Evaluation of Prebiotic Potential of Refined Psyllium (*Plantago ovata*) Fiber in Healthy Women

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Goal: To assess the effects of the consumption of psyllium seed husk on fecal bifidobacteria in healthy women and the ability of fecal bifidobacteria to metabolize psyllium seed husk in vitro.

Background: Poor microbiologic evidences are nowadays available concerning the ability of psyllium seed husk to promote the growth of bifidobacteria in human gut.

Study: Eleven healthy women consumed 7.0 g/d of psyllium seed husk for 1 month. Viability of bifidobacteria in feces was assessed at different time points.

Results: In vivo results showed that the average fecal content of viable bifidobacteria was not significantly affected even if fecal counts were found to increase significantly after treatment in 6 out of 11 women having low initial concentration. In vitro trials conducted on bifidobacteria strains isolated from treated women failed to confirm the prebiotic potential of undigested psyllium seed husk, whereas treatment with simulated gastric and pancreatic juices and mimicking physical and chemical alterations during human gut transit allowed fecal *Bifdobacterium* isolates to metabolize psyllium seed husk as carbon source in a growth medium deprived of sugar.

Conclusions: Psyllium seed husk can be metabolized by bifidobacteria only after partial hydrolysis. Bifidogenic potential can be detected in healthy women only in case of low level of fecal bifidobacteria before treatment.

Key Words: psyllium seed husk, ispaghula husk, water-holding capacity, prebiotic, *Bifidobacterium*

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Plantago ovata is an annual plant cultivated postmonsoon season in the north-western region of India. Different refinement levels lead from seed to purified seed husk, also known as ispaghula husk, containing a bioactive water-soluble mucilage polysaccharide. Psyllium seed husk is chemically composed of arabinoxylans that consist of β-xylan chains forming a backbone with arabinose side-chains.[1] It was compared with other dietary fibers for the ability to support the fermentative activity of fecal flora in vitro, concluding that ispaghula husk is only partially and slowly fermented by colonic flora.[2–4] Marlett and Fisher[5] and Marlett et al[6] recognized that psyllium seed husk is largely constituted of 3 fractions with only 1 of them degraded by colonic bacteria. Nevertheless, xylan fraction of psyllium seed husk has been demonstrated to be fermented by bifidobacteria after hydrolyzation.[7] Xylan was also found to act as a substrate for *Bifidobacterium adolescentis* and *Bifidobacterium infantis*.[8]

Psyllium seed husk is usually listed among the prebiotics for the treatment of inflammatory bowel disease, ulcerative colitis, and Crohn's patients[9,10] and in patients resected for colonic cancer.[11] Fujimori et al[12] observed the positive impact of the treatment of Crohn's patients with symbiotic therapy (probiotics plus psyllium fiber) for more than 12 months, with reduced incidence of flatulence and bloating symptoms.

Most of the evidences related to the positive impact of psyllium fiber on gut functions are not currently supported by measurement of microbiologic parameters such as viability of selected bacterial group like *Bifidobacterium* spp. The aim of the present study was to fulfill the lack of microbiologic investigations related to the bifidogenic effects of psyllium fiber by in vitro and in vivo trials.

MATERIALS AND METHODS

Experimental Design

Eleven healthy women with ages ranging from 35 to 47 years (mean age: 39.7 y) consumed 3.5 g of psyllium seed husk (Psyllogel, Nathura S.r.l., Montecchio Emilia, Italy) twice a day per 30 days. A washout period of 30 days was scheduled after the end of the consumption phase. Fecal samples were collected at the beginning of the trial (day 0) and after 15 (day 15), 30 (day 30), and 60 days (day 60). Viable bifidobacteria were enumerated by plating fresh fecal samples onto trypticase-peptone-yeast extract medium, prepared as described by Beerens,[13] and anaerobically incubated at 37°C for 72 hours. Bifidobacterial shape of plate colonies grown was confirmed by microscopy observation.

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Isolation, Identification, and Growth Trials of *Bifidobacterium* Spp. Strains

Colonies from 1 fecal sample of the volunteer showing the most significant increase in bifidobacteria counts at day 30 (subject no. 6) were subcultured in trypticase-peptone-yeast extract broth. Two isolates were first lysed by means of MicroLYSIS (Microzone Ltd, Haywards Heath, West Sussex, UK) and then amplified by polymerase chain reaction using species-specific primers for *B. adolescentis* and *Bifidobacterium longum*.[14,15] These strains were first assayed for their ability to metabolize intact psyllium seed husk by plating them onto deMan Rogosa Sharpe (MRS) fermentation medium (ADSA-Micro, Barcelona, Spain) supplemented with cysteine-HCl (Sigma-Aldrich, San Louis, MO) 0.5 g/L and 1% glucose (final concentrations), as positive control, and onto modified MRS fermentation medium obtained by replacement of glucose with 1% psyllium seed husk final concentration *Bifidobacterium* spp. strains were also plated onto MRS medium containing partially digested psyllium seed husk, obtained by incubation with simulated gastric and pancreatic juices,[16] as sole carbon source. Standard agar medium supplemented with 1% glucose was used as positive control. Plates were incubated at 37°C for 72 hours in anaerobic conditions.

RESULTS

In Vivo Assessment of the Bifidogenic Potential of Psyllium Seed Husk

Study results failed to demonstrate any prebiotic effect of psyllium seed husk on fecal *Bifidobacterium* spp. Statistical analysis of the overall data revealed that the variations observed among treated subjects were not statistically significant ($P > 0.05$). More detailed examination of the results suggested that volunteers could be divided into 2 subgroups differing for the concentration of viable bifidobacteria in feces before the beginning of the trial. Five volunteers had in fact 10 or more $\log_{10}$ colony forming units (CFUs) *Bifidobacterium* spp. per gram of wet feces before the beginning of the assumption period (subgroup 1), whereas the remaining 6 volunteers had fecal counts under 10 $\log_{10}$ CFUs/g of wet feces at day 0 (subgroup 2). During and after treatment (day 15 and day 30), subgroup 1 showed a slight decrease in bifidobacteria with average counts of 9.40 ± 0.98 and $9.62 \pm 0.33 \log_{10}$ CFUs, respectively, compared with day 0 (mean value $10.1 \pm 0.12 \log_{10}$ CFUs) ($P < 0.05$). On the contrary, subgroup 2, presenting an average count of viable bifidobacteria of $8.32 \pm 1.01 \log_{10}$ CFUs at day 0, showed a statistically significant ($P < 0.05$) increase at day 15 and at day 30 with mean values of 8.84 ± 0.96 and 9.72 ± 0.54, respectively. None of the considered subgroups evidenced significant variation during the washout period, as showed by mean values of fecal bifidobacteria at day 30 and at day 60 (subgroup 1 ranging from 9.62 ± 0.33 to $9.66 \pm 0.95 \log_{10}$ CFUs/g of wet feces and subgroup 2 from 9.72 ± 0.54 to $9.86 \pm 1.10 \log_{10}$ CFUs).

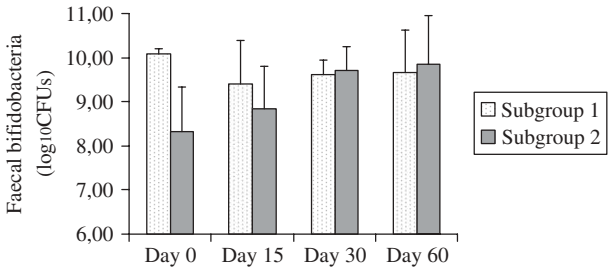

FIGURE 1. Comparison of the mean bifidobacteria fecal counts (in $\log_{10}$ CFUs) of the 2 subgroups of psyllium seed husk-treated volunteers at 4 different time points (day 0, day 15, day 30, and day 60). Gray bars refer to women presenting less than 10 $\log_{10}$ CFUs at day 0 (subgroup 2). Dot bars show average fecal bifidobacteria counts of subjects presenting equal or more than 10 $\log_{10}$ CFUs/g of wet feces at day 0 (subgroup 1). CFU indicates colony forming unit.

A graphical presentation of the results described above is given in Figure 1.

Fermentation of Intact and Partially Digested Psyllium Seed Husk by *Bifidobacterium* Strains Isolated From Human Feces

B. adolescentis and *B. longum* strains, isolated from volunteer no. 6 after treatment, were used to perform growth trials aimed to confirm the prebiotic potential of psyllium fiber in vitro. No colonies were retrieved from psyllium-supplemented medium, indicating that bifidobacteria were not able to ferment this fiber. Psyllium seed husk was therefore treated with simulated gastric and pancreatic juices before performing growth trials. Partially gastric-digested commercial preparation allowed bifidobacteria isolates to achieve growth results comparable with those obtained with glucose as sole energy source (Table 1).

DISCUSSION

Our in vivo study conducted on healthy women indicated that the consumption of psyllium seed husk for 1 month had no impact on fecal bifidobacterial content as statistically insignificant variations were detected. Broad differences in fecal bifidobacteria were detected among the 11 tested volunteers at day 0. This observation induced to divide the population at day 0 in 2 subgroups; women showing fecal bifidobacteria under 10 $\log_{10}$ CFUs took advantage from psyllium seed husk consumption, whereas subjects with higher initial counts confirmed what was previously stated by Morelli and Callegari[17] reporting about the difficulties in increasing the presence of lactic acid bacteria population in healthy subjects.

In vitro trials conducted with natural isolates failed to confirm the ability of bifidobacteria to ferment intact psyllium seed husk as sole carbon source, as *Bifidobacterium* colonies were only retrieved from plates containing pepsin-treated or pancreatin-treated psyllium. We can, therefore, speculate that modifications undergone during gastric transit could be responsible for the fractionation

TABLE 1. Growth of *Bifidobacterium* spp. Strains, Isolated at Day 30 From Volunteer No. 6, on Intact and Partially Digested Psyllium Seed Husk

		Growth (CFU/mL of Washed Culture)			
		Standard Control Medium	Supplemented FB Medium		
Isolate No.	Identification	FB Glucose	Psyllium Seed Husk	Pepsin-treated Psyllium Seed Husk	Pancreatin-treated Psyllium Seed Husk
6/2	*Bifidobacterium adolescentis*	5.70	nd	5.30	5.23
6/3	*Bifidobacterium longum*	5.78	nd	5.48	5.70

CFU indicates colony forming unit; FB, fermentation broth; nd, not detected, absence of growth.

of psyllium seed husk, therefore making the prebiotic fraction available for bifidobacteria. However, the chemical nature of the molecules produced from the pepsin-digested psyllium seed husk still remains to be identified.

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