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Biotransformation of Common Bean (*Phaseolus vulgaris* L.) Flavonoid Glycosides by *Bifidobacterium* Species from Human Intestinal Origin

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Bifidobacteria strains from human origin were screened for the specific activity (β-glucosidase activity) involved in the metabolism of dietary flavonoids. Five strains with high β-glucosidase activity were selected for further metabolism analyses (high-performance liquid chromatography separations) of flavonoid glycosides occurring in *Phaseolus vulgaris* L. (common bean) seeds and seedlings. All selected strains were found to be active in the conversion of kaempferol 3-*O*-glucoside, daidzin, genistin, and glycitin into their aglyconic forms. No metabolites were detected after the fermentation tests with the diglucosidic compound kaempferol 3-*O*-xylosylglucoside. In addition, to verify the effective bioavailability of flavonoid aglycones, the degradation rates of daidzein, genistein, glycitein, and kaempferol, following incubation with selected strains, were monitored. The results showed that the five selected strains of bifidobacteria, being active in the biotranformation of flavonoid glycosides occurring in common bean seeds and seedlings, could be considered as probiotic dietary adjuncts to improve the nutritional and health properties of flavonoid-based products, comprising hypothetical common bean food derivatives.

KEYWORDS: **β-Glucosidase activity; bifidobacteria; common bean; isoflavones; kaempferol; seed; seedling**

INTRODUCTION

Plants not only form the basis of the human food chain but they are also an important means to improve human health and well-being. Flavonoids are biologically active polyphenolic compounds found in plants and present in plant-derived foods that are intrinsic components of human diets (*1*, *2*). A large body of literature exists regarding flavonoid content in various plant foods. Among these, soy foods have been investigated intensively during the past 5−10 years, especially because soybean is considered the main dietary source of a group of flavonoids called isoflavones, exhibiting a wide range of biological activities (*3*). However, many other legumes have been reported to contain flavonoids as well, even if in concentration 6−10-fold lower than the isoflavone amounts generally found in soybeans. As an example, several glycosidic forms of quercetin and kaempferol have been identified in seeds of common bean (*Phaseolus vulgaris* L.) (*1*, *4*−*8*). In addition, earlier studies by our group showed that some Italian common bean ecotypes contained three glycosidic forms of the flavonoid kaempferol at the seed level (*9*), and conspicuous amounts of isoflavones phytoestrogens (daidzein, genistein, and glycitein), along with kaempferol, were detected in acid-hydrolyzed extracts of 2−3-day-old seedlings (*10*).

Flavonoids are found in foods mainly as *O*-glycosides. Glucose is the most common sugar moiety, but other glycosidic units can include galactose, rhamnose, arabinose, and xylose. The *O*-β-glucosidic bonds of flavonoids including the isoflavone daidzin (daidzein-7-*O*-β-D-glucopyranoside), genistin, and glycitin are hydrolyzed in the gut by microbial and mammalian β-glucosidases to their aglucons, daidzein, genistein, and glycitein, respectively (*11*−*15*).

An increasing number of scientific papers point to the importance of the intestinal microbiota in the degradation and bioavailability of dietary flavonoids (*16*).

Several groups of bacteria are known to possess β-glucosidase activity, including bifidobacteria (*17*), which are a major component of the human gastrointestinal tract (*18*). So far, certain strains of *Bacteroides* (*19*), *Clostridium* (*20*−*22*), *Eubacterium* (*13*, *20*), and *Bifidobacterium* (*23*) are known to convert flavonoids into the bioactive aglycones. However, many of those studies focused mainly on the bacterial deconjugation capabilities of soybean isoflavones. To the contrary, to our best knowledge, no studies have been performed about the conversion of flavonoids commonly found in legumes other than soybean such as the common bean.

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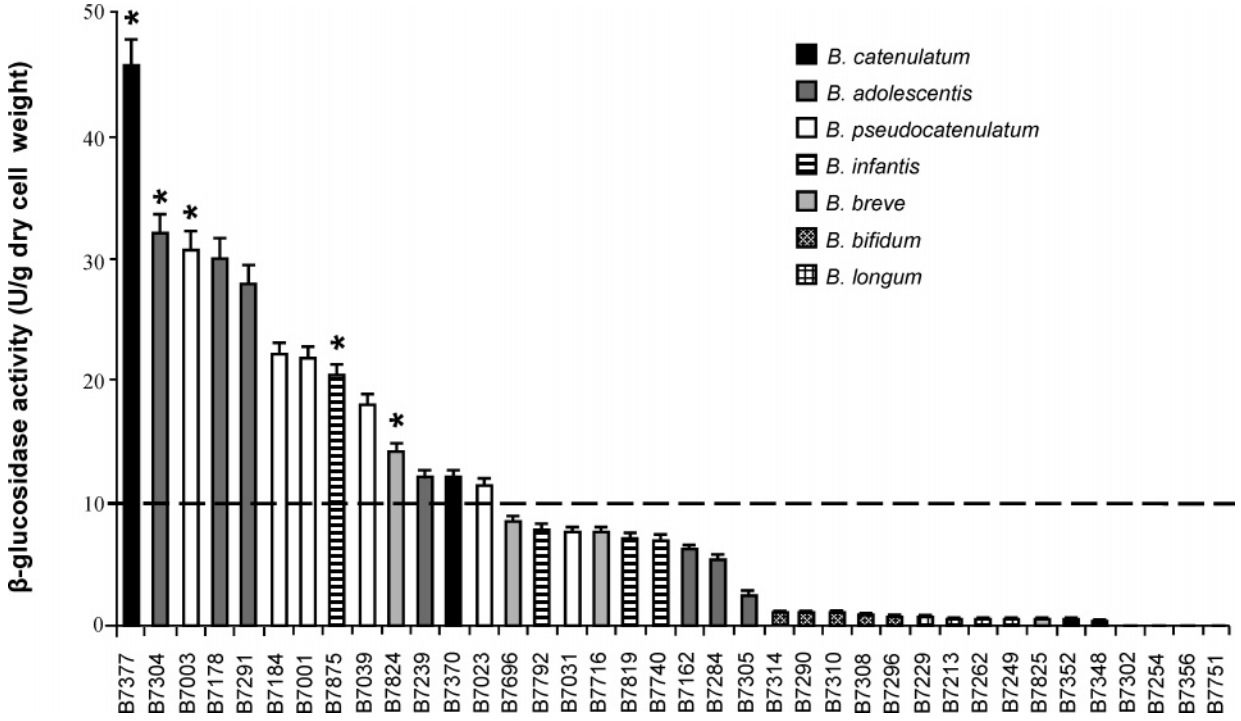

Figure 1. β-Glucosidase activity of tested *Bifidobacterium* spp. strains expressed as unit (U) of enzyme (nmol of *p*-nitrophenol/min) per gram of dry cell weight. Error bars represent standard deviation from the mean of six replicates. Dotted line indicates the threshold limit (10.0 U/g of dry cell weight) arbitrarily chosen for the selection of strains employed in further investigations (asterisks).

The objectives of this study were to screen strains of *Bifidobacterium* spp. from human origin for β-glucosidase activity and to examine their ability to convert the flavonoid glycosides, previously identified in Italian common bean ecotypes (*9*, *10*), to bioactive aglycones.

MATERIALS AND METHODS

Chemicals. Authentic standards of kaempferol 3-*O*-glucoside and kaempferol were purchased by Indofine Co. (Hillsborough, NJ). Kaempferol 3-*O*-xylosylglucoside standard was kindly provided by the Department of Pharmaceutical Science, University of Ferrara, Italy (Vincenzo Brandolini). Isoflavone glycosides (daidzin, genistin, glycitin) and aglycones (daidzein, genistein, glycitein) were from Extrasynthese (Genay, France). Methanol, hydrochloric acid, acetic acid, and all solvents used for high-performance liquid chromatography (HPLC) separations were of analytical or HPLC grade from Carlo Erba, Milan, Italy.

Bacteria and Culture Conditions. The *Bifidobacterium* strains *B. longum* (B7213, B7249, B7254, B7262, B7229), *B. catenulatum* (B7348, B7352, B7370, B7356, B7377), *B. pseudocatenulatum* (B7001, B7003, B7023, B7031, B7039, B7184), *B. adolescentis* (B7162, B7239, B7291, B7304, B7305, B7178, B7284), *B. bifidum* (B7290, B7296, B7302, B7308, B7310, B7314), *B. infantis* (B7740, B7751, B7792, B7819, B7875), and *B. breve* (B7696, B7824, B7825, B7716) originated from the collection of the Department of Agroenvironmental Science and Technology, University of Bologna, Italy. Tested strains were obtained from fecal samples of human origin and were identified according to their deoxyribonucleic acid (DNA)−DNA homology characteristics (*18*) and complex carbohydrate fermentation patterns (*24*). Purity of cultures was checked, and strains were stored at −80 °C in 12% (w/v) sterile reconstituted skim milk supplemented with D-glucose (1% w/v), yeast extract (0.05% w/v), and glycerol (40% v/v). Strains were activated by two successive transfers in Trypticase−phytone−yeast extract (TPY) medium (*25*). An inoculum level of 5% (v/v) was used for strain activation, and incubation was carried out anaerobically at 37 °C for 24 h, using Oxoid gas jars and anaerobic gas paks (*26*). Viable cell counts of the strains were determined in duplicate by using the pour plate method on TPY agar and were calculated as colony-forming units (CFU) per milliliter of culture.

Sample Preparation for HPLC. Sep-Pak C18 cartridges (Agilent Technologies, Milan, Italy) were activated by 2 mL of ethyl acetate, 2 mL of methanol, and 2 mL of water. Each supernatant, obtained from bacterial culture grown in the presence or absence of isoflavone and flavonol glycosides, was added to the column, and then the columns were washed with 2 mL of ethyl acetate to collect the metabolites for HPLC analysis. Each extract was vacuum-dried, dissolved in 0.5 mL of 80% methanol solution, and stored at −20 °C until analysis.

***β*-Glucosidase Activity.** All 38 *Bifidobacterium* strains were tested for β-glucosidase activity following the procedure described by Hur et al. (*15*) with minor modifications. In brief a stationary phase (16−18 h), culture of each strain was inoculated (5%, v/v, inoculum optical density at 600 nm was 0.7) into the test tube consisting of 10 mL of TPY medium and in the presence or absence of 400 μg/mL of the substrate *p*-nitrophenyl-β-D-glucopyranoside (pNPG). The optical density at 600 nm (O.D. 600) of the cultures at the end of the incubation was 0.7. For the control, TPY medium was incubated with a 400 μM concentration of the substrate for the same duration. The cultures were centrifugated at 10000*g* for 10 min, and the amount of *p*-nitrophenol (pNP) released in the supernatants was quantified by HPLC separations (see below).

One unit (U) of the enzyme activity was defined as the amount of β-glucosidase that released 1 nmol of pNP from the substrate per minute under the assay conditions. The specific activity for each tested strain was expressed as units of enzyme per gram of dry cell weight. Among the bacterial strains showing a β-glucosidase activity higher than 10 U/g of dry cell weight (threshold arbitrarily chosen), five strains, each representative of a different *Bifidobacterium* species, were selected for further study of the metabolism of flavonoid glycosides.

Standard Flavonoid Fermentations. All standard flavonoids were prepared in methanol/water (80:20, v/v) as individual solutions. The flavonoid glycosides kaempferol 3-*O*-glucoside, kaempferol 3-*O*-xylosylglucoside, daidzin, genistin, and glycitin and the aglycones daidzein, glycitein, genistein, and kaempferol were added to the incubation test tubes for a final concentration of 400 μmol/L and incubated anaerobically at 37 °C for 24 h in the presence and absence of the selected bifidobacteria strains. This concentration did not have any effect on cell growth. The fermentations were performed in duplicate. One milliliter was taken aseptically from each test tube immediately for time 0 and frozen on dry ice. One milliliter aliquots

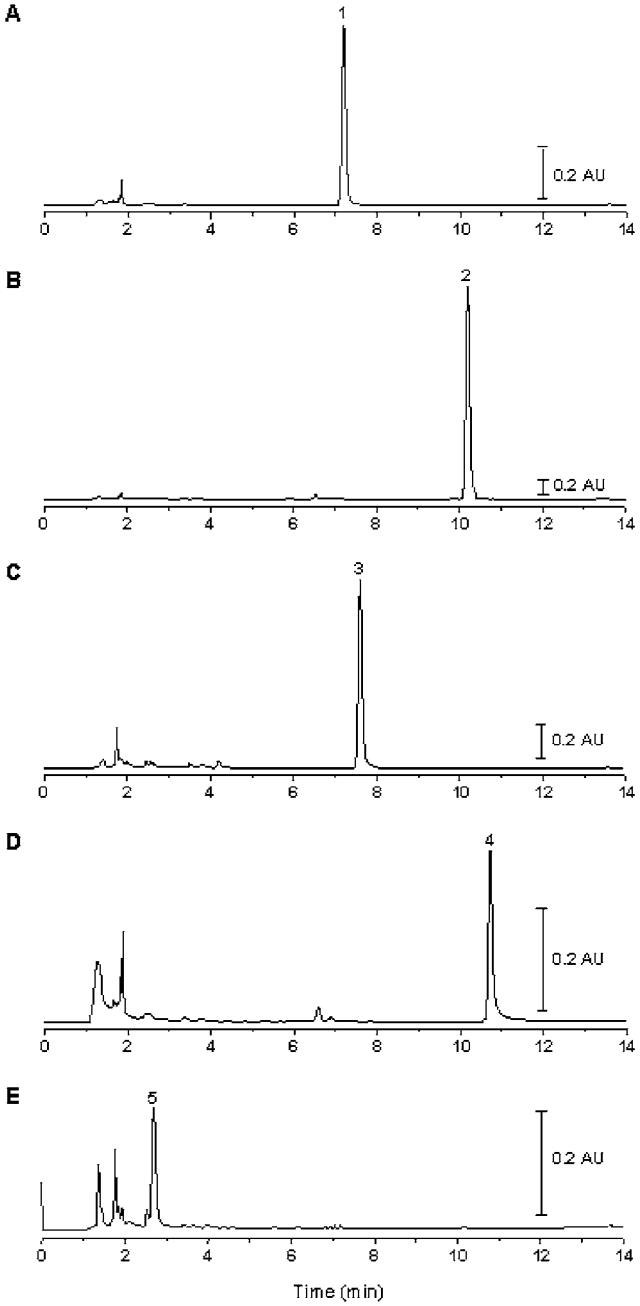

Figure 2. HPLC elution profiles (λ = 260 nm) of the supernatant of growing bifidobacteria cultures (B7003, *B. pseudocatenulatum*) incubated for 24 h with 400 μM daidzin (**A**), genistin (**B**), glycitin (**C**), kaempferol 3-*O* glucoside (**D**), and kaempferol 3-*O*-xylosylglucoside (**E**). Peaks: 1, daidzein; 2, genistein; 3, glycitein; 4, kaempferol; **5**, kaempferol 3-*O*-xylosylglucoside. AU, absorbance units.

were sampled from the incubation test tubes with aglycones at 4, 8, 12, and 24 h and frozen. Negative controls consisted of the bifidobacteria suspension without flavonoids. Microbial degradation by bifidobacteria was confirmed by positive controls, which consisted of TPY medium and flavonoids without inoculum.

Flavonoids from *Phaseolus vulgaris*: Extraction and Fermentations. The Italian *P. vulgaris* landrace "Zolfino del Pratomagno" was employed for fermentation analyses with selected bifidobacteria strains. The choice of this landrace was based on our previous findings, highlighting the presence of relevant flavonoid amounts at the seed and seedling levels (*9*, *10*). In our earlier studies, the flavonoid contents of Zolfino bean seeds and seedlings was assessed and found to be 160 and 13.4 μg/g of fresh weight, respectively. In addition, HPLC analyses highlighted the presence of three flavonoid glycosides (kaempferol 3-*O*-glucoside, kaempferol 3-*O*-xylosylglucoside, and a kaempferol monoglucoside not yet identified) at the seed level, whereas daidzein, genistein, glycitein, and kaempferol were detected in 2−3-day-old seedlings.

Zolfino bean seeds were placed in Petri dishes on moist sterile sand and incubated in the dark at 25 °C in a growth chamber for 2−3 days. Flavonoids were extracted from 500 mg of ground seeds and 3 g of seedlings, following the procedure described by Dinelli et al. (*9*), and added to the incubation tubes. The fermentation tests were performed as described for flavonoid authentic standards.

HPLC Analysis of Metabolites. *p*-Nitrophenol and flavonoids resulting from bifidobacteria metabolism were analyzed by RP-HPLC (Beckman) consisting of a Gold 126 multisolvent pump, photodiode array detector Beckman 168, and a Spark Holland autosampler. For the separations a Waters XTerra MS reverse-phase C18 column (2.1 × 150 mm, 5 μm) operating at 25 °C was employed. For *p*-nitrophenol analyses aqueous 0.1% phosphoric acid (solvent system A) and methanol (solvent system B) served as the mobile phase. The HPLC was run in gradient mode (solvent B from 5 to 30% in 20 min, from 30 to 50% in 5 min, from 50 to 80% in 10 min, and from 80 to 100% in 4 min) at a flow rate of 1 mL/min and detection at 280 nm. In addition, UV spectra were recorded in the range of 200−350 nm. For detection of flavonoids the eluent was composed of (A) H_2O/CH_3COOH (99.9:0.1) and (B) ACN/ H_2O/CH_3COOH (80:19.9:0.1). A two-step linear solvent gradient system was used starting from 20 to 40% of solution B for 14 min at a flow rate of 0.2 mL/min. The percentage of solution B reached 30% from 5 to 9 min and then 40% from 10 to 12 min. The injection volume was 10 μL. UV−vis spectra were recorded in the 200−500 nm range, and the chromatograms were recorded at 260 nm. The limit of detection was defined as the amount of pNP or flavonoids resulting in a peak height 3 times higher than the standard deviation of the baseline noise. Peak identification was based on the comparison of retention time and diode array scans of pure standards. Quantification of pNP and flavonoids was by peak area measurement. Calibration curves of pNP and flavonoid standards were made over a range of 0.5−30 μg/mL. Detector response was linear over the concentration range used. For all standards r^2 was >0.998.

Data Analysis. Assay for β-glucosidase activity, cell dry weight determinations, enumeration of bifidobacteria, and quantification of flavonoids were performed in duplicate and are presented as mean ± standard deviation of six replicates.

The rate of disappearance of flavonoids in the fermentation tubes was estimated by plotting ln(% remaining flavonoid) versus time. The negative slope of this line was the apparent first-order degradation rate constant. A standard curve was employed to estimate the concentration of each flavonoid in the fermentation tubes. Statistical evaluation of degradation rate differences was performed using the Statistica system (version 6, StatSoft Inc., Tulsa, OK). The statistical differences of all analyses was set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

***β*-Glucosidase Activity of Bifidobacteria.** The β-glucosidase activity of 38 bifidobacteria strains was determined using the substrate pNPG. The β-glucosidase activity in TPY medium reached a maximum after 18−24 h of cultivation, which corresponded to the stationary phase of growth based on the O.D. 600 of the cultures (data not shown). As shown in **Figure 1**, besides four strains (B7302, B7254, B7356, B7751) with not detectable β-glucosidase activity, all other tested bifidobacteria showed a wide variability ranging from 0.4 U/g of dry cell weight for B7348 (*B. catenulatum*) to 45.6 U/g of dry cell weight for B7377 (*B. catenulatum*). Significant differences were also observed among the various bifidobacteria species. Although the genus *Bifidobacterium* in general is reported to produce β-glucosidase (*16*), *B. bifidum* and *B. longum* exhibited very low activity under the assay conditions with mean values of 0.8 ± 0.4 and 0.5 ± 0.3 U/g of dry cell weight, respectively.

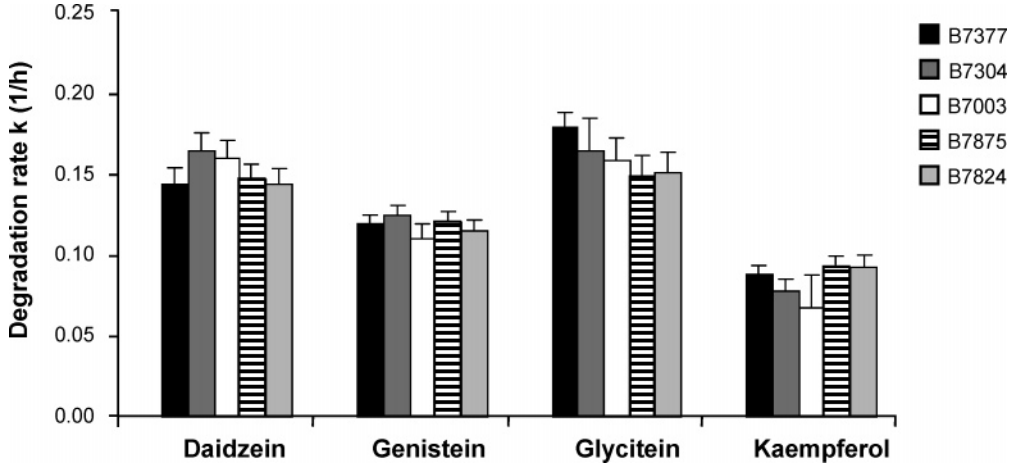

Figure 3. Bifidobacteria degradation rates of daidzein, genistein, glycitein, and kaempferol in selected bifidobacteria strains. Error bars represent standard deviation from the mean of six replicates.

On the contrary, the highest activities were detected in *B. pseudocatenulatum*, *B. adolescentis*, and *B. catenulatum* with mean values of 18.6 ± 7.0, 16.5 ± 12.3, and 12.1 ± 19.7 U/g of dry cell weight, respectively. Intermediate activities were present in *B. infantis* and *B. breve*, with mean values of 8.4 ± 7.4 and 7.2 ± 4.8 U/g of dry cell weight, respectively. These findings are in general agreement with a similar study by Tsangalis et al. (*23*) reporting the high variability in β-glucosidase activity among four different bifidobacteria species (*B. pseudolongum*, *B. longum*, *B. animalis*, and *B. infantis*). However, the abovementioned study highlighted *B. longum* and *B. infantis* as the species with the highest and lowest levels of β-glucosidase activity, respectively. This discrepancy may be attributed to the differences in the growth medium and bacterial strains used. Choi et al. (*27*) reported that the level of β-glucosidase activity by *Bifidobacterium* varies significantly depending on the type of growth medium. Tsangalis et al. (*23*) also observed that the level of β-glucosidase activity in bifidobacteria depended mostly on the formulation of the growth medium. To select among tested bacterial strains for further analyses, a threshold value of β-glucosidase activity equal to 10.0 U/g of dry cell weight was arbitrarily chosen. Thirteen of the 38 investigated strains had a β-glucosidase activity value that fell above the threshold limit. Among them, five strains (B7003, B7875, B7304, B7377, B7824), each representative of one *Bifidobacterium* species and with the highest β-glucosidase activity within the species, were used for flavonoid metabolism analyses.

Metabolism of Flavonoids by Selected Strains. Strains B7003 (*B. pseudocatenulatum*), B7875 (*B. infantis*), B7304 (*B. adolescentis*), B7377 (*B. catenulatum*), and B7824 (*B. breve*) were incubated with daidzin, genistin, kaempferol 3-*O*-glucoside, and kaempferol 3-*O*-xylosylglucoside for 24 h. For the five selected bifidobacteria strains, HPLC analysis detected metabolites eluting at 7.2 ± 0.1 min from the cultures incubated with daidzin (peak 1, **Figure 2A**), at 10.3 ± 0.1 min from the cultures incubated with genistin (peak 2, **Figure 2B**), at 7.6 ± 0.1 min from the cultures incubated with glycitin (peak 3, **Figure 2C**), and at 10.7 ± 0.2 min from the cultures incubated with kaempferol 3-*O*-glucoside (peak 4, **Figure 2D**). These metabolites were absent in the TPY medium incubated with daidzin, genistin, glycitin, and kaempferol 3-*O*-glucoside and in control bacterial cultures incubated without flavonoids for the same duration. The retention time and UV spectrum of the metabolite eluting at 7.2 ± 0.1 min in daidzin cultures corresponded with those of standard daidzein. Similarly, the retention times and UV spectra of the metabolites eluting at 10.3 ± 0.1 min in genistin cultures and at 7.6 ± 0.1 min in glycitin cultures corresponded with those of standard genistein and glycitein, respectively. Finally, the compound eluting at 10.7 ± 0.2 min from the cultures incubated with kaempferol 3-*O*-glucoside was identified as kaempferol on the basis of UV spectrum and retention time of authentic standard. No metabolites were detected after incubation of the cultures with kaempferol 3-*O*-xylosylglucoside (peak 5, **Figure 2E**). This was not surprising as the selection of bifidobacteria was based on their capability of converting a monoglucosidic compound (pNPG) to its agluconic form (pNP). Thus, their inactivity with a diglucosidic compound, as is kaempferol 3-*O*-xylosylglucoside, was expected. However, as intestinal bacteria are known to be equipped with a vast array of enzymes of hydrolysis of various dietary flavonoid glycosides (*20*), it is not improbable that other bifidobacteria strains, not directly investigated in the present study, could succeed in the biotransformation of kaempferol 3-*O*-xylosylglucoside.

To verify the effective bioavailability of flavonoid aglycones, the degradation rates of daidzein, genistein, glycitein, and kaempferol, following incubation with selected strains, were monitored (**Figure 3**). The incubation of 400 μM concentrations of the aglycones daidzein, genistein, glycitein, and kaempferol for 4, 8, 12, and 24 h with selected bifidobacteria strains highlighted a correlation between chemical structures and microbial degradation of flavonoids.

The degradation rates of genistein (5,7,4′-trihydroxyisoflavone) and kaempferol (3,5,7,4′-tetrahydroxylflavone) with average $k = 0.12 \pm 0.01$ h^{-1} and $k = 0.08 \pm 0.02$ h^{-1}, respectively, were significantly faster than those of daidzein (7,4′-dihydroxyisoflavone) and glycitein (7,4′-dihydroxy-6-methoxyisoflavone) with average $k = 0.15 \pm 0.01$ h^{-1} and $k = 0.16 \pm 0.01$ h^{-1}, respectively. Data suggested that the number and position of hydroxyl groups are important structural characteristics for flavonoid degradation by human bifidobacteria. In particular, flavonoids with hydroxyl groups at the 5-, 7-, and 4′-positions (genistein and kaempferol) were found to be more rapidly degraded than the other examined flavonoids (daidzein and glycitein). This was in agreement with literature data. Previous findings by Griffiths and Smith (*28*) showed that isoflavones possessing a 5-hydroxyl group were much more susceptible to cleavage of the central ring system by rat intestinal bacteria, and the same capability was observed for several strains isolated from the human gut (*20*, *29*). More recently, Simons et al. (*30*) observed that all of the flavonoids with 5-, 7-, and 4′-hydroxyl

groups were degraded more quickly by human gut microflora than the flavonoids lacking any one of these hydroxyl groups.

Because kaempferol and genistein were the most rapidly degraded flavonoids, they may not be as bioavailable in the colon as compared to more slowly degraded flavonoids, as they have less time to be absorbed before being metabolized by gut microflora. However, the degradation products of kaempferol and genistein may potentially be bioactive metabolites of interest. Several studies reported that the major metabolites produced following flavonoid aglycone fermentation with human fecal flora were phenolic compounds in which, as the flavonoid precursors, may reside antioxidant capacities (*31*, *32*).

Transformation of Common Bean Flavonoids by Selected Strains. The effective capability of selected bifidobacteria strains of transforming flavonoids directly extracted from bean matrix was also assessed. Following results achieved in our earlier studies (*9*, *10*), flavonoids from seeds and 2−3-day-old seedlings of the Italian common bean landrace "Zolfino del Pratomagno" were extracted and added to the selected strain cultures. Following 24 h of incubation under the conditions previously described for flavonoid standards, the metabolites were analyzed by HPLC. All five selected bifidobacteria strains showed the same capability in converting the flavonoid glycosides occurring in common bean matrices. As an example, in **Figure 4** the HPLC elution profiles of flavonoids extracted from bean seeds (**Figure 4A**,**B**) and seedlings (**Figure 4C**,**D**), incubated without (**Figure 4A**,**C**) and with (**Figure 4B**,**D**) the bifidobacteria strain B7003 (*B. pseudocatenulatum*), are reported. The HPLC profile of *P. vulgaris* seed extracts incubated in the absence of the B7003 strain revealed the presence of three main peaks (**Figure 4A**), identified as kaempferol 3-*O*-xylosylglucoside (peak 5) kaempferol 3-*O*-glucoside (peak 6), and an unidentified monoglucoside derivative of kaempferol (peak 7), on the basis of our previous findings (*9*). After the incubation with B7003 culture, peak 5 was still detected, confirming the incapability of selected bifidobacteria in hydrolyzing the diglucosidic compound kaempferol 3-*O*-xylosylglucoside. On the other hand, peaks 6 and 7 disappeared following fermentation and a new peak was detected (peak 4, **Figure 4B**) and identified as kaempferol on the basis of the comparison of retention time and UV/DAD spectrum with those of authentic standard. Analogously, the incubation of 2−3-day-old seedling extracts with B7003 culture highlighted the presence of four main peaks (**Figure 4D**), identified as daidzein (peak 1), glycitein (peak 3), genistein (peak 2), and kaempferol (peak 4) on the basis of retention times and UV/DAD spectra with authentic standards. These metabolites were not present in the HPLC elution profile of seedling extracts incubated without the B7003 strain (**Figure 4C**). On this basis, the presence of monoglucoside *O*-derivatives (peaks eluting between 1.8 and 2.7 min, **Figure 4C**) of detected flavonoid aglycones in the seedling extracts could be inferred.

Conclusions. In this study, the conversion of flavonoid glycosides by human bifidobacteria strains was investigated. In particular, the biotransformation into aglyconic forms was assessed for flavonoid glycosides such as kaempferol 3-*O*-xylosylglucoside, kaempferol 3-*O*-glucoside, daidzin, genistin, and glycitin, previously detected by our group in some Italian common bean ecotypes (*9*, *10*). Our earlier findings showed that the investigated common beans contain three glycosidic forms of the flavonol kaempferol at the seed level (*9*), and the isoflavones daidzein, genistein, and glycitein, along with kaempferol, were detected in 2−3-day-old seedling extracts after acid hydrolysis (*10*). Generally, among legume crops, soybean is considered to be the most relevant source of flavonoids

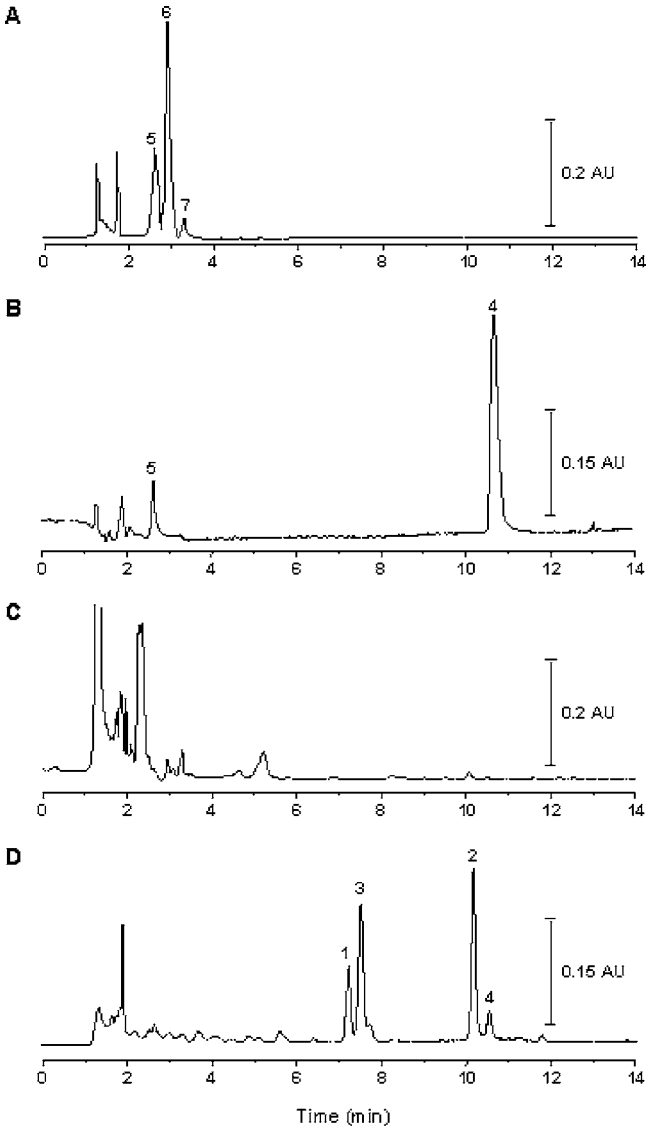

Figure 4. HPLC chromatogram (λ = 260 nm) of common bean extracts from seeds (**A**, **B**) and seedlings (**C**, **D**) incubated without (**A**, **C**) and with (**B**, **D**) B7003 (*B. pseudocatenulatum*) strain. Peaks: 1, daidzein; 2, genistein; 3, glycitein; 4, kaempferol; 5, kaempferol 3-*O*-xylosylglucoside; 6, kaempferol 3-*O*-glucoside; 7, unidentified monoglucoside derivative of kaempferol. AU, absorbance units.

(isoflavone phytoestrogens) with beneficial health effects, and therefore a huge range of soy products, both traditional and nontraditional, are available on the market today as healthy foodstuffs (*33*). The literature presents many reports of the biotransformation of isoflavones in soybean-derived products by lactic acid bacteria and bifidobacteria (*23*, *34*−*39*). In this context many *Lactobacillus* spp. and *Bifidobacterium* spp. strains were proposed to have potential as functional starter cultures for developing fermented soybean-derived products with higher estrogenicity and better absorption, facilitating the bioavailability of isoflavones. However, soybean is traditionally consumed in Asian countries, and fermented products based on substrates of soybeans and soybean milk have had little application in Western countries. On the other hand, the existence of a wide variety of bean-based dishes in the traditional cooking of many Mediterranean people testified that nonsoybean legumes, such as common beans, play an important role in the diet of those countries. With these premises common bean could be consid-

ered as a valuable and "Mediterranean" alternative to soybean to obtain plant-derived products with health benefits. As a matter of fact, the idea of preparing dairy products with legumes other than soybean is not new. Jiménez-Martinez et al. (*40*), for example, successfully obtained a yogurt-like product from lupin seeds.

In addition, the five strains of bifidobacteria identified in the present study, being active in the biotranformation of flavonoid glycosides occurring in common bean seeds and seedlings, could be considered as probiotic dietary adjuncts to improve the organoleptic properties, nutritional value, and health beneficial effects of a vast array of flavonoid-based products, comprising hypothetical common bean food derivatives.

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