Isoflavones and Functional Foods Alter the Dominant Intestinal Microbiota in Postmenopausal Women[1]

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ABSTRACT Dietary phytoestrogens, such as isoflavones, are used as food additives to prevent menopause-related disorders. In addition to other factors, their bioavailability strongly depends on the activity of intestinal bacteria but the underlying interactions remain poorly understood. A randomized, double-blind, placebo-controlled study was undertaken with 39 postmenopausal women to characterize changes in the dominant microbial communities of the intestinal tract after 2 mo of isoflavone supplementation with and without pro- or prebiotic. The diversity and composition of the dominant microbiota were analyzed by temporal temperature-gradient gel electrophoresis (TTGE) and fluorescent in situ hybridization. Isoflavones alone stimulated dominant microorganisms of the *Clostridium coccoides-Eubacterium rectale* cluster, *Lactobacillus-Enterococcus* group, *Faecalibacterium prausnitzii* subgroup, and *Bifidobacterium* genus. The stimulation of the *Clostridium coccoides-Eubacterium rectale* cluster depended on the women's equol excretion and was transient, with the exception of a prolonged bifidogenic effect. Lasting changes in the diversity of the dominant species were also observed. The probiotic strain supplied could be detected by TTGE during its passage through the intestinal tract, and ingestion of fructooligosaccharides triggered a marked and specific bifidogenic effect. In conclusion, this is the first human study that shows changes in the diversity and composition of dominant bacterial communities in response to dietary supplementation with hormone-related compounds combined with functional foods. J. Nutr. 135: 2786–2792, 2005.

KEY WORDS: • *isoflavone metabolism* • *intestinal microbiota* • *functional foods* • *postmenopausal disorders*

Bacteria of the human intestinal tract form a complex ecosystem that remains poorly described despite its probable implication for health. In recent years, analyses of the 16S ribosomal RNA gene sequences have extended our knowledge of the composition and phylogenetic diversity of this ecosystem (1,2). A few bacterial groups make up 50–70% of the dominant microbiota, comprising mainly the *Clostridium coccoides-Eubacterium rectale* cluster (Erec),[3] the genus *Bacteroides* (and relatives) (Bac), the *Clostridium leptum* subgroup (including the genus *Faecalibacterium*), and the genus *Bifidobacterium* (Bif) (3–8). Moreover, the distribution of microbial species within these dominant groups is relatively specific and stable over time for healthy adults (9–11). In spite of its stability, this bacterial ecosystem must be viewed as a dynamic system, which constantly interacts with food components.

Intestinal bacteria can metabolize unabsorbed food components. Isoflavones, for example, are estrogen-related compounds found in plants, especially soy; their bioavailability depends upon gut bacteria (12,13). The bacterial transformations exerted toward isoflavones include deglycosylation (14), demethylation (15), reduction (16), and ring fission (17,18), which lead to changes in their biological activities. Relevant activities include estrogen agonism and antagonism, and antioxidant and enzyme-inhibiting properties (19). Thus, isoflavones are thought to prevent hormone-related diseases, such as osteoporosis (20,21), which is relevant to both public health and economy; it is also a disease of postmenopausal women in particular (22). One of the major isoflavones in the human

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[3] Abbreviations used: Ato, *Atopobium* group; Bac, *Bacteroides* and relatives; Banim, *Bifidobacterium animalis* and related species; Bif, *Bifidobacterium* species; Erec, *Clostridium coccoides-Eubacterium rectale* cluster; FISH, fluorescent in situ hybridization; FOS, short-chain fructooligosaccharides; Fprau, *Faecalibacterium prausnitzii* subgroup; Lab, *Lactobacillus-Enterococcus* group; TTGE, temporal temperature-gradient gel electrophoresis.

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diet is daidzein (4′,7-dihydroxyisoflavone). It can be metabolized to the end products equol or *O*-desmethylangolensin whose biological activities differ markedly (23). A growing number of intestinal strains were found to convert daidzein (15–18,24,25). The occurrence of these and related organisms within the intestinal ecosystem might explain the large interindividual variations in equol production, which is detected in only approximately one-third of the human population (26).

Food components can act as modulating factors of the intestinal ecosystem. The recent concept of functional foods refers to the use of nutritional supplements that may lead to alteration of the microbiota and exert beneficial health effects. Originally defined by Fuller in 1989 (27), probiotics are "live microbial food supplements that beneficially affect the host animal by improving its intestinal microbial balance." Prebiotics are "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (28). Finally, the term synbiotic defines "a product that contains both prebiotics and probiotics with expected synergistic effects" (29). Probiotics were used successfully in several clinical studies for the treatment of lactose intolerance and diarrhea (30). Certain prebiotics are known for their bifidogenic effect, as well as their positive influence on calcium availability (31,32).

To date, few studies have focused on the interactions between intestinal bacterial communities, isoflavone metabolism, and functional foods (33–38), and data for humans are limited (39). However, it is crucial to elucidate these interactions to better evaluate the possible health effects of dietary isoflavones. In the present study, we investigated the influence of isoflavones and functional foods on both the composition and diversity of the dominant intestinal microbiota in humans.

SUBJECTS AND METHODS

Participants. The randomized, double-blind, placebo-controlled study with parallel groups stratified by equol production level included 39 French postmenopausal women. The subjects (60.4 ± 7.1 y old) were selected after interviews and a health screening that included medical history and physical examination. Exclusion criteria were: vegetarian, high fiber or soy diet; nutritional supplementation, antibiotic, laxative or hormone replacement therapy up to 3 mo before the study; menstrual bleeding up to 12 mo before the study; surgically induced menopause or surgery of the digestive tract; history of chronic digestive disorders; involvement in any clinical trial up to 6 mo before the study; aversion or allergy to dairy or soy products. The study was approved by the local "Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale" in July 2001. The volunteers were fully informed of the aims of the study and gave their written consent. They were followed up during the study without any measured protocol deviation. Routine clinical laboratory tests were performed to monitor the subjects' health. A nutrition history questionnaire was used to elicit usual intake pattern.

Study design. After a 2-wk isoflavone, pro- and prebiotic run-in period and throughout the 2-mon challenge, subjects received 1 gelified milk (Danone Vitapole) and 1 cereal bar (Nutrition & Santé), twice a day, in addition to their usual diet. They were asked to exclude from their diet specific food items containing isoflavones, pro-, or prebiotics. During month 1 (d 0 to 30), the gelified milk and cereal bars together provided 100 mg/d of isoflavones aglycon equivalents (Prevastein®HC, Eridania Béghin-Say). On d 5, subjects were stratified according to their urinary equol production levels. Equol was measured in blood and urine samples using immunological methods (40). On d 30, subjects were randomly assigned to 1 of the 3 supplementation groups described below so that each group included high (>9 μmol/24 h, n = 16), low (<5 μmol/24 h, n = 8) and nonproducers of equol (0 μmol/24 h, n = 15). High and low producers of equol made up 41 and 20.5% of the cohort, respectively. Subjects maintained their equol production status throughout the study, with the exception of 3 individuals who were low producers at d 5 and for whom equol was not detected in urine at the end of the study, and 1 individual who was nonproducer at d 5 and became a low producer. During mo 2 (d 30 to 60), gelified milk and cereal bars provided 100 mg/d of isoflavones alone for the control group (n = 12), 100 mg of isoflavones combined with 125 mg of yogurt cultures (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and ~10^9 viable cells of the probiotic strain *Bifidobacterium animalis* DN-173 010) for the probiotic group (n = 14), or 100 mg of isoflavones combined with 7 g of short-chain fructooligosaccharides (FOS) (Actilight, Béghin-Meiji Industries) for the prebiotic group (n = 13). Fecal samples were collected on d 0, 30, and 60. The study design is summarized in **Figure 1**.

Collection and processing of fecal samples. Fecal samples were collected in plastic boxes, kept under anaerobic conditions using an anaerocult®A (Merck) and stored at 4°C for a maximum of 4 h before processing. For temporal temperature-gradient gel electrophoresis (TTGE), 0.2 g of feces (wet weight) were frozen and kept at −80°C for DNA extraction. For fluorescent in situ hybridization (FISH), 1-g fecal aliquots were fixed with paraformaldehyde as described previously (7).

Extraction of total genomic DNA and 16S ribosomal DNA amplification. DNA was extracted from 200 mg of feces as described previously (41). To amplify the V_6-V_8 region specific for the bacterial domain or the V_2-V_3 region specific for bifidobacteria, we used either primers U968-GC-f (5′ GC Clamp-AA CGC GAA GAA CCT TAC) and L1401-r (5′ GCG TGT GTA CAA GAC CC) (9) or Bif164-f (5′ GGG TGG TAA TGC CGG ATG) and Bif662-GC-r (5′ GC Clamp-CCA CCG TTA CAC CGG GAA) (42), respectively. PCR was performed as described previously (43). A modified PCR program was used for V_2-V_3 amplification: 95°C for 15 min, 27 cycles of 97°C for 1 min, 58.7°C for 1 min, and 72°C for 1 min 30 s, and finally, 72°C for 15 min. If no amplification was observed with a 10^{-1} dilution of template DNA, several concentrations (1 and 3 μL undiluted DNA, 1 μL of 10^{-2} or 10^{-3} dilutions) were tested and the lowest concentration giving a positive PCR was used for further analyses. PCR products were analyzed by electrophoresis on a 1% agarose gel (wt/v) containing ethidium bromide (Eurobio) in Tris-Borate-EDTA buffer (Amresco) to control their size (434 and 499 bp, respectively) and estimate their concentration.

TTGE procedure and similarity measurement. Electrophoreses were run in a Dcode Universal Mutation Detection System (Bio-Rad) that contained 2 polyacrylamide gels (43) immersed in TAE-buffer

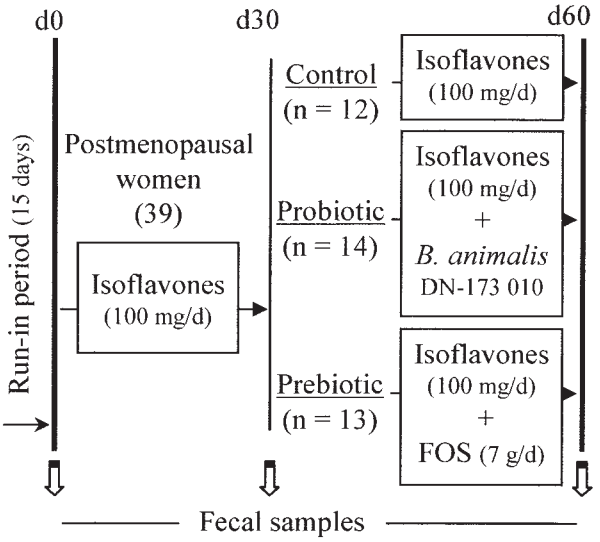

FIGURE 1 Study design. Isoflavones and functional foods were supplemented in cereal bars and gelified milk as described in the Materials and Methods section.

(50 mmol/L tris, 25 mmol/L acetate, 1.25 mmol/L EDTA, pH 8.3), at a initial intensity of 64 mA, an initial temperature of 66°C, a ramp rate of 0.3°C/h for 13.5 h and, after a short run of 20 min, at 20 V. Gels were loaded, stained and read as described previously (11). TTGE profiles were compared as described previously (44). Only intragel comparisons were performed. To estimate the reproducibility of the method, fecal aliquots from 5 individuals collected on d 0, 30, and 60 were analyzed using 8 independent PCR-TTGE analyses, which had a CV of 5.9%.

FISH and flow cytometry analyses. The specific probes used in this study are listed in **Table 1**. The abbreviations used in the text and the figures relate to the nomenclature of the probes (46). The specific probe Banim targets organisms of the species *Bifidobacterium animalis*, *B. lactis*, *B. gallicum* and *B. pseudolongum*. The EUB-338–5′ GCT GCC TCC CGT AGG AGT, conserved within the bacterial domain (47), and the NON-EUB-5′ ACA TCC TAC GGG AGG C were used as positive and negative controls, respectively. Hybridization of fixed bacterial suspensions and data acquisition were performed as described previously (7). Cell granularity was measured with the side scatter channel and assigned as the primary acquisition parameter. The voltage setting of the corresponding photomultiplier tube was 458 V with a threshold of 253 V. The multiplying factor of the photodiode used for detection of forward scatter signals was 10^2. Voltage settings for the FL1 and FL4 fluorescence were 649 and 800 V, respectively. All amplifiers were set to logarithmic mode. Cell enumeration was performed as described previously (7). Samples were analyzed in duplicate and bacterial percentages expressed as the means. Analyses were repeated if the CV was >10%. Because we systematically observed high CV with the Enterobacteriaceae-specific probe, the corresponding bacterial percentages were not included in the statistical analyses.

Statistical analyses. TTGE prevalences were the percentages of subjects for whom the double-band of the probiotic strain appeared at d 60 on the *Bifidobacterium*-specific PCR-TTGE gels. FISH results were expressed either as means ± SD or as prevalences. SD were calculated as follows: $SD = [\Sigma(x_i - \text{mean})^2/n]^{1/2}$, where x_i is the given value of sample i and n is the total number of samples. For a defined bacterial group, the FISH prevalences were the percentages of subjects who showed at least a 50% increase in the corresponding bacterial proportions over the month of dietary supplementation. Data were analyzed with the statistical software SPSS 11.5 (SPSS). The Shapiro-Wilk test was used to assess normal distributions. Variances were checked for their uniformity by means of *F*-tests. For the isoflavone supplementation (d 0–30), parametric data were analyzed by Student tests and nonparametric data by Wilcoxon tests. χ^2 analyses were performed: *1*) to compare the bacterial compositions with data from a previous study; and *2*) to compare equol production levels, as well as functional food supplementation groups, with respect to changes in bacterial prevalences. Data for the functional food supplementation (d 30–60) were analyzed by ANOVA and Kruskal-Wallis tests to compare the 3 groups. These tests were applied on delta values. For all of the tests, the bilateral α risk was $\alpha = 0.05$. Principal component analyses were performed with the statistical software SPAD 4.01 (Cisia Ceresta). This multivariate strategy was used to point out and correlate relevant variables, such as bacterial groups or equol excretion levels, that could partly explain the variations observed in the composition of dominant fecal microbiota.

RESULTS

Isoflavone dietary supplementation

Assessment of bacterial composition using FISH. In spite of large interindividual differences, average microbiota compositions at d 0 did not differ from bacterial proportions measured by our laboratory in 91 adults from 5 different European countries ($P > 0.10$) (48).

Isoflavones markedly altered dominant bacterial communities. After 1 mo of supplementation with 100 mg/d of isoflavones aglycon equivalents, the percentages of the Erec cluster ($P = 0.021$), the *Lactobacillus-Enterococcus* group ($P = 0.049$), the *Faecalibacterium prausnitzii* subgroup ($P = 0.004$) and the genus *Bifidobacterium* ($P = 0.014$) ($n = 39$) (**Table 2**) were significantly increased. In contrast, the supplementation did not affect the percentages of *Atopobium*- and *Bacteroides*-related organisms.

During mo 2 of isoflavone supplementation, bacterial percentages for the Erec cluster ($P = 0.017$), the *Lactobacillus-Enterococcus* group ($P = 0.005$), and the *F. prausnitzii* subgroup ($P = 0.034$) ($n = 12$) (Table 2) decreased significantly in control subjects. However, percentages of bifidobacteria tended to decrease ($P = 0.158$).

A principal component analysis showed positive correlations between the Erec cluster, the *Lactobacillus-Enterococcus* group and the *F. prausnitzii* subgroup: r(Erec, Lab) = 0.69 ($P < 0.01$), r(Erec, Fprau) = 0.57 ($P < 0.01$), r(Lab, Fprau) = 0.53 ($P < 0.01$) (**Fig. 2**). During mo 1, 13 individuals, including 12 equol producers, had simultaneous increases by > 50% in at least 2 of those bacterial groups (data not shown). Interestingly, changes in the percentages of *Bifidobacterium* species were not correlated with the other bacterial groups.

Changes in microbiota composition were associated with the equol excretion levels of the subjects; 9 of 15 nonproducers (60%) had at least a 50% increase in percentages of bifidobacteria, whereas only 8 of 24 producers (30%) did ($P > 0.10$). Conversely, the quantitative increase observed for the Erec cluster was dependant on equol excretion at d 5. Of the equol producers, 12 of 24 (50%) had at least a 50% increase in the percentages of the Erec cluster, whereas none of the nonproducers did ($P \leq 0.01$).

Assessment of bacterial diversity using TTGE. Isoflavones markedly altered the distribution of dominant bacterial species. Based on comparisons of intraindividual TTGE-pat-

TABLE 1

16S ribosomal RNA probes used in the FISH experiment

Target	Nomenclature	Sequence (5′-3′)	Reference
Bifidobacterium spp.	S-G-Bif-0164-a-A-18	5′ CAT CCG GCA TTA CCA CCC	(4)
Bacteroides and relatives	S-*-Bacto-303-a-A-17	5′ CC AAT GTG GGG GAC CTT	(6)
Clostridium coccoides-Eubacterium rectale cluster	S-*-Erec-0482-a-A-19	5′ GCT TCT TAG TCA RGT ACC G	(3)
Lactobacillus-Enterococcus group	S-G-Lab-0158-a-A-20	5′ GGT ATT AGC AYC TGT TTC CA	(45)
Atopobium group	S-*-Ato-0291-a-A-17	5′ GGT CGG TCT CTC AAC CC	(45)
Faecalibacterium prausnitzii subgroup	S-*-Fprau-0645-a-A-23	5′ CCT CTG CAC TAC TCA AGA AAA AC	(8)
Bifidobacterium animalis, B. gallicum, B. lactis and *B. pseudolongum* species	S-*-Banim-1167-a-A-18	5′ TGA CCC CGG CGG TCC CAC	Rochet et al., unpublished
Enterobacteriaceae	S-G-Enter-1418-a-A-15	5′ CTT TTG CAA CCC ACT	(7)

TABLE 2

Bacterial proportions of the dominant fecal microbiota in French postmenopausal women over the 2-mo challenge

		Proportions of dominant bacterial groups[1]						
Subject	Day	Bac	Lab	Erec	Fprau	Ato	Bif	Banim
All	0	7.7 ± 7.1	3.2 ± 2.8	17.6 ± 8.7	9.1 ± 5.2	3.8 ± 3.1	3.1 ± 2.0	ND
(n = 39)	30	7.8 ± 6.5	4.5 ± 3.3*	20.3 ± 8.8*	12.1 ± 6.1**	3.8 ± 2.9	4.3 ± 3.1*	1.1 ± 2.2
Control	0	7.0 ± 3.6	2.8 ± 2.0	17.3 ± 8.9	12.3 ± 3.7	3.7 ± 3.0	3.4 ± 2.0	ND
(n = 12)	30	8.0 ± 5.4	5.8 ± 3.8*	23.1 ± 10.0*	12.6 ± 4.9	4.4 ± 2.6	5.4 ± 3.8	1.8 ± 3.2
	60	5.7 ± 4.6	2.4 ± 1.5††	16.2 ± 7.5†	8.8 ± 2.4†	3.8 ± 3.1	4.4 ± 3.3	1.4 ± 2.6
Probiotic	0	7.3 ± 6.7	4.3 ± 3.5	19.1 ± 9.2	9.8 ± 6.3	4.0 ± 3.1	3.2 ± 2.4	ND
(n = 14)	30	7.8 ± 3.1	4.3 ± 3.1	19.4 ± 8.7	14.2 ± 6.9	3.4 ± 2.9	3.0 ± 2.1	0.3 ± 0.5
	60	6.7 ± 5.3	2.8 ± 2.6	17.6 ± 13.0	9.2 ± 7.4	3.7 ± 3.5	3.3 ± 3.7	0.8 ± 1.4
Prebiotic	0	8.8 ± 9.3	2.5 ± 2.1	16.1 ± 7.6	7.2 ± 4.5	3.8 ± 3.2	2.6 ± 1.3	ND
(n = 13)	30	7.5 ± 8.7	3.5 ± 2.5	18.9 ± 7.1	9.4 ± 5.3	3.7 ± 2.9	4.7 ± 2.8	1.3 ± 2.1
	60	5.1 ± 3.3	2.7 ± 2.1	16.7 ± 7.7	8.9 ± 5.4	3.5 ± 2.7	7.3 ± 5.3	3.3 ± 3.8

[1] Values are means ± SD. Bacterial groups are abbreviated as in Table 1. Asterisks indicate different from d 0: * $P \leq 0.05$; ** $P \leq 0.01$; daggers indicate different from d 30: † $P \leq 0.05$; †† $P \leq 0.01$ (two-tailed paired Student tests).

terns, the microbial diversity had 73% similarity for the whole cohort when comparing d 0 with 30. Twenty-five subjects displayed a similarity value < 80% without correlation to equol excretion (data not shown). The control group had 75% ± 12 similarity when comparing d 0 with 60, including 7 individuals with < 80%. Interindividual differences with respect to changes in microbial diversity were also present.

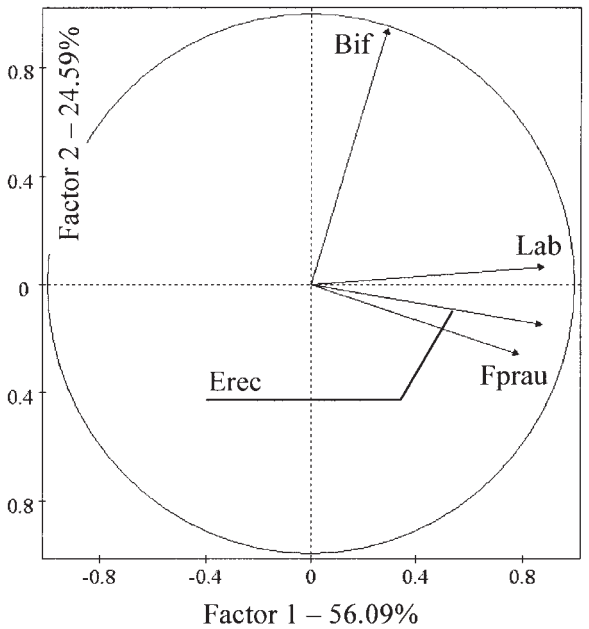

FIGURE 2 Principal Component Analysis: Quantitative variables (i.e., proportion of bacterial groups of the dominant intestinal microbiota) during mo 1 of isoflavone supplementation of postmenopausal French women (n = 39). Factor 1 is a projection of the Erec cluster, the *Lactobacillus-Enterococcus* group and the *F. prausnitzii* subgroup. Factor 2 is a projection of the genus *Bifidobacterium*. These factors illustrate 56.09 and 24.59%, respectively, of the quantitative variations within the aforementioned dominant bacterial groups. The proximity of 1 vector to the rim of the circle indicates a good projection of the corresponding variable. The proximity of 1 vector to another, as well as to the axes, shows the correlation between the 2 variables that are considered.

Functional food dietary supplementation

Functional foods triggered dynamic changes in dominant intestinal communities to a greater extent than the isoflavone supplementation. Between d 30 and 60, the microbial diversity had percentages of similarity of 71 ± 18 and 68 ± 16% for the probiotic and the prebiotic groups, respectively, as determined by TTGE.

Functional foods had an effect on total bifidobacteria (P = 0.030) and *B. animalis* and related species (P = 0.014), as determined by ANOVA. Individuals of the prebiotic group had increases in the corresponding bacterial proportions (Table 2). The probiotic group had a marked increase in TTGE prevalence (P = 0.003), and the prebiotic group was characterized by FISH prevalences of *B. animalis* and related species > 50% (P = 0.051) (**Fig. 3**). Urinary equol production did not differ among the groups.

DISCUSSION

For the first time in a human intervention study, the combined use of TTGE and FISH allowed an examination of the effects of nutritional supplementations on both qualitative and quantitative modifications of the dominant intestinal microbiota, thus providing complementary information on interactions between intestinal microbes and health-related dietary compounds. We showed that isoflavone, probiotic, and FOS ingestion led to significant dynamic changes in the composition and diversity of dominant microbial communities. The data on bacterial composition suggest the absence of a clear-cut relation between menopause and changes in dominant bacterial groups. Because of large interindividual differences, this observation warrants confirmation by intraindividual analyses over a long period of time in perimenopausal women.

Isoflavones altered the dominant microbiota, both qualitatively and quantitatively. Microbial patterns after 1 mo of isoflavone supplementation had 73% similarity with those at d 0. Moreover, the control group had 75% similarity between d 0 and 60. This reflects lasting changes within dominant bacterial species distributions compared with characteristic intraindividual stabilities illustrated by 83–96% similarity over a period of 3 mo (10). On the other hand, bacterial compositions returned to near original values during mo 2 of isoflavone supplementation, with the exception of *Bifidobacterium* spe-

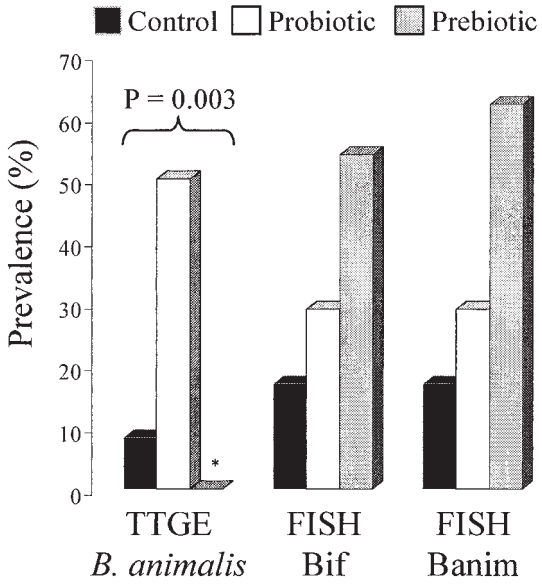

FIGURE 3 Prevalence of *Bifidobacterium* species during the 1-mo functional food dietary supplementation of French postmenopausal women for the control, pro-, and prebiotic groups. The P-value was obtained by χ^2 analysis. Prevalences are: percentages of subjects characterized by the appearance of the probiotic-specific double-band on the d 60-TTGE gels (TTGE *B. animalis*); percentages of subjects who showed at least a 50%-increase of total bifidobacteria (FISH Bif) or *B. animalis* and related species (FISH Banim); *the corresponding value is 0.

cies. This transient quantitative stimulation of the intestinal ecosystem does not exclude reorganization of bacterial species, such as apparition of organisms within the dominant microbiota. However, we did not systematically observe atypical new bands in TTGE patterns of control subjects at d 30 or 60.

The significant stimulation of Erec-related organisms after 1 mo of isoflavone supplementation agrees with the tendency toward higher clostridial numbers previously noted in vitro in response to a soygerm powder treatment (37). In addition, in early life, the late establishment of clostridia within the intestinal microbiota on the one hand, and the delayed establishment of daidzein conversion to equol on the other hand, are consistent with our finding (39). We did find an association between the ability of the subjects to produce equol, i.e., activate daidzein, and the stimulation of bacteria that belong to the Erec cluster. The stimulation was observed for equol producers only. Organisms of the Erec cluster such as *Peptostreptococcus productus* and *E. ramulus* (18) are involved in the metabolism of isoflavones. Thus, their specific stimulation likely influences the availability and biological properties of isoflavones. However, because the metabolism of isoflavones is a sensitive equilibrium between activation and inhibition of daidzein and genistein (18), it is difficult to determine to what extent the stimulation benefits the host. Nevertheless, because daidzein may be more available (49) and more efficient in preventing bone loss (50) than genistein, its activation by members of the Erec cluster is important. The relative stability of Erec percentages for nonproducers of equol suggests that it may be difficult to convert a nonproducer to an equol producer (23). The same authors pointed out the importance of dividing subjects between equol producers and nonproducers to clearly assess the effect of isoflavone supplementations. Our results suggest that the concentration of Erec-related organisms might be an indicator for equol production. This also sheds light on the link between the interindividual variations that we observed in intestinal microbiota compositions and the interindividual variations in isoflavones metabolism (51).

In addition, isoflavones displayed a relatively wide spectrum of responsive bacterial populations. Thus, the Erec stimulation was associated with quantitative increases of the *F. prausnitzii* subgroup and the *Lactobacillus-Enterococcus* group, and the latter increase was consistent with recent in vitro data (37,38). The fact that a combination of those 3 dominant bacterial groups explained 56.09% of the quantitative modifications between d 0 and 30 supports the idea of bacterial interactions in response to isoflavone ingestion. It is conceivable that isoflavones indirectly affect members of the dominant microbiota. In addition to the selective pressure they exert on intestinal microorganisms involved in their metabolism, they may modify environmental conditions of the intestinal tract with an ensuing effect on sensitive bacterial communities. Quantitative increases in bacterial population after 1 mo of isoflavone supplementation also occurred for the genus *Bifidobacterium*. Interestingly, this increase in bifidobacterial proportions was not associated with the other bacterial groups. It preferentially occurred in nonproducers of equol, whereas, as already demonstrated, the Erec stimulation was clearly linked to equol production. Thus, the bifidogenic effect of isoflavones might be rather specific. Overall, the bacterial changes observed during the isoflavone supplementation tended to show that the influence of hormone-related factors on bacterial composition would be relevant to the intestinal tract environment (52).

For the prebiotic group, based on increases in the percentage of total bifidobacteria and of *B. animalis* and related species, we confirmed the expected effect of FOS (31). Because the species *B. animalis* is not yet recognized as a member of the commensal dominant microbiota (53), the molecular strategy used here may have allowed the detection of FOS-stimulated endogenous species closely related to *B. animalis*. Because the subjects in the present study were all postmenopausal women $>$ 50 y old, the bifidogenic effect of FOS might be relatively independent of the age and hormonal status of the population. Because FOS are also known for their positive effects on calcium availability in humans (32), these observations are important for the further use of prebiotics in the elderly who usually have lower concentrations of bifidobacteria (54) and tend to suffer from osteoporosis. Moreover, it is conceivable that daidzein activation by Erec members and FOS ingestion act synergistically against bone disorders (36). However, recent studies suggested that FOS inhibit equol production (33,38). For the probiotic group, the strain *B. animalis* was detected in feces during supplementation. Interestingly, it was not accompanied by large increases in FISH prevalences. However, an overall quantitative stability does not exclude specific qualitative and quantitative adjustment within bacterial groups due to the probiotic ingestion.

In conclusion, simultaneous consumption of isoflavones and functional foods led to marked changes in the dominant intestinal ecosystem of postmenopausal women. Stimulation of the *C. coccoides-E. rectale* cluster and the underlying production of equol, as well as stimulation of *Bifidobacterium* species were of particular interest. In the future, it might be valuable to consider the bacterial indicators of daidzein activation pointed out in the present study and investigate the effects of such dietary supplementations over a longer time to appraise the alleged health consequences for the host.

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