked with 3-hydroxy-3-methylglutaric acid and other phenolic compounds (e.g., *p*-coumaric and/or ferulic acid glycosides) in the form of SDG oligomers.[50,51] SDG and the other lignans are not active in the forms as they occur in plants, and their beneficial effects greatly depend on bioconversion into the aglycons and further reactions.[52,53]

Hydroxycinnamic acids (e.g., cinnamic, coumaric, caffeic, and ferulic acids) are one of the major classes of dietary phenolic compounds, occurring in a number of fruits, vegetables, and grains.[5,54] They may be found in foods as free carboxylic acids, esterified with flavonoids, carbohydrates, and quinic and tartaric acid, and, to a minor extent, as amides (with amines or amino acids). Free hydroxycinnamic acids are promptly absorbed through the intestine via both passive and active mechanisms, whereas esters (such as chlorogenic acid) present markedly reduced absorption rates.[54]

GENETIC AND ENZYMATIC CHARACTERS OF PROBIOTIC BACTERIA INVOLVED IN TRANSFORMATIONS OF 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. From this perspective, information concerning the potential interaction of probiotic bacteria with the dietary compounds is of great interest.

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, β-glucosidases (EC 3.2.1.21) are the most pertinent for the release of the aglycones, because, as formerly described, many phytochemicals are glucoconjugates. In particular, the initial hydrolysis of soy isoflavone glucosides to their respective aglycones is the rate-limiting step in isoflavone absorption, and β-glucosidase activity has been claimed as relevant in relation to isoflavone bioavailability.[45,55−57]

Bifidobacteria are known to produce β-glucosidases. The analysis of *Bifidobacterium* genome and nucleotide sequences for predictable β-glucosidases suggests that *B. adolescentis*, *B. animalis*, *B. bifidum*, *B. breve*, *B. longum* subsp. *infantis*, *B. longum* subsp. *longum*, and *B. pseudocatenulatum* possess two to nine genes encoding β-glu with cytoplasmic or membrane location (http://www.ncbi.nlm.nih.gov/gene; http://www.cazy.org; http://www.cbs.dtu.dk/services/SignalP). The production of β-glucosidases by *B. animalis*, *B. adolescentis*, *B. catenulatum*, *B. pseudocatenulatum*, *B. breve*, and *B. infantis* was correlated to the hydrolysis of glucoconjugate forms of phytochemicals, even though a wide diversity in the activity was found among species and strains and a quantitative relationship with hydrolysis yield could not be established.[58−61] As a matter of fact, cultures of selected members of the genus *Bifidobacterium* are capable of hydrolyzing the glucose moiety from glucoconjugates of isoflavones, flavonols, and lignans.[59,61,62] In particular, β-glucosidase-positive bifidobacteria were active in the hydrolysis of daidzin, genistin, glycitin, kaempferol 3-*O*-glucoside, and SDG into their aglyconic forms.[58,59,61,62]

Lactobacilli are also known to produce β-glucosidase activity.[13,56,63] The analysis of genome and nucleotide sequences of the main probiotic species of *Lactobacillus* revealed that *L. reuteri* and *L. salivarius* lack any sequence annotated as β-glucosidase (http://www.ncbi.nlm.nih.gov/gene; http://www.cazy.org; http://www.cbs.dtu.dk/services/SignalP). Otherwise, a variable number of sequences encoding cytosolic β-glucosidases was found in the genomes of *L. acidophilus* (5−8 sequences), *L. casei* and *L. paracasei* (4−6), *L. plantarum* (9−11), and *L. rhamnosus* (6−8). β-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.

Up to now, the ability of diverse species of bifidobacteria and lactobacilli to produce β-glucosidase has been mostly applied in food technologies for the production of fermented soy-based foods, enriched in soy isoflavones, as described in several studies.[10,41,58,64−71]

Apart from β-glucosidase, bifidobacteria and lactobacilli are known to produce a number of glycolytic activities that may be potentially involved in phytochemicals activation. However, information on the hydrolysis of conjugated phytochemicals by enzymes other than β-glucosidases is still very scarce and deserves deeper investigation. Rhamnosidases from *L. acidophilus* and *L. plantarum* efficiently hydrolyzed rutinosides and neohesperidosides of flavonols and flavanones such as rutin, hesperidin, and naringin.[72,73] 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. No information is available in the literature about the capacity of *Bifidobacterium* species to hydrolyze the rutinosides rutin and hesperidin. Preliminary results indicate that no bifidobacteria are able to convert rutin into its aglycone quercetin, and only *B. pseudocatenulatum* could transform hesperidin into its aglycone hesperetin to some extent (unpublished data). 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, α-arabinopyranosidase, β-xylosidase, and β-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.[74−77]

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 absorbed in the gut.[54,78−81] Lactobacilli capable of performing this transformation were found within the species *L. johnsonii* and *L. gasseri*.[82,83] Among the most common species of probiotic bifidobacteria, the hydrolysis of chlorogenic acid was found only in *B. animalis* subsp. *lactis* and *B. animalis* subsp. *animalis*.[16,83]

SOY ISOFLAVONES AND LIGNANS: TRANSFORMATIONS BY THE GUT MICROBIOTA AND IN VIVO EFFECTS OF PROBIOTICS ON BIOAVAILABILITY

Soy isoflavones and lignans are emblematic examples of phytochemicals that are subjected to bacterial transformations yielding molecules with enhanced biological activity. In fact, colonic bacteria are responsible for the transformation of these molecules into very effective phytoestrogens, which mimic the action of estrogens on target receptors and exert many health benefits against hormone-dependent diseases such as protection against breast and prostate cancers, prevention and treatment of osteoporosis, lowering of hematic cholesterol and lipids, cardiovascular protection, antioxidant activity, and alleviation of menopausal symptoms.[84−86] Specific bacterial groups were demonstrated to be responsible for definite reactions in the biotransformation route of soy isoflavones and lignans toward active phytoestrogens.

One of the major soy isoflavones is daidzein. It is mostly found in the glucoconjugated form daidzin, which is poorly absorbed in the intestine and undergoes extensive transformation in the colon. The first step of daidzin activation is the hydrolysis and the release of the aglycone daidzein, mainly carried out by hydrolytic enzymes from colonic bacteria, such as lactobacilli, bifidobacteria, coliforms, and *Bacteroides*.[15,40,52,87,88] Daidzein can be absorbed through the gut epithelium; otherwise, it can be further transformed by intestinal bacteria into a variety of metabolites with improved or decreased biological activity.[10,11,89,90] In particular, a bacterial reductive pathway could yield *S*-equol, which is the most effective in stimulating an estrogenic response among the isoflavone derivatives (Figure 1). This pathway proceeds with the

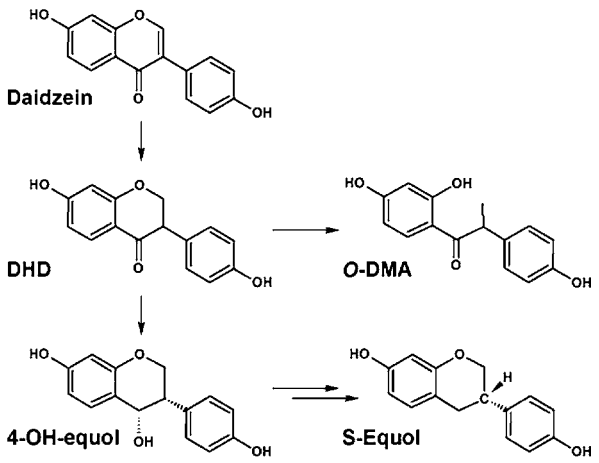

Figure 1. Intestinal bacterial metabolism of the isoflavone daidzein to *O*-desmethylangolensin (*O*-DMA) and *S*-equol.

hydrogenation of daidzein to dihydrodaidzein, carried out by several *Clostridium*-like strains.[11] Dihydrodaidzein constitutes a branch point of two divergent routes of bacterial transformation. One route gives *O*-desmethylangolensin (*O*-DMA), originated by C-ring cleavage, which primes the breakdown of the molecule. The other route proceeds through keto group reduction to 4-hydroxyequol, followed either by reductive rearrangement or by dehydration and subsequent reduction, yielding *S*-equol as the end-product.[89] The role of colonic bacteria in both pathways is well documented, and the composition of the microbiota is responsible for interindividual differences in the capacity to produce *S*-equol and *O*-DMA. In fact, production of *S*-equol occurs only in 30−40% of people, whereas approximately 80−90% can produce *O*-DMA. The species *Eubacterium ramulus*, belonging to *Clostridium* cluster XIVa, can accomplish the C-ring cleavage and is regarded as one of the major isoflavones degrading bacteria in the human gastrointestinal tract.[15] On the other hand, nearly all of the equol-producing bacterial isolates, such as those belonging to the genera *Eggerthella*, *Slackia*, and *Adelcreutzia*, have been classified in the family of Coriobacteriaceae within the high G +C content Gram-positive Actinobacteria.[90−96]

The activation of lignans also depends on the release of the aglycone followed by other bacterial reactions (Figure 2). The

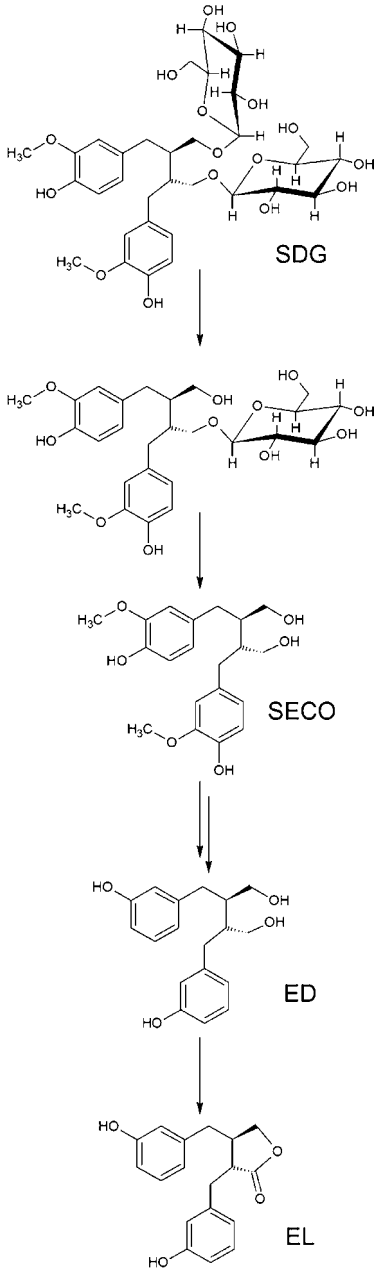

Figure 2. Intestinal bacterial metabolism of secoisolariciresinol diglucoside (SDG) to secoisolariciresinol monoglucoside (SMG), secoisolariciresinol (SECO), enterodiol (ED), and enterolactone (EL).

dx.doi.org/10.1021/jf402722m | *J. Agric. Food Chem.* 2013, 61, 9551−9558

release of SECO from SDG occurs in two steps, with the consecutive removal of the two glucose moieties by bacterial β-glucosidases.[51,62] Bacteriodetes, Clostridiales, and the novel species *Clostridium saccharogumia* are involved in glycoconjugate hydrolysis with release of SECO.[97] Then SECO is further transformed into ED and EL, which exert estrogen-dependent and -independent activity. Production of ED requires demethylation followed by dehydroxylation.[9,98] The species *Peptostreptococcus productus* seems crucial for SECO demethylation, but bacterial isolates capable of this reaction were identified also within other Clostridiales (e.g., *Eubacterium limosum*, *Eubacterium callanderi*, and *Butyribacterium methylotrophicum*); dehydroxylation to ED is a common feature of the species *Eggerthella lenta* within Actinobacteria, but is performed by some Clostridiales as well (e.g., *Clostridium scindens*).[9] The dehydrogenation converting ED into EL is carried out by subdominant species, such as *Lactonifactor longoviformis* in the phylum of Clostridiales, occurring at lower concentrations (in the magnitude order of 10^5 bacteria/g of intestinal content).[9]

For both *S*-equol and ED/EL production, the composition of the gut microbiota is at the basis of interindividual differences in the ability to produce these metabolites.[9,11,15] From this perspective, the impact of probiotic consumption on the transformation of isoflavones and lignans into *S*-equol and ED/EL is of great interest.

The effect of probiotic consumption on the bioavailability of active metabolites of isoflavones and lignans has been investigated. The rationale of these studies always rested on the β-glucosidase activity ascribed to probiotic strains, in agreement with experimental data that consumption of probiotics alters fecal enzymatic activities both in animals and in humans.[99] It has been hypothesized that an increase of β-glucosidase activity, due to probiotic consumption, could improve the aglycone release and increase the flux toward other reactions yielding the bioactive compounds. It is necessary to highlight that probiotic lactobacilli and bifidobacteria seem to be involved only in deconjugation reactions, whereas they do not take part in the reactions transforming the aglycones daidzein and SECO into *S*-equol and ED/EL, respectively.

A few in vivo trials determined whether the consumption of probiotics together with soy-based supplements might improve the bioavailability of active metabolites, enhancing the intestinal absorption and enteroepathic recirculation. In these trials, the subjects were fed soy proteins or soy foodstuffs and treated for 5 weeks with a probiotic yogurt containing 10^8 cfu/100 g daily serving of each of *Lactobacillus* GG, *L. acidophilus*, and *Bifidobacterium bifidus*,[57] for 6 weeks with 3 capsules/day containing 10^9 cfu of *L. acidophilus* DDS+1 and *B. longum* and 15−30 mg of fructooligosaccharide,[100] or for a month with a high load (at least 8×10^{10} cfu/day) of *Lactobacillus* GG.[96] None of these studies established a positive correlation between probiotic consumption and urinary and/or plasma isoflavone metabolite concentrations. In particular, probiotic supplementation did not significantly modify the levels of plasma and urinary isoflavone metabolites, or even negatively affected them.[57,100,101] These results suggest that the transient colonization by different probiotic bacteria can alter the overall microbiota composition and its metabolic activities in a manner that can be hardly predicted and may differ among individuals. Interestingly, in the trial carried out with the highest load of probiotics, levels of isoflavone metabolites were lower than control.[101] Furthermore, although the effect of probiotic consumption did not significantly affect the bioavailability of isoflavone metabolites, in a few subjects their concentration increased or decreased by approximately 9- and 7-fold, respectively, assessing a large interindividual variability in terms of response to the same probiotic formulation.[100]

The effect of probiotic consumption, alone and together with galacto-oligosaccharides, on EL level has been investigated.[102] The probiotic formulation contained a total amount of 2×10^{10} cfu/day of *Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freudenreichii* ssp. *shermanii* JS, and *B. breve* Bb99 and was associated with a minimum of 120 g/day whole-grain rye bread in addition to the normal diet. Probiotics alone negatively affected the serum EL concentration, whereas the association of probiotics and galacto-oligosaccharides (3.8 g/day) did not determine significant changes. Because this specific combination of strains did not contribute to transformations resulting in EL production, it can be established that the colonization of these probiotic strains likely repressed the growth and metabolic activity of species involved in transformations of lignans. The presence of the prebiotic likely changed the relative amounts of the diverse microbial groups, giving different results than the probiotic alone. Furthermore, these results are consistent with previous evidence that excluded the capability of any of the strains combined into the probiotic preparation to transform the plant lignan 7-hydroxymatairesinol.[103]

DISCUSSION

The food industry continually offers innovative products that satisfy consumer needs and, in some cases, actually persuade the consumer that they have a need. Functional foods containing probiotic microorganisms with scientifically supported health claims for improving the state of well-being and reducing the risk of diseases already constitute a growing market. Because the metabolism of the phytochemicals in the colon is influenced by many factors of the gut environment, exploitation of probiotic bacteria to modify the bioavailability of bioactive compounds can provide new perspectives for nutraceutics.

The in vivo studies herein discussed were based on the assumption that aglycone release could be accelerated by β-glucosidase activity of the probiotic strains. Because the probiotic strains exploited for these trials belong to species that bear genes encoding β-glucosidase, it is conceivable that they produce the enzyme, even though data about activity are not available. However, the presence of several different β-glucosidases in both lactobacilli and bifidobacteria does not ensure the capability of the corresponding enzymes to carry out the hydrolysis reaction of the diverse glycosylated phytochemicals. In particular, steric hindrance can result as a main determinant of substrate reactivity. Enzyme location is probably another aspect that is potentially involved in the difficulties encountered by these probiotic bacteria to hydrolyze the conjugated forms. None of the β-glucosidases annotated in the genome of bifidobacteria and lactobacilli are predicted to be extracellular. Hence, membrane transporters are likely involved in making the substrate available to cytosolic β-glucosidase. Consistently, the inability of certain β-glucosidase-positive bifidobacterial strains to hydrolyze SDG was ascribed to the lack of a transport system enabling SDG to enter the cell and encounter cytosolic enzyme.[62] Similar information is not available for lactobacilli, for which an extensive investigation deserves to be accomplished to conclusively assess their

potential role in aglycone release. In addition, the gut microbiota has intrinsically very high hydrolytic activities,[19,104] and it cannot be excluded that the contribution of the probiotic strains, if any, would be inconsistent.

This overview provides a picture of the potentiality of probiotic bifidobacteria and lactobacilli to take part in the release of aglycones, improving the levels of bioactive metabolites of phytochemicals. However, the outcomes of the few studies carried out administering associations of probiotics and phytochemicals are discouraging. Attention should be given to the evidence that the proposed probiotic strains have not been validated for the major genetic and enzymatic features that can affect phytochemical metabolism. The utilization of probiotic strains, selected for the hydrolysis and activation of specific phytochemicals, needs substantial efforts to be validated.

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Notes

The authors declare no competing financial interest.

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